A DEFICIENCY in vascular responsiveness to vasodilator substances is an attractive hypothesis to explain the development of hypertension. Unfortunately, experimental hypertension has been reported to increase, not change or decrease, the sensitivity of blood vessels to vasodilator substances. Much of this variation in findings may be due to the type of hypertension (deoxycorticosterone acetate-salt, renovascular, genetic), the type of preparation (vascular strip or perfused vasculature), and the methods of analysis. An important new observation by Furchgott and colleagues may shed light on another mechanism for altered sensitivity to vasodilator agents. They found that the presence of endothelial cells was obligatory for the vasodilator response to some (e.g., acetylcholine) but not all dilator substances (e.g., adenosine). Thus, they suggested that acetylcholine stimulated endothelial cells to release an unknown, short-lived relaxing factor termed endothelium-derived relaxing factor (EDRF). The physiological role EDRF plays in hypertension and in reactive hyperemia following ischemia is unclear. The endothelium may release less EDRF in hypertension, so that vasodilatation to some stimuli is diminished.

We have studied the effects of intravenous infusions of acetylcholine, adenosine, and serotonin and the reactive hyperemic response to ischemia in the intact hindquarter circulation of the conscious, chronically instrumented rabbit. Our aim was to investigate the effects of 5 weeks of cellophane perinephritic hypertension on the reactivity of a resistance bed to ischemia and to endothelium-dependent (acetylcholine) and endothelium-independent (adenosine) vasodilator substances. In addition, we prepared separate groups of rabbits that had spent 4 weeks on a high cholesterol diet.
Materials and Methods

Animals and Operations

Male and female adult rabbits developed from a colony of New Zealand white and English multicolored strains were used in this study. Rabbits weighed 1.9 to 2.7 kg (average, 2.3 kg). All animals were kept in individual cages on a 12-hour light/dark cycle and had free access to food and water. A preliminary operation was performed with the rabbits under open circuit halothane (Fluothane) anesthesia, after induction with propanidid (Epontol), 30 mg/kg i.v. A midline abdominal incision was made, and a continuous wave ultrasonic blood flow transducer (4 mm inside diameter) was placed around the lower abdominal aorta just above the iliac bifurcation. A Silastic balloon (for obtaining reference zero flow) was placed around the aorta just proximal to the flow transducer. The balloon catheter and the Doppler lead wires were led subcutaneously to the back of the neck. Through the abdominal incision, both kidneys then were exposed and wrapped in cellophane. The renal fat was separated from the capsule, the kidney gently lifted, and a cellophane square (approximately 12 × 12 cm) was wrapped around the kidney. The ends of the cellophane were gathered at the hilum and secured loosely with a silk tie. Sham operations were performed in a second group of rabbits. The kidneys were exposed but not disturbed. A sham operation or renal wrapping was performed alternately. Rolitetracycline (Reverin), 40 mg/kg i.m., was administered at the end of the operation.

On the day of the experiment, the lead wires from the flow transducer and the balloon catheter were retrieved from below the skin of the neck and the central ear artery and vein were cannulated with the rabbits under local anesthesia (0.5% lignocaine [Xylocaine]). The arterial catheter was connected to a Statham P23Db pressure transducer (Gould, Saddle Brook, NJ, USA), and the mean and phasic blood pressure recorded on a Grass polygraph (Model 7; Grass Instrument, Quincy, MA, USA). Variables recorded were heart rate, blood pressure, and heart period recorded for 30 minutes through a catheter inserted in a central ear artery with the rabbits under local anesthesia (0.5% lignocaine). On each occasion, 2 ml of blood was withdrawn from the arterial catheter for determination of plasma cholesterol concentration. After the preliminary operative procedures (see Animals and Operations), the rabbit then underwent pharmacological autonomic effector blockade to prevent reflexes complicating hemodynamic responses to vasodilator drugs. The first step in autonomic blockade was achieved by intravenously infusing guanethidine sulfate (Ciba-Geigy, Basel, Switzerland), 10 mg/kg in 10 ml of dextran 40 (Rheomacrodex), slowly over 1 hour. This dose blocks constrictor responses to lumbar sympathetic nerve stimulation for 4 to 6 hours but does not interfere with secretion of adrenal catecholamines. At the end of this infusion the following drugs were given intravenously: phenolamine mesylate (Regitine) 1 mg/kg bolus and 1.5 mg/kg/hr infusion, and propranolol hydrochloride (ICI, Macclesfield, Cheshire, England), 0.5 mg/kg bolus and 2.4 mg/kg/hr infusion. These doses were adequate to block heart rate and peripheral ß-adrenergic and ß-adrenergic receptor mediated responses to intravenous doses of isoprenaline, 1.5 µg/kg, and norepinephrine, 2 µg/kg. Following the dose-response curve to acetylcholine,
autonomic effector blockade was completed by intravenous administration of methscopolamine bromide (Upjohn, Kalamazoo, MI, USA), 50 μg/kg bolus and 50 μg/kg/hr infusion.

Infusion of Vasodilator Drugs
Fifteen to twenty minutes after "partial" autonomic blockade, a dose-response curve was constructed to a steady state intravenous infusion (Harvard infusion pump, Millis, MA, USA) of acetylcholine bromide (Sigma) in the range of 0.3 to 60 μg/kg/min. Each infusion level was maintained for 3 minutes or until the parameters had reached plateau. A higher infusion rate was then given and so on until increments in infusion rate did not lower the resistance any further. The maximum infusion rate necessary to cause the minimum resistance varied as much as threefold among rabbits. Volumes infused were 0.08 to 3.24 ml/min. Intravenous infusion of saline over this range had no effect on resting hemodynamics. Following the acetylcholine infusion, methscopolamine bromide was administered as described to complete the autonomic effector blockade. The rabbit then rested for 30 minutes. Responses to adenosine infusion, 30 to 3000 μg/kg/min, were tested after this rest period and followed 30 minutes later by infusion of serotonin, 3 to 300 μg/kg/min. Higher doses of serotonin (600 and 1200 μg/kg/min) began to cause constriction. Reactive hyperemic responses to timed periods of aortic occlusion were measured 30 minutes after the serotonin infusion ended. In early experiments a second EDRF-dependent vasodilator, substance P, was used. The responses to bolus injection (5-20,000 μg/kg) were inconsistent and showed a high degree of tachyphylaxis.

Reactive Hyperemia
Local regulation of blood flow was measured as the reactive hyperemic response to periods of hindlimb ischemia. The lower aortic balloon catheter was inflated to lower iliac bed flow to zero for periods of 5, 10, 20, 40, and 80 seconds. The chart paper speed was increased to 100 mm/min, and the hyperemic response was recorded on abrupt release of the balloon. Iliac bed flow was allowed to return to the resting value before applying subsequent periods of ischemia. Periods of hindlimb ischemia were completed in ascending order of duration.

Assay and Staining Techniques
Serum cholesterol concentrations were determined enzymatically (Boehringer, Mannheim, W. Germany) using high and low sera standards with a Technicon Autoanalyzer II (Tarrytown, NY, USA). In 10 rabbits (approximately 7 weeks after operation), aortas were dissected free from the aortic root to the iliac bifurcation. The aortas were cut longitudinally, pinned out on wax blocks, and fixed for 1 hour in 10% formalin. The specimens were then stained with Sudan IV (Herzheimer's solution).

Drugs
In addition to those that have already been mentioned, drugs used included adenosine (Sigma) and 5-hydroxytryptamine creatinine sulfate (Sigma). All drugs were freshly prepared on the day of the experiment. Drugs were diluted in 0.9% saline solution for intravenous administration with the exception of guanethidine, which was diluted in 10% dextran 40 in dextrose. All drug concentrations were calculated from the weight of the salt.

Parameter Measurements and Statistical Analysis
At weekly intervals each rabbit had its heart period and ear artery blood pressure measured for 30 minutes, after which time 10 measurements (approximately 15 seconds apart) were averaged to give a resting value (see Figure 1). On the experimental day (Day 35), the rabbit rested quietly for approximately 30 minutes after the preliminary operative procedures, and resting values for cardiovascular parameters then were measured. During the sequential development of pharmacological autonomic blockade, parameters were measured when they had reached steady state (approximately 10-15 minutes; see Figure 2). During infusions of the vasodilator drugs, parameters were measured at the plateau response to each dose (approximately 3 minutes). The reactive hyperemic response to hindlimb ischemia was measured by calculating the area (Complot 7000 [Bausch & Lomb, Austin, TX, USA] digitizing tablet attached to a microcomputer) under the mean blood flow trace on abrupt release of the aortic cuff until flow had returned to rest (termed repayment in Figure 7). The flow debt (during occlusion) was measured as the area of the fall in hindlimb flow from rest to zero until release of the balloon cuff (see Figure 7).

Average standard error of the mean within animals for each parameter was calculated from two-way analysis of variance (ANOVA) as (error mean square/ number of animals) after subtracting the sums of squares between animals and between times from the total sums of squares for each drug time. When shifts in sensitivity (location of 50% effective dose [ED50]) values) to the vasodilator agents were compared, the changes in iliac vascular resistance (IVR) for each rabbit were first computer-fitted to a sigmoid curve function and the data expressed as a percentage of the maximum fall in IVR for each agonist. From each curve, dose values for ED10, ED50, ED90, and ED100 were taken. For each treatment group, average curves were constructed from the mean values of these doses at the different response levels (10-90%). Comparisons of hemodynamic changes, ED90 values, and so on for different agonists among the four treatment groups were performed by three-way ANOVA statistics. Where appropriate, two-way ANOVA with orthogonal partitioning was also used. Significance was accepted as a p level of less than 0.05.
Results

Mean blood pressure did not change significantly throughout the 5-week period in the sham-operated rabbits fed either the normal diet or the 1% cholesterol diet (80 ± 2 and 84 ± 2.2 mm Hg, respectively, Day 35; Figure 1). Cellophane perinephritis caused a similar increase in mean blood pressure over the 5-week period in the wrap-operated rabbits on either normal or 1% cholesterol diet (134.7 ± 2.4 and 133 ± 3.6 mm Hg, respectively, Day 35; see Figure 1). Heart period fell significantly 7 days after operation in all four treatment groups (paired t test; \( p < 0.05 \); see Figure 1). During the ensuing 4 weeks, heart period returned to control values in the sham-operated rabbits on normal diet but remained low in the renal-wrap animals on normal or 1% cholesterol diet and in the sham-operated animals on 1% cholesterol diet (2-way ANOVA; \( p < 0.05 \)).

Rabbits in all groups gained little weight in the first 7 postoperative days (Table 1). In the ensuing 4 weeks all rabbits gained weight at approximately the same rate with the exception of the renal-wrap rabbits receiving 1% cholesterol, which began to lose weight during the final 7 days.

Plasma cholesterol concentrations did not change significantly during the 5-week period in the sham-operated and renal-wrap rabbits on normal diet (see Table 1). In the two groups on 1% cholesterol diet, plasma cholesterol concentrations were greatly elevated at the first measurement 7 days after commencement of the diet (2-way ANOVA; \( p < 0.05 \); Day 14 in Table 1), and plasma cholesterol levels continued to rise throughout the study.

On Day 35, the IVR in each group was (relative to sham-operated rabbits) renal wrap and cholesterol diet, 229%; renal wrap, 223%; sham-operated and cholesterol diet, 121%; sham-operated, 100%. Of these, the IVR of hypertensive rabbits was elevated significantly (Table 2 and Figure 2). Total autonomic effector blockade was developed in stages. Hemodynamic responses to this serial blockade are given in Figure 2. Overall comparisons of these parameters before and after total autonomic blockade (within rabbit) indicate that the IVR rose substantially above predrug values only in the normotensive rabbits (by 50%), principally after the administration of propranolol and phentolamine (see Table 2 and Figure 2). Following the infusion of guanethidine, heart period was not different from control values in the four treatment groups. Mean blood pressure fell significantly in all groups except for the sham-operated animals on normal diet (see Figure 2). The IVR fell significantly in the hypertensive rabbits (2-way ANOVA; \( p < 0.05 \)). Following administration of phentolamine and propranolol, heart period increased significantly in the sham-operated animals on normal and 1% cholesterol diets (2-way ANOVA; \( p < 0.05 \)). Mean arterial blood pressure was not different in any group following \( \alpha \)-adrenergic and \( \beta \)-adrenergic receptor blockade. The IVR increased above resting values in the two sham-operated groups following phentolamine and propranolol (2-way ANOVA; \( p < 0.05 \)). These antagonists caused a rise in IVR in the two wrapped groups to levels that were not significantly different from those at rest. After completion of the autonomic effector blockade with administration of methscopolamine, heart period was significantly decreased in all groups (2-way ANOVA; \( p < 0.05 \); see Table 2 and Figure 2). Mean blood pressure increased in all groups except for the wrapped animals on 1% cholesterol diet, in which the average value was not different from that at rest. Although muscarinic blockade further increased IVR to levels well above resting values in the two sham-operated groups (2-way ANOVA; \( p < 0.05 \)), in the renal-wrap rabbits the IVR rose only to levels similar to the resting values (see Table 2 and Figure 2).

There were no significant differences among the IVR values at rest (within rabbit) just before infusion of each of the three vasodilator drugs in the four groups (3-way ANOVA); however, the IVR did not return to resting values 30 minutes after cessation of the serotonin infusion. Before the start of the reactive hyperemia experiments, IVR values in each group were about 18% lower than equivalent values before the serotonin infusion (Table 3).

During maximum dilatation with acetylcholine, mean arterial pressure fell to as low as 54% of resting values in the sham-operated rabbits on 1% cholesterol diet (Table 4). Values in the two renal-wrap groups fell

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**Figure 1.** Heart period (HP) and mean arterial pressure (MAP) values measured in conscious rabbits during a 5-week period. Day 0 was just before renal cellophane wrap or sham operation; Day 7 indicates commencement of cholesterol or normal diet; Day 35 was the day of the experiment. Groups were sham operation + normal diet (○; \( n = 7 \)); sham operation + 1% cholesterol diet (○; \( n = 6 \)); renal wrap + normal diet (△; \( n = 7 \)); and renal wrap + 1% cholesterol diet (Δ; \( n = 6 \)). Error bars are average SEM (see Methods).
Table 1. Plasma Cholesterol Levels and Weight Changes Over 5 Weeks in Renal-Wrap and Sham-Operated Rabbits on Normal or 1% Cholesterol Diet

<table>
<thead>
<tr>
<th>Group</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>35</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operation + normal diet (n = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma cholesterol (mg/dl)</td>
<td>119.4±15.5</td>
<td>107.8±21.1</td>
<td>97.1±13.4</td>
<td>109.3±11.7</td>
<td>112.6±11.9</td>
<td>114.3±17.4</td>
</tr>
<tr>
<td>Δ body weight (g)</td>
<td>—</td>
<td>39±24</td>
<td>189±33</td>
<td>246±45</td>
<td>371±22</td>
<td>393±31</td>
</tr>
<tr>
<td>Sham operation + 1% cholesterol diet (n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma cholesterol (mg/dl)</td>
<td>78.6±10.0</td>
<td>76.1±11.4</td>
<td>848±95.7</td>
<td>1155±117</td>
<td>1491±186</td>
<td>1319±182</td>
</tr>
<tr>
<td>Δ body weight (g)</td>
<td>—</td>
<td>40±29</td>
<td>228±61</td>
<td>300±67</td>
<td>410±82</td>
<td>507±74</td>
</tr>
<tr>
<td>Renal wrap + normal diet (n = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma cholesterol (mg/dl)</td>
<td>87.9±17.2</td>
<td>101.6±11.4</td>
<td>83.2±13.9</td>
<td>107.1±22.6</td>
<td>116.8±24.2</td>
<td>107.1±23.6</td>
</tr>
<tr>
<td>Δ body weight (g)</td>
<td>—</td>
<td>5±46</td>
<td>141±38</td>
<td>209±44</td>
<td>239±40</td>
<td>308±44</td>
</tr>
<tr>
<td>Renal wrap + 1% cholesterol diet (n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma cholesterol (mg/dl)</td>
<td>121±14.7</td>
<td>113.4±22.1</td>
<td>744±51.4</td>
<td>909±102</td>
<td>1049±69</td>
<td>951±91</td>
</tr>
<tr>
<td>Δ body weight (g)</td>
<td>—</td>
<td>1±48</td>
<td>88±67</td>
<td>243±67</td>
<td>367±60</td>
<td>322±98</td>
</tr>
</tbody>
</table>

Values are mean ± 1 SEM. Δ body weight = the change from Day 0 measurement.

Changes in heart rate occurred only with the highest dose of the vasodilator: heart rate fell by 22 and 38 beats/min in the sham-operated groups on normal and cholesterol diet, respectively, and by 23 and 45 beats/min in the renal-wrap groups on normal and cholesterol diet, respectively. Changes in IVR in the sham-operated rabbits on either diet and renal-wrap rabbits on cholesterol diet showed a similar sensitivity (ED$_{50}$) to adenosine (Figure 5). Sensitivity to adenosine decreased significantly (almost twofold) in the wrapped animals on normal diet compared with that in the sham-operated animals on normal diet (see Table 5). The IVR at maximum adenosine-induced dilatation was similar in all groups except for the renal-wrap animals on cholesterol diet, which showed a significantly higher value compared with that in the sham-operated animals on normal diet (unpaired t test; p < 0.05).

During maximum serotonin-induced dilatation, hindlimb flow increased to 182% of the resting averages in both sham-operated treatment groups and to 198% and 204% of resting values in the renal-wrap animals on normal and cholesterol diet, respectively (see Table 4). With the highest dose of serotonin, heart rate increased by 21 and 17 beats/min in the sham-operated rabbits on normal and cholesterol diet, respectively, and by 5 and 9 beats/min in the wrapped rabbits on normal and cholesterol diet, respectively. Changes in IVR in the sham-operated group on 1% cholesterol diet showed an almost twofold increase in sensitivity (ED$_{50}$) to serotonin compared with those in the sham-operated rabbits on normal diet (Figure 6; see Table 5). There was no significant difference in ED$_{50}$ in the other groups. The IVR at maximum serotonin-induced dilatation was similar in all groups (see Table 5).

No between-group difference was observed when
Table 2. Hemodynamics Before and After Pharmacological Autonomic Effector Blockade in Renal-Wrap and Sham-Operated Rabbits on Normal or 1% Cholesterol Diet

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP (mm Hg) Before</th>
<th>MAP (mm Hg) After</th>
<th>SED</th>
<th>HP (msec) Before</th>
<th>HP (msec) After</th>
<th>SED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operation + normal diet</td>
<td>80 ± 2</td>
<td>106.8 ± 4</td>
<td>4.27*</td>
<td>322.3 ± 20</td>
<td>241 ± 9</td>
<td>20.51*</td>
</tr>
<tr>
<td>Sham operation + 1% cholesterol diet</td>
<td>84 ± 2.2</td>
<td>106.2 ± 4</td>
<td>5.41*</td>
<td>285.6 ± 16</td>
<td>216.7 ± 5.1</td>
<td>13.9*</td>
</tr>
<tr>
<td>Renal wrap + normal diet</td>
<td>134.7 ± 2.3</td>
<td>143.7 ± 2.1</td>
<td>2.39*</td>
<td>275.7 ± 12</td>
<td>222 ± 14.3</td>
<td>22.25</td>
</tr>
<tr>
<td>Renal wrap + 1% cholesterol diet</td>
<td>133 ± 3.6</td>
<td>139.5 ± 7.2</td>
<td>6.19</td>
<td>247 ± 13</td>
<td>217 ± 6.7</td>
<td>11.72*</td>
</tr>
</tbody>
</table>

Values are mean ± 1 SEM. MAP = mean arterial pressure; HP = heart period; Q = mean hindlimb blood flow; IVR = iliac vascular resistance; SED = standard error of the difference.

*p < 0.05 (paired t test).

Figure 2. Responses of heart period (HP), mean arterial pressure (MAP), and iliac vascular resistance (IVR) to sequential development of pharmacological autonomic blockade, which consisted of rest (R), guanethidine (G) administration, propranolol and phentolamine (P) administration, and methscopolamine (Me) administration in sham-operated rabbits on normal diet (◊; n = 7); sham-operated rabbits on 1% cholesterol diet (○; n = 6); renal-wrap rabbits on normal diet (▲; n = 6); and renal-wrap rabbits on 1% cholesterol diet (△; n = 6). Error bars are average SEM.

Discussion

In the present study, 4 weeks of experimental hypertension and hypercholesterolemia alone or together raised hindlimb vascular resistance and reduced the maximum vasodilator response to the endothelium-dependent agent acetylcholine but not to serotonin, adenosine, or reactive hyperemia following ischemia. After pharmacological autonomic blockade, IVR was elevated above control levels in rabbits fed a high cholesterol diet. This finding may have been due to raised blood viscosity, especially with "milky plasma" noted in cholesterol levels greater than 700 mg/dl. Hypertension raised IVR to even higher levels than did cholesterol diet alone, which is consistent with the finding of Folkow et al. 13 in the hindquarters of spontaneously hypertensive rats, in which geometrical considerations of medial hypertrophy can account for the observations. Rabbits subjected to both cholesterol diet and bilateral renal wrapping had the highest IVR levels and did not become hypertensive at a faster rate than renal-wrap rabbits on control diet.

Against these alterations in resting hindlimb resistance we sought to examine how the hindlimb bed responded to vasodilator stimuli. Two parameters were used to define the vasodilator response to a stimu-
### Table 2. (continued)

<table>
<thead>
<tr>
<th>Group</th>
<th>Q (kHz)</th>
<th>IVR (mm Hg/kHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After SED</td>
</tr>
<tr>
<td>2.11 ± .21</td>
<td>1.83 ± .16</td>
<td>0.21</td>
</tr>
<tr>
<td>1.88 ± .25</td>
<td>1.57 ± .24</td>
<td>0.10*</td>
</tr>
<tr>
<td>1.85 ± .33</td>
<td>1.58 ± .16</td>
<td>0.26</td>
</tr>
<tr>
<td>1.71 ± .26</td>
<td>1.62 ± .20</td>
<td>0.10</td>
</tr>
</tbody>
</table>

### Table 3. Stability of Preparation in Renal-Wrap and Sham-Operated Rabbits

<table>
<thead>
<tr>
<th>Group</th>
<th>Acetylcholine</th>
<th>Adenosine</th>
<th>Serotonin</th>
<th>Reactive hyperemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operation + normal diet (n = 8)</td>
<td>49.1 ± 3.2</td>
<td>56.1 ± 3.3</td>
<td>54.1 ± 4.5</td>
<td>40.8 ± 4.3</td>
</tr>
<tr>
<td>Sham operation + cholesterol diet (n = 7)</td>
<td>61.7 ± 8.4</td>
<td>60.5 ± 9.1</td>
<td>66.8 ± 11.1</td>
<td>46.7 ± 9.5</td>
</tr>
<tr>
<td>Renal wrap + normal diet (n = 7)</td>
<td>73.8 ± 10.9</td>
<td>79.1 ± 13.3</td>
<td>68.9 ± 11.0</td>
<td>53.2 ± 9.4</td>
</tr>
<tr>
<td>Renal wrap + cholesterol diet (n = 7)</td>
<td>93.4 ± 11.8</td>
<td>81.4 ± 11.7</td>
<td>81.5 ± 13.5</td>
<td>56.4 ± 11.8</td>
</tr>
</tbody>
</table>

Values are mean ± 1 SEM. IVR = iliac vascular resistance.

### Table 4. Mean Arterial Pressure and Hindquarter Blood Flow During Maximum Dilatation in Renal-Wrap and Sham-Operated Rabbits

<table>
<thead>
<tr>
<th>Group</th>
<th>Acetylcholine</th>
<th>Adenosine</th>
<th>Serotonin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operation + normal diet (n = 8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>78 ± 2.3</td>
<td>1.62 ± 0.08</td>
<td>99.7 ± 2.8</td>
</tr>
<tr>
<td>Max. dilatation</td>
<td>48.8 ± 2.3</td>
<td>2.72 ± 0.16</td>
<td>27 ± 1.1</td>
</tr>
<tr>
<td>Sham operation + cholesterol diet (n = 7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>83.7 ± 4.2</td>
<td>1.55 ± 0.24</td>
<td>100.7 ± 6.2</td>
</tr>
<tr>
<td>Max. dilatation</td>
<td>45.5 ± 2.4</td>
<td>1.94 ± 0.30</td>
<td>38.3 ± 2.9</td>
</tr>
<tr>
<td>Renal wrap + normal diet (n = 7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>123.3 ± 5.9</td>
<td>1.92 ± 0.28</td>
<td>130.4 ± 3.9</td>
</tr>
<tr>
<td>Max. dilatation</td>
<td>70.4 ± 6.0</td>
<td>2.04 ± 0.36</td>
<td>43.6 ± 3.8</td>
</tr>
<tr>
<td>Renal wrap + cholesterol diet (n = 7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>128.3 ± 4.6</td>
<td>1.51 ± 0.18</td>
<td>127.9 ± 7.3</td>
</tr>
<tr>
<td>Max. dilatation</td>
<td>73.2 ± 6.8</td>
<td>2.24 ± 0.31</td>
<td>49.2 ± 1.7</td>
</tr>
</tbody>
</table>

Values are mean ± 1 SEM. Maximum concentrations of acetylcholine, adenosine, and serotonin were 30 to 60, 3000 to 6000, and 120 to 300 μg/kg/min, respectively. MAP = mean arterial pressure; Q = mean hindlimb blood flow.
**FIGURE 3.** A. Average dose-response curves to acetylcholine. Ordinate: iliac vascular resistance (IVR); abscissa: acetylcholine i.v. (log scale); R = resting value. Groups were sham operation + normal diet (•; n = 9); sham operation + 1% cholesterol diet (○; n = 7); renal wrap + normal diet (△; n = 7); and renal wrap + 1% cholesterol diet (△; n = 7). Error bars are average SEM. The average IVR values shown at the highest infusion rates of acetylcholine are not representative of the whole group since individual rabbits varied in the concentration of dilator that gave maximum response. B. Effect of acetylcholine on IVR with the response expressed as percent maximum dilatation on the ordinate (see Methods). Abscissa: acetylcholine i.v. (log scale). Error bars are SEM of the ED$_{50}$ values calculated from logistic fitted curves.

**TABLE 5.** ED$_{50}$ Values and Iliac Vascular Resistance During Maximum Dilatation with the Vasodilator Drugs in Renal-Wrap and Sham-Operated Rabbits

<table>
<thead>
<tr>
<th>Group</th>
<th>Acetylcholine</th>
<th>Adenosine</th>
<th>Serotonin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ED$_{50}$ (µg/kg/min)</td>
<td>IVR ($\text{mm Hg/kHz}$)</td>
<td>ED$_{50}$ (µg/kg/min)</td>
</tr>
<tr>
<td>Sham operation + normal diet (n = 9)</td>
<td>0.34 ± 0.08</td>
<td>20.0 ± 2.0</td>
<td>2.36 ± 0.04</td>
</tr>
<tr>
<td>Sham operation + cholesterol diet</td>
<td>0.40 ± 0.11</td>
<td>31.1 ± 5.7</td>
<td>2.44 ± 0.10</td>
</tr>
<tr>
<td>(n = 7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal wrap + normal diet (n = 7)</td>
<td>0.49 ± 0.14</td>
<td>38.2 ± 5.4</td>
<td>2.59 ± 0.05</td>
</tr>
<tr>
<td>Renal wrap + cholesterol diet (n = 7)</td>
<td>0.32 ± 0.07</td>
<td>34.9 ± 4.1</td>
<td>2.38 ± 0.09</td>
</tr>
</tbody>
</table>

Values are mean ± 1 SEM. ED$_{50}$ = effective dose, 50% (log$_{10}$); IVR = iliac vascular resistance (during maximum dilatation).

**FIGURE 4.** Hemodynamic changes during adenosine infusion in a sham-operated rabbit on control diet. HP = heart period; BP = mean blood pressure; Q = phasic iliac bed blood flow; V = phasic iliac bed blood flow; IVR = iliac vascular resistance.

**FIGURE 5.** A. Dose-response curves to adenosine. Ordinate: iliac vascular resistance (IVR); abscissa: adenosine i.v. (log scale). See Figure 3 for key to abbreviations. Error bars are average SEM. B. Effect of adenosine on IVR with the response expressed as percent maximum dilatation on the ordinate. Abscissa: adenosine i.v. (log scale). Error bars are SEM of the ED$_{50}$ values. Star indicates ED$_{50}$ value significantly different from that of the sham-operated group on normal diet (unpaired t test; p < 0.01).
Vasodilatation in Hypertension and Hypercholesterolemia

1) Maximum effect, defined as minimum resistance, and 2) sensitivity, defined as ED50 (the infusion concentration that caused half-maximum response).

The sensitivity of the rabbit iliac vascular bed to acetylcholine, serotonin, and adenosine was altered less than twofold by hypertension or high plasma cholesterol levels, but the maximum acetylcholine-induced dilatation was significantly less in hypertensive rabbits and in rabbits fed cholesterol (Figure 8; see Table 5). That this finding was restricted only to acetylcholine, the endothelium-dependent vasodilator agent, suggests that there may be some defect in the release of EDRF, or decrease in response of the smooth muscle of resistance vessels to EDRF, in hypertension or hypercholesterolemia.

Many factors interact at the vascular smooth muscle in the conscious rabbit preparation. On the day of the experiment, we eliminated neural effector reflexes and circulating catecholamines as much as possible with a pharmacological cocktail. The preparation remained stable for 5 to 6 hours, as observed by the return to resting values of hindlimb resistance, blood pressure, and heart rate before the start of a new infusion curve of another dilator drug (see Table 3). Thus, we are confident that the dose-response curves to acetylcholine, serotonin, and adenosine can be compared among drugs and among rabbit treatment groups.

Addition of cholesterol to the diet did not appear to alter the onset or magnitude of the hypertension over 5 weeks. The heart rate trends during the same period were similar across treatments except for the significantly higher rates toward 5 weeks for the hypertensive rabbits. This trend in rabbits with perinephritic hypertension has been observed previously.14,15

### Figure 6

A. Dose-response curves to serotonin. Ordinate: iliac vascular resistance (IVR); abscissa: serotonin i.v. (log scale); R = resting value. Groups were sham operation + normal diet (○; n = 8); sham operation + 1% cholesterol diet (□; n = 7); renal wrap + normal diet (▲; n = 6); renal wrap + 1% cholesterol diet (△; n = 7). Error bars are average SEM. B. Effect of serotonin on IVR with the response expressed as percent maximum dilatation on the ordinate. Abscissa: serotonin i.v. (log scale). Error bars are SEM of the ED50 values. Star indicates ED50 value significantly different from that of the sham-operated group on normal diet (unpaired t test; p < 0.01).

### Figure 7

A. Reactive hyperemia response to hindlimb ischemia. Ordinate: repayment of hindlimb flow on release of balloon cuff (log area, arbitrary units; see Methods). Abscissa: period of hindlimb ischemia (i.e., occlusion time in seconds, log scale). Groups were sham operation + normal diet (○; n = 5); sham operation + 1% cholesterol diet (□; n = 5); renal wrap + normal diet (▲; n = 4); and renal wrap + 1% cholesterol diet (△; n = 5). Error bars are average SEM. B. Reactive hyperemic response (repayment) plotted on the ordinate versus the hindlimb flow debt (see Methods) on the abscissa (log area, arbitrary units). Error bars are average SEM. Inset shows effect of lower aortic balloon inflation on mean hindlimb blood flow (Q). Flow debt and repayment are indicated during and after balloon inflation. Ordinate: time (seconds).

### Figure 8

Iliac vascular resistance (IVR) at rest (just before agonist infusion; solid symbols) and during maximum dilatation with each of the vasodilator drugs (open symbols) in the four treatment groups. Drugs were acetylcholine (○); adenosine (△); and serotonin (□); groups were sham operation + normal diet (S; n = 9); sham operation + 1% cholesterol diet (S + C; n = 7); renal wrap + normal diet (W; n = 7); and renal wrap + 1% cholesterol diet (W + C; n = 7). Error bars are SEM.
The combination of guanethidine, propranolol, phentolamine, and methscopolamine provides an adequate regimen for neural and circulating catecholamine blockade. Our goal was to maintain a stable hemodynamic pattern for 4 to 5 hours with minimal influences of autonomic reflexes. Comparison of the preblockade IVR with that after the total autonomic blockade had settled down, clearly shows that the regimen had the same effect on all treatment groups (see Figure 2). Perhaps the only drawback was that phentolamine raised the IVR in the normotensive groups proportionately more than in the hypertensive groups, thus reducing the magnitude of the separations of the resting IVR values before the start of the vasodilator drug infusions. In this context, it is worth noting that phentolamine not only antagonizes α-adrenergic receptors but probably acts as a partial α, adrenergic receptor agonist in the rabbit to raise blood pressure and peripheral resistance.

Confirmation that acetylcholine-induced vasodilatation in the resistance circulation is indirect (by the release of EDRF) comes from rat mesenteric microvessels and rabbit leg skin and muscle microvessels studied in vitro (J.A. Angus and C.E. Wright, unpublished observations, 1985). In these experiments, removal of endothelium by saponin or mechanical abrasion abolished the response to acetylcholine; these findings are analogous to those for large conduit vessels in vitro or in vivo.

There is a difference in the experimental conditions under which the three dilator agents were tested. Acetylcholine was always infused first before completion of total autonomic block with methscopolamine, which allowed the vascular effects of infused acetylcholine to be tested. Under these conditions, the peripheral vasodilatation and hypotension caused cardiac vagal withdrawal and tachycardia. In the sham-operated group on normal diet, the minimum resistance at maximum dilatation was similar for acetylcholine and serotonin infusion, which indicates that the lack of cardiac vagal blockade for the acetylcholine responses may not have affected this measure of vascular responsiveness. However, the minimum resistance to adenosine was significantly less (see Table 5).

The maximum dilatation in the cholesterol-fed rabbits was significantly less (i.e., higher resistance) during acetylcholine infusion compared with that during adenosine or serotonin infusion (see Figure 8). This difference may be explained by a decrease either in EDRF release or in response of the underlying smooth muscle to EDRF in the resistance vasculature. This effect of cholesterol on the maximum response to acetylcholine was relatively small and did not affect the sensitivity (i.e., ED50) of the vasculature to acetylcholine. The 4-week cholesterol diet was effective in producing a high plasma cholesterol level (>700 mg/dl) and obvious atherosclerotic plaque in the thoracic and abdominal aortas as stained by Sudan IV. Plaque formation, however, does not occur further distal from the aorta than large branch arteries. In this respect, rings of aorta from cholesterol-fed rabbits (2% for 11 weeks) recently were reported to be about eightfold less sensitive to acetylcholine on ED50 values, and the decreased sensitivity was related to the degree of atherosclerotic plaque. Thus, plaque formation rather than hypercholesterolemic plasma may be the key to the altered sensitivity to EDRF-dependent vasodilator agents.

A similar decrease in maximum response without change in sensitivity was found for acetylcholine in hypertensive rabbits and for rabbits with hypertension and hypercholesterolemia (see Figure 8). Thus, the loss of EDRF appears to be similarly affected by either treatment and is not enhanced by the combination. DeMey and Gray reported that the magnitude (but again not the sensitivity) of the EDRF-dependent relaxation to acetylcholine in isolated mesenteric resistance vessels was much reduced in spontaneously hypertensive rats compared with Wistar-Kyoto rats. Interestingly, partial mechanical removal (up to 90%) of the lumen surface area of endothelial cells in large coronary arteries still leaves the segment capable of relaxing to near normal in response to acetylcholine (J.A. Angus and T.M. Cocks, personal observation, 1983). Total (100%) removal of endothelial cells will abolish the response. Since adenosine is now considered to relax vascular smooth muscle directly without releasing EDRF, it was used as the benchmark for comparison with acetylcholine in assessing the functional integrity of EDRF release in hypertensive and hypercholesterolemic rabbits. Serotonin, on the other hand, has been shown to relax pig and dog coronary arteries by an endothelium-dependent mechanism as well as to contract underlying smooth muscle. Thus, removal of endothelium was found to enhance the constrictor action of this autacoid. However, this EDRF-dependent dilator response does not occur in the dog carotid artery (J.A. Angus and K. Satoh, personal observation, 1985), which makes extrapolation from one site to another or across species hazardous. The mechanism by which serotonin dilates the intact but autonomically blocked hindlimb bed of the rabbit is still unknown. We were unable to find a consistent relaxation to serotonin in precontracted isolated skin and muscle microvessels, 150–250 μm inside diameter (J.A. Angus and C.E. Wright, unpublished observation, 1985), which precluded us from testing the role of endothelium in this action of serotonin. Nevertheless, we did observe small increases in sensitivity (left shift in ED50) to serotonin in both sham-operated and renal-wrap rabbits on a 1% cholesterol diet. In large coronary arteries of rabbits that had hereditary hyperlipidemia or were fed cholesterol, the sensitivity of serotonin-induced contraction was markedly enhanced, particularly in areas of atheromatous plaque. Similar findings have been reported from experiments in the hindlimb of atherosclerotic monkeys. While the rabbit coronary artery response to serotonin (contraction) is different from that seen in the hindlimb bed, the cholesterol-induced increase in sensitivity to serotonin in...
both vascular beds warrants further study. This small
sensitization by cholesterol in the hindlimb circulation was quite different from that found for either acetyl-
choline or adenosine in the same animals.

The reactive hyperemia test was designed to evalu-
ate the vasodilator response to the local metabolites
generated during short periods of ischemia. No evi-
dence was found to suggest that hypertension or hyper-
cholesterolemia altered the response to this stimulus. A
proper evaluation of the importance of EDRF in
reactive hyperemia must await the discovery of an
EDRF antagonist. Judging by the small reduction in
the acetylcholine maximum response induced by hy-
pertension or a cholesterol diet, it is unlikely that we
would be able to measure any alteration in reactive
hyperemia if EDRF was only one of several factors
contributing to the response to ischemia. Because the
occluding cuff was located proximal to the flow trans-
ducer on the abdominal aorta, there is little chance that
collateral flow distorted the hyperemia during the 80-
second occlusion periods.

In summary, 1 month of hypertension and high plasma
cholesterol levels altered the sensitivity of the rab-
bit hindlimb by less than twofold to vasodilator stimuli
either from local tissue metabolites generated by isch-
emia or from intravenous infusions of acetylcholine,
adenosine, or serotonin. Some reduction in the magni-

dude of the acetylcholine response was found during both
treatments, which suggests that release of EDRF
may have been reduced or that there was a decrease in
the response of the smooth muscle of the resistance
vasculature to EDRF.

Acknowledgment
We acknowledge the excellent secretarial assistance of Juliana
Wong in the preparation of this manuscript.

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Hypertension. 1986;8:361-371
doi: 10.1161/01.HYP.8.5.361

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
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