Development of Collateral Function with Repetitive Coronary Occlusion in a Canine Model Reduces Myocardial Reactive Hyperemia in the Absence of Significant Coronary Stenosis

Hideo Yamamoto, Hitonobu Tomoike, Hiroaki Shimokawa, Shozo Nabeyama, and Motoomi Nakamura

From the Research Institute of Angiocardiology and Cardiovascular Clinic, Faculty of Medicine, Kyushu University, Fukuoka, Japan

SUMMARY. The present study was designed to elucidate the role of collateral development, per se, on reactive hyperemia without persistent coronary stenosis in instrumented conscious dogs. Functional states of coronary collaterals were augmented by repetitive 2 minutes of coronary occlusion every 30 minutes for 2-9 days. Regional shortening measured sonomicrometrically recovered from −1.2 ± 6.5% of the preocclusive state at the end of the first coronary occlusion to 100.5 ± 1.2% (n = 8, P < 0.01) after repeated coronary occlusions. Before and after collateral development, transient coronary occlusions of 5, 10, 20, 30, 60, 90, and 120 seconds were randomly performed. The degree of regional dysfunction and the following reactive hyperemic response were measured. Up to 20 seconds of coronary occlusion, the flow ratio and duration of coronary reactive hyperemia increased similarly, both before and after collateral development. However, when the duration of coronary occlusion was over 30 seconds, flow ratio and debt repayment ratio were reduced progressively after the collateral development. Among the indices exhibiting reactive hyperemia, debt repayment ratio decreased initially and correlated well with the recovery of regional dysfunction during coronary occlusion. Thus, the augmentation of collateral function after repetitive coronary occlusion reduces reactive hyperemia even in the absence of significant coronary stenosis. (Circ Res 55: 623-632, 1984)

REACTIVE hyperemia reflects the degree of myocardial ischemia during coronary occlusion (Olsson and Gregg, 1965) and has been used as an index of collateral development (Elliott et al., 1968). Tomoike et al. (1981) evaluated the functional state of collateral vessels, comparing regional shortening before and after coronary occlusion, and noted an inverse relation between the functional state of collateral development and the reduction of reactive hyperemia during the gradual progress of coronary stenosis produced by an ameroid constrictor. However, it has been shown that the extent of reactive hyperemia is also determined by the degree of coronary stenosis remaining during reperfusion (Gould et al., 1974; Hills and Friesinger, 1976).

The greater the extent of reactive hyperemia, the more viable was the rendered ischemic tissue (Matthews et al., 1971; Bitter et al., 1972). Wright et al. (1980) applied a miniaturized Doppler probe at the time of aortocoronary bypass surgery to assess the hemodynamic significance of coronary occlusive disease and to determine the need for a bypass graft. Although these clinical studies pointed out the possible role of collateral circulation on the apparent reduction of reactive hyperemia in the presence of significant coronary stenosis, quantitative evaluations of the extent to which the net collateral anastomosis attenuated the reactive hyperemia have not been rigorously analyzed.

Accordingly, to evaluate the role of collateral function, per se, on the reactive hyperemia, it is necessary to prepare an animal model with collateral development but without persistent coronary stenosis. Recent clinical observations on collaterals during coronary spasm (Takeshita et al., 1982; Tada et al., 1983) and an animal model of collateral development proposed by Franklin et al. (1981) suggested the usefulness of repeated and transient myocardial ischemia for development of collateral circulation. Therefore, in the present study, consecutive 2 minutes of coronary occlusion were elicited every 30 minutes for 24 hours/day for 2–9 days (Shimokawa et al., 1983) to produce collateral development, and the effects of collaterals on the reactive hyperemia were examined.

Methods

An Animal Model

Twenty-six adult mongrel dogs, weighing 18–32 kg, were anesthetized with the intravenous administration of
pentobarbital sodium (25 mg/kg) and ventilated with room air using a positive pressure respirator. With a sterile surgical procedure, a left thoracotomy was performed in the 4th intercostal space, and a No. 8F polyvinyl chloride catheter filled with heparin was inserted into the aortic arch through the left internal thoracic artery. The pericardium was incised, and a miniature pressure gauge (P-22, Konigsberg Instrument) was placed in the left ventricular cavity through a stab incision at the apex.

The left circumflex coronary artery was dissected free near its origin, and a 20-MHz pulsed Doppler flow probe or a 10-MHz Doppler flow probe fabricated in our laboratory (diameter = 3 mm, 2 × 2 mm crystal attached at a 45° angle to a shell) or an electromagnetic flow probe (Nihon Kohden) and a hydraulic cuff occluder were placed around it. Two pairs of 5-MHz ultrasonic crystals (Murata) (diameter 2 mm) were placed subendocardially in the left ventricular wall, as described previously (Tomoike et al., 1983a). Briefly, each pair of ultrasonic crystals was inserted into the subendocardial portion, 1-2 cm apart, to measure the regional myocardial length; one pair was placed in the left anterior descending coronary artery (LAD) area as a nonischemic segment length, and the other was placed in the center of the left circumflex coronary artery (LCX) area to measure the extent of regional ischemia. We ascertained proper placement of the crystals in the ischemic area by abruptly occluding the LCX. The pericardium was left open and all wires and tubings were passed subcutaneously to the back of the dog and secured between the scapulae. The chest then was closed, the air evacuated, and penicillin G (1.0 million U) and streptomycin (0.3 g) were administered intramuscularly for 4 weeks (n = 13), and that the instrumentation for 7 days after the instrumentation.

Thirteen of 26 dogs were used to verify the stability of regional shortening. The values for segment lengths were normalized to an initial EDL as 10 mm by dividing the measured EDL and ESL by the initial control EDL and multiplying by 10 (Tomoike et al., 1983a).

Flow debt and reactive hyperemic flow were measured on records of mean flow. Calculations of flow debt, reactive hyperemic flow, repayment of flow debt, and flow ratios were done according to the method of Coffman and Gregg (1960), as follows: (1) flow debt = (control flow rate) X (duration of occlusion); (2) reactive hyperemic flow = (total flow during reactive hyperemia) − (control flow rate X duration of reactive hyperemia); (3) debt repayment ratio = reactive hyperemic flow/flow debt; (4) flow ratio = peak reactive hyperemic flow/control resting flow.

The duration of reactive hyperemia was taken as the time required for hyperemic flow to revert within 5% of control level. Two persons independently measured these indices, and the values were averaged.

### Measurements

The miniature pressure gauge was calibrated in vivo by directly measuring the left ventricular pressure with a catheter attached to a calibrated strain-gauge manometer (Statham P-23Db). Coronary blood flow of the LCX was measured with a flowmeter (545C-3, directional-pulsed Doppler flowmeter, Marcus et al., 1981) or a square wave electromagnetic flowmeter (Nihon Kohden). The aortic pressure was measured by a calibrated strain gauge manometer (Statham P-23Db). Left ventricular pressure, left ventricular dp/dt, aortic pressure, ischemic, and nonischemic segment length, heart rate, and phasic and mean coronary blood flow of the LCX were recorded continuously at a paper speed of 50 mm/sec on a pen recorder (Sanei) and stored on a magnetic tape (TEAC model 280LT FM data recorder) for subsequent analysis. Mean coronary blood flow was obtained using a 2-second time constant filter. Systolic regional shortening was calculated by the formula:

\[
\text{End-diastolic length} - \text{end-systolic length} = \frac{\text{EDL}}{\text{EDL}}
\]

the result being termed "percent shortening." The values for segment lengths were normalized to an initial EDL as 10 mm by dividing the measured EDL and ESL by the initial control EDL and multiplying by 10 (Tomoike et al., 1983a).

<table>
<thead>
<tr>
<th>Days after instrumentation</th>
<th>Analysis of variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>89 ± 4</td>
</tr>
<tr>
<td>LVSP (mm Hg)</td>
<td>110 ± 9</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>LV dp/dt (+) (mm Hg/sec)</td>
<td>3048 ± 445</td>
</tr>
<tr>
<td>LAD segment</td>
<td></td>
</tr>
<tr>
<td>EDL (mm)</td>
<td>10</td>
</tr>
<tr>
<td>%AL</td>
<td>21.3 ± 2.7</td>
</tr>
<tr>
<td>LCX segment</td>
<td></td>
</tr>
<tr>
<td>EDL (mm)</td>
<td>10</td>
</tr>
<tr>
<td>%AL</td>
<td>20.2 ± 2.0</td>
</tr>
</tbody>
</table>

Values are means ± SE. HR = heart rate; LVSP = peak left ventricular systolic pressure; LVEDP = left ventricular end-diastolic pressure; LV dp/dt (+) = first derivative of left ventricular pressure; LAD = left anterior descendence; EDL = end-diastolic length; LCX = left circumflex; %AL = percent shortening.
Collateral Development with Repetitive Short Occlusion of the LCX

Studies were carried out 7–10 days after the initial surgery. All dogs recovered full activity, appeared to be in good health, and were afebrile. Restraints or medications were avoided, and noise was kept at a minimum. Collateral development was elicited with repetitive 2 minutes of coronary occlusion every 30 minutes (Franklin et al., 1981), continuously, day and night (Shimokawa et al., 1983). When the regional shortening at the end of 2-minute coronary occlusion recovered to the preocclusive state, repetitive short coronary occlusion was stopped, and a reactive hyperemia study of the condition of collateral development was performed.

Experimental Protocol

The reactive hyperemia study was performed in 13 dogs under two conditions; one without collateral development (n = 13) before repetitive coronary occlusions, and the other after collateral development (n = 8). In five of 13 dogs, ventricular fibrillation developed immediately after the short coronary occlusion, and these dogs died.

After resting control recordings, the LCX was occluded with an implanted hydraulic occluder for a predetermined length of time. Recordings were continued for at least 2 minutes after the reflow and other hemodynamics had returned to the control level. The duration of coronary occlusion was 5, 10, 20, 30, 60, 90, and 120 seconds. The sequences of these occlusions were performed randomly. Each intervention was interposed with at least 15 time intervals of the duration of preceding coronary occlusion, when the regional shortening returned to the preocclusive state.

After the end of the reactive hyperemia study, dipyridamole (0.5 mg/kg) was administered intravenously to test whether there was any change in the coronary vasodilating capacity, before and after collateral development. Six of eight dogs, in which fully functional collaterals had developed, were reanesthetized with the intravenous administration of sodium pentobarbital, and coronary cineangiography was performed with the Kifa catheter in a left anterior oblique view before coronary occlusion to evaluate the degree of coronary stenosis of the LCX due to instrumentation (Inou et al., 1980), and again at 2 minutes after the occlusion of the LCX to ascertain the developed collaterals. The x-ray tube with a 0.6-mm focal spot (Toshiba) was placed beneath the catheterization table, and the output phosphor of the image intensifier (Toshiba 6/9-inch II) was recorded at 42 frames/sec on 35-mm cine film (CFS 746, Kodak) loaded in an Arriflex camera. The resolution of our x-ray cine angiographic system was 0.1 mm. Cineframes were projected on a viewing screen (model 35CX, Tagarno). The degree of narrowing was expressed as a percent stenosis, determined from the diameter reduction of the LCX around the cuff occluder, as compared with the LCX diameter in its normal proximal or distal portion. Reproducibility of the coronary diameter measurements was examined by comparing the data obtained by individual observers (r = 0.975, P < 0.001 between repeated measurements and r = 0.985, P < 0.001 between different observers). The degree of collateralization was graded as follows: 0, no collaterals visible; 1, collaterals faintly seen; 2, collaterals well-visualized, but the occluded vessel remained small; and 3, occluded vessel stained to normal size (Nolewajka et al., 1979).

At the end of the study, the heart was removed and a barium gelatin mixture was perfused into the left and right coronary arteries at a perfusion pressure of 140 mm Hg while the left circumflex coronary artery was occluded. The right ventricular free wall and the ventricular septum were separated from the left ventricle at the anterior border and radiographed stereoscopically, as described previously (Tomoike et al., 1983b). Then, the collateral anastomosis was documented angiographically.

![Figure 1](http://circres.ahajournals.org/external_ref.php?content_type=html&doi=10.1161/01.RES.75.11.625)  
**Figure 1.** Effects of 90-second coronary occlusion before (panel A) and after (panel B) collateral development in the same conscious dog, on left ventricular pressure (LV pressure), left ventricular dP/dt, ischemic segment length, control segment length, aortic pressure (AoP), phasic flow and its mean flow, and heart rate (HR). Note that, after collateral development, regional hyposhortening in an ischemic area progressively recovered before the release of LCX occlusion, and the resultant reactive hyperemia was markedly attenuated.
Statistics

All values are presented as mean ± se. Data were analyzed by Student’s t-test for paired data on hemodynamics, regional performance, and reactive hyperemia between each occlusion, with and without collateral development. The chronic time course of hemodynamics and regional performance, or change in reactive hyperemia and regional myoccardial shortening. The level of statistical significance was P < 0.05.

Results

Hemodynamics and Regional Wall Motion

Typical tracings of the measured variables during transient coronary occlusion before and after collateral development are illustrated in Figure 1. Before collateral development, coronary artery occlusion resulted in gradual increases in heart rate (HR) and left ventricular end-diastolic pressure (LVEDP), and gradual decreases in left ventricular systolic pressure (LVSP) and left ventricular dP/dt (Fig. 1, Table 2). Peak negative left ventricular dP/dt was reduced transiently at the early phase of coronary occlusion.

The change of mean aortic pressure (mAoP) during coronary occlusion was not statistically significant (Table 2). Percent shortening of the control segment during coronary occlusion increased transiently (Fig. 1). Percent shortening of the segment of the LCX area decreased from 100% to −1.2 ± 6.5% (P < 0.01) after 2 minutes of the first coronary occlusion (Table 3).

After applying the procedure of repeating 2 minutes of coronary occlusion every 30 minutes for 2–9 days, we found that regional shortening in the ischemic area decreased transiently after abrupt coronary occlusion, and then gradually recovered to near the control level (100.5 ± 1.2%) at the end of coronary occlusion (Fig. 1; Tables 2 and 3). These findings indicate that the functional state of coronary collaterals was well augmented. HR, LVSP, LVEDP, mAoP, and regional shortening in the nonischemic area during coronary occlusion did not change, except for a negative left ventricular dP/dt, which decreased transiently (Fig. 1; Table 2).

Reactive Hyperemic Response

Reactive hyperemia after 5, 10, 20, 30, 60, and 120 seconds of coronary occlusion is traced before and after collateral development in the same dog.

| TABLE 2 | Hemodynamics and Regional Shortenings Prior to Reperfusion before and after Collateral Development |
|-----------------|---------------------------------|-----------------|-----------------|-----------------|
| Duration of coronary occlusion (sec) | HR (beat/min) | mAoP (mm Hg) | LVSP (mm Hg) | LVEDP (mm Hg) |
| Before C.O. | | | | |
| Collateral − | 99 ± 6 | 94 ± 4 | 109 ± 4 | 8.0 ± 0.8 |
| Collateral + | 96 ± 6 | 98 ± 8 | 116 ± 6 | 6.5 ± 0.8 |
| Prior to reperfusion | | | | |
| 5 | Collateral − | 104 ± 7 | 94 ± 4 | 107 ± 4 | 8.6 ± 0.9 |
| Collateral + | 94 ± 4 | 104 ± 9 | 116 ± 5 | 6.9 ± 1.1 |
| 10 | Collateral − | 105 ± 9 | 94 ± 3 | 107 ± 3 | 7.9 ± 0.7 |
| Collateral + | 100 ± 6 | 98 ± 7 | 117 ± 6 | 7.4 ± 1.1 |
| 20 | Collateral − | 111 ± 7 | 95 ± 3 | 107 ± 3 | 11.9 ± 1.2 |
| Collateral + | 95 ± 4 | 103 ± 7 | 120 ± 5 | 9.1 ± 1.0 |
| 30 | Collateral − | 111 ± 7 | 94 ± 3 | 106 ± 3 | 12.0 ± 1.2 |
| Collateral + | 99 ± 5 | 101 ± 8 | 117 ± 5 | 8.2 ± 0.95 |
| 60 | Collateral − | 126 ± 8 | 92 ± 3 | 103 ± 3 | 12.5 ± 1.25 |
| Collateral + | 100 ± 5 | 97 ± 8 | 120 ± 7 | 8.2 ± 1.0 |
| 90 | Collateral − | 122 ± 8 | 95 ± 5 | 106 ± 4 | 14.1 ± 1.65 |
| Collateral + | 97 ± 5 | 100 ± 8 | 116 ± 6 | 8.2 ± 1.3 |
| 120 | Collateral − | 129 ± 8 | 95 ± 5 | 105 ± 4 | 13.9 ± 1.45 |
| Collateral + | 89 ± 3 | 98 ± 13 | 119 ± 7 | 5.9 ± 0.65 |

Values are means ± se. n = 8 dogs. HR = heart rate; mAoP = mean aortic pressure; LVSP = peak left ventricular systolic pressure; LVEDP = left ventricular end-diastolic pressure; LV dP/dt = first derivative of left ventricular pressure; C.O. = coronary occlusion; EDL = end-diastolic length; %ΔL = percent shortening.

* P < 0.05; † P < 0.01 between before collateral development (collateral −) and after collateral development (collateral +).

† P < 0.05; § P < 0.01 between before and during coronary occlusion, in which the level of regional shortening before reperfusion was compared with the control recordings before each respective coronary occlusion.
(Fig. 2). The level of resting coronary blood flow remained the same before and after collateral development (Table 3). Before collateral development, the indices of reactive hyperemia such as flow ratio tended to increase, and duration of reactive hyperemia increased progressively with prolongation of the coronary occlusion ($P < 0.01$ by a repeated-measures analysis of variance), while debt repayment ratio was fairly constant during 30–120 seconds of coronary occlusion (Fig. 3).

Reactive hyperemic flow of less than 30 seconds of coronary occlusion after collateral development appeared grossly similar to the state before collateral development (Fig. 2). However, as shown in Figure 3, the flow ratio and debt repayment ratio of reactive hyperemic flow after more than 60 seconds of coronary occlusion were reduced from the levels of 5 seconds of coronary occlusion ($P < 0.01$ by a repeated-measures analysis of variance). Overpayment disappeared when the occluding time lasted for more than 60 seconds. A comparison of the degree of reactive hyperemic response before and after collateral development indicated that debt repayment ratio was the most sensitive among the indices of reactive hyperemia (Fig. 3).

To determine whether repeated coronary occlusion would modify the vascular reactivity to a vasodilator substance, we tested responses of coronary blood flow to intravenous administration of dipyridamole before and after collateral development. As summarized in Table 3, coronary blood flow during dipyridamole increased to almost the same extent before (461 ± 28%) and after (422 ± 45%) the collateral development.

![Figure 2. Relationship between the duration of coronary occlusion and the degree of reactive hyperemia before (panel A) and after (panel B) collateral development.](http://circres.ahajournals.org/)

**TABLE 3**

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>% Shortening at 2 min of C.O.</th>
<th>Rest flow (ml/min)</th>
<th>Response to DIP (ml/min)</th>
<th>Coronary arteriograph</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coll -</td>
<td>Coll +</td>
<td>Coll -</td>
<td>Coll +</td>
</tr>
<tr>
<td>1</td>
<td>-0.7 (-7.0)</td>
<td>14.7 (92.9)</td>
<td>36</td>
<td>ND ND</td>
</tr>
<tr>
<td>2</td>
<td>4.0 (13.7)</td>
<td>25.0 (100.8)</td>
<td>43</td>
<td>ND ND</td>
</tr>
<tr>
<td>3</td>
<td>-4.0 (-29.9)</td>
<td>13.9 (103.7)</td>
<td>35</td>
<td>ND ND</td>
</tr>
<tr>
<td>4</td>
<td>-0.4 (-2.5)</td>
<td>27.0 (99.3)</td>
<td>25</td>
<td>27 ND</td>
</tr>
<tr>
<td>5</td>
<td>4.6 (27.7)</td>
<td>16.7 (102.0)</td>
<td>ND 56*</td>
<td>ND 220*</td>
</tr>
<tr>
<td>6</td>
<td>1.4 (8.8)</td>
<td>18.2 (102.3)</td>
<td>50</td>
<td>47 202</td>
</tr>
<tr>
<td>7</td>
<td>0.0 (0.0)</td>
<td>20.5 (101.5)</td>
<td>41</td>
<td>53 198</td>
</tr>
<tr>
<td>8</td>
<td>-3.5 (-20.2)</td>
<td>20.7 (101.3)</td>
<td>40</td>
<td>36 198</td>
</tr>
</tbody>
</table>

Coll = collateral; Coll — = before collateral development; Coll + = after collateral development; C.O. = coronary occlusion; DIP = dipyridamole; ND = not determined; 3+ = Angiographically well opacified collaterals. The degree of coronary stenosis was determined by coronary arteriography. The numbers in parentheses are the percentage change from control state (=100%). When a Doppler flow probe was applied, volume flow was calculated as the product of cross-sectional area and blood velocity. Cross-sectional area was assessed by coronary arteriography in vivo, or vessel wall thickness was measured at autopsy.

* Measured by electromagnetic flowmeter.

Collateral and Reactive Hyperemia

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Relationship between Regional Shortening during Coronary Occlusion and Subsequent Reactive Hyperemia

The relationship between regional shortening in the ischemic area and debt repayment ratio is shown in Figure 4. Before collateral development, the regional shortening in the ischemic area decreased progressively up to 60 seconds, and, thereafter, it remained stably akinetic or dyskinetic. Debt repayment ratio was fairly constant, even with an increase in the duration of coronary occlusion.

After collateral development, regional shortening in the ischemic area decreased transiently (0–20 sec) and then recovered progressively, even in the presence of coronary occlusion (Table 2). There was an inverse linear relation between debt repayment ratio and the regional shortening at the end of 20–120 seconds of coronary occlusion ($r = -0.60, y = 3.78 - 0.032x$, $P < 0.001, n = 35$).

Effects of Collateral Development on the Profile of Reactive Hyperemia

To elucidate the role of collateral development, per se, on reactive hyperemia, we compared the reactive hyperemic response with the same peak level of flow following 5–20 seconds of coronary occlusion in six dogs, before and after collateral development (Table 4), and found some recovery of regional shortening in the LCX area at 20 seconds of coronary occlusion. As shown in Figure 5, 20 seconds of coronary occlusion before and after collateral development resulted in a similar peak level of reactive hyperemia. However, the hyperemic flow elicited after collateral development returned more rapidly to the control level. The duration of 50%
level of peak hyperemic flow (half time) after 20 seconds of coronary occlusion decreased from 23.0 ± 4.4 seconds before collateral development to 12.8 ± 0.6 seconds ($P < 0.05$) after collateral development (Table 4).

### Angiographic Assessment of Coronary Collaterals and Coronary Stenosis

Before occlusion of the LCX, no collateral vessels were observed, and there was no significant coronary stenosis around a flow probe and a hydraulic cuff occluder (Fig. 6, A and C; Table 3). After collateral development, distal portions of the occluder were clearly visible during LCX occlusion by the flow via collateral vessels (Fig. 6D; Table 3). Post-mortem angiography also demonstrated the presence of vascular anastomosis between the LCX and LAD (Fig. 6E).

### Discussion

A model of collateral development without persistent coronary stenosis, as proposed by Franklin et al. (1981) and our group (1983), was applied to eliminate the effects of coronary stenosis on coronary blood flow. Functional states of coronary collaterals were assessed by regional shortening recovered during 2 minutes of complete coronary occlusion (Fig. 1; Table 3). It has been shown that the degree of regional myocardial hypokinesia in conscious dogs correlated directly with the amount of regional blood flow measured by a microsphere technique during acute coronary stenosis (Vatner, 1980) and during collateral development in an ame-roid model (Tomoki et al., 1983a). Advantages of the present model were as follows: (1) coronary collaterals developed without coronary stenosis, (2) normal antegrade flow was well maintained to the LCX area before coronary occlusion, (3) functional

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**TABLE 4**

Changes in Half-Time of Reactive Hyperemia with the Same Peak Level before and after Collateral Development ($n = 6$)

<table>
<thead>
<tr>
<th>Duration of coronary occlusion</th>
<th>5 sec</th>
<th>10 sec</th>
<th>20 sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coll (−)</td>
<td>3.2 ± 0.5</td>
<td>4.2 ± 0.8</td>
<td>4.3 ± 0.6</td>
</tr>
<tr>
<td>Coll (+)</td>
<td>3.5 ± 0.7</td>
<td>NS</td>
<td>4.5 ± 1.2</td>
</tr>
<tr>
<td>Half-time (sec)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coll (−)</td>
<td>10.5 ± 2.2</td>
<td>14.2 ± 2.8</td>
<td>23.0 ± 4.4</td>
</tr>
<tr>
<td>Coll (+)</td>
<td>8.1 ± 0.9</td>
<td>NS</td>
<td>10.0 ± 0.8</td>
</tr>
</tbody>
</table>

Values are mean ± SE. Coll = collateral. A half-time of the reactive hyperemia was measured as the duration of 50% level of peak hyperemic flow. NS = not significant. Statistical significance in difference of flow ratio and half-time between before [Coll (−)] and after [Coll (+)] collateral development was tested by paired t-test at the respective durations of coronary occlusion.

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**FIGURE 5.** Effect of collateral development on the reactive hyperemic response to 20 seconds of coronary occlusion. The two panels at the left depict hyperemic responses before (panel A) and after (panel B) collateral development. Note that reactive hyperemic flow returns faster after collateral development. The two coronary flow patterns shown in the left panel are superimposed for comparison in the panel C.
FIGURE 6. Coronary arteriography was performed 24 days apart in the same dog before (panels A and B) and after (panels C and D) and coronary collateral development induced by repetitive coronary occlusions for 8 days. Cineangiograms were taken before (panels A and C) and during coronary occlusion (panels B and D) to document the degree of coronary stenosis due to instrumentation and to evaluate the collateral development angiographically. Magnification of photographs was different, before and after collateral development, due to a different magnification ratio of the image intensifier applied. The big arrow on the left circumflex coronary artery indicates the area of placement of a cuff occluder, and small or open arrows indicate the direction of collateral flow. Note that no collaterals are stained before the repetition of short coronary occlusion (panel B). Collateral channels between the LAD and LCX were also demonstrated by a postmortem angiogram by occluding the LCX with a cuff after removing a pressure transducer and wires as much as possible, as shown in circles in panel E. Abbreviation: DFP = a Doppler flow probe.
state of the collateral channels was quantified by the degree of regional hypokinesia during transient coronary occlusion (Tomoike et al., 1981), and (4) the model had a close clinical relevance to coronary spasm (Takeshita et al., 1982; Tada et al., 1983).

There are, however, criticisms of the present animal model. First, instrumentation of both a hydraulic cuff occluder and a flow probe may induce a coronary stenosis due to tissue reaction to a foreign substance around the coronary artery (Inou et al., 1980) and may bend the coronary artery due to the weight of the instruments. However, coronary cineangiography at the end of the study showed less than 40% stenosis (Table 3), a degree which is too small to influence the physiological reserve of the coronary circulation (Gould et al., 1974; Hills and Friesinger, 1976). The lack of significant coronary stenosis due to instrumentation was also supported by the evidence that the resting coronary blood flow, as well as the percent increase in flow level after administration of dipyridamole or the flow ratio after 5–20 seconds of coronary occlusion (Fig. 3), did not differ, before and after collateral development. Before collateral development, reactive hyperemia following various durations of coronary occlusion (Fig. 3) was qualitatively and quantitatively the same as in the classical reports (Coffman and Gregg, 1960; Olsson and Gregg, 1965; Bach et al., 1974; Marcus et al., 1981). Thus, the stenosis inherent to instrumentation was too slight to produce physical limitations to a high flow phenomenon or reactive hyperemia.

Second, repetitive 2-minute coronary occlusion every 30 minutes for 2–9 days might also reduce the vascular reactivity. However, the resting coronary blood flow, flow ratio after 5–20 seconds of coronary occlusion (Fig. 3) and the response of the coronary blood flow to dipyridamole were similar, before and after collateral development. Therefore, the reactivity of coronary vessels was considered to be unaltered by chronic repetition of mechanical coronary occlusion.

Third, reactive hyperemia is related to the level of myocardial metabolic activity (Pauly et al., 1973; Bach et al., 1973), as well as to the level of coronary artery pressure (Dole et al., 1981). In the present study, heart rate increased by 30 beats/min during coronary occlusion before collateral development, whereas the mean arterial pressure was unchanged (Table 2). Accordingly, increased myocardial metabolic activity during coronary occlusion resulted in an enhancement of reactive hyperemia. After collateral development, hemodynamic changes after coronary occlusion were minimal (Table 2). Therefore the differences in degree of reactive hyperemia, before and after collateral development, was influenced in part by the difference in the metabolic state.

After collateral development, the functional state of the collaterals was well augmented to the level of almost complete recovery of regional hypokinesia from −1.2 ± 6.5% before induction of collateral development to 100.5 ± 1.2% at 2 minutes of coronary occlusion. Despite the presence of these potential collaterals, coronary occlusion for up to 10 seconds (Fig. 4; Table 2) produced a similar degree of wall motion abnormalities seen before collateral development. Although flow ratio of reactive hyperemia to less than 30 seconds of coronary occlusion increased similarly, before and after collateral development, half-time of reactive hyperemia was curtailed progressively after collateral development (Table 4). These findings suggest that collateral channels became functionally effective after coronary occlusion, and that a lag time of about 30 seconds was necessary for full function of the collateral channels developed after repetitive short coronary occlusions. This was confirmed by the progressive amelioration of regional hypokinesia during a sustained coronary occlusion of longer than 30 seconds (Fig. 4; Table 2).

The mechanisms of collateral development are still debated. Repeated transient ischemia was experimentally effective for the development of collateral function (Franklin et al., 1981; Shimokawa et al., 1983), as well as clinically (Takeshita et al., 1982; Tada et al., 1983). However, why collateral function develops during repetitive short coronary occlusion remains to be determined. In the present study, we did not evaluate quantitatively each role of pressure gradient and myocardial ischemia appearing during coronary occlusion for the stimulation of collateral development.

A more rapid return of coronary reactive hyperemia to control and a reduction of the peak level of reactive hyperemia after collateral development caused a reduction of debt repayment ratio and disappearance of overpayment, when the coronary occlusion lasted for more than 60 seconds. As shown in Figure 5 and Table 4, when collaterals developed, reactive hyperemia returned more rapidly, despite the fact that the peak hyperemic flow was the same as the state without collateral development. This peculiar phenomenon agrees well with reduction of reactive hyperemia following short coronary occlusion with intracoronary administration of adenosine deaminase (Saito et al., 1981). Duration of reactive hyperemia and debt repayment ratio was reduced markedly when the coronary occlusion lasted for more than 20 seconds (Figs. 2–4). Thus, possibly the vasodilator substance produced during myocardial ischemia was washed out by collateral blood flow before release of coronary occlusion.

The present study has important clinical implications. During aortocoronary bypass surgery, reactive hyperemia following transient occlusions of vein grafts has occasionally been tested to assess the presence of critical stenosis distal to the graft, the vasodilator capacity of the coronary circulation, and viability of the distal myocardium supplied by the
bypass graft. As evidenced in the present study, when the distal myocardium is well supplied by collateral flow, the degree of reactive hyperemia decreases, depending on the maturity of collateral function. Because reactive hyperemia of the coronary bypass graft will be determined not only by the presence of distal coronary stenosis and myocardial viability, but also by the functional state of collaterals, failure to elicit reactive hyperemia is not related solely to the severity of the coronary artery disease.

Coronary collateral development could be produced when the coronary artery was repeatedly occluded. Therefore, even if a significant coronary stenosis is not evident angiographically, this does not necessarily indicate the absence of functionally effective collateral anastomosis. Changes in regional wall motion during coronary occlusion or the level of debt repayment ratio should be tested to assess the final benefit of the bypass graft.

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Development of collateral function with repetitive coronary occlusion in a canine model reduces myocardial reactive hyperemia in the absence of significant coronary stenosis.
H Yamamoto, H Tomoike, H Shimokawa, S Nabeyama and M Nakamura

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