Blood Supply of the Myocardium after Temporary Coronary Occlusion

By Arno Krug, M.D., Wolfgang du Mesnil de Rochemont, M.D., and Gerhard Korb, M.D.

ABSTRACT
A coronary artery and the accompanying vein were ligated temporarily for 30, 60 and 120 minutes in 39 cats. By labelling the blood with the fluorescent dye acridine orange, the intravital blood supply of the myocardium was investigated 5 minutes to 6 hours after release of the ligature. The region supplied by the temporarily ligated coronary artery (RSCA) was demonstrated by postmortanal perfusion of this vessel using the dye light green. Resumption of circulation in the deeper layers of the RSCA was delayed after a prior coronary occlusion, even occasionally after occlusions lasting only 30 minutes. After a coronary ligation of 60 to 120 minutes the inner layers of the RSCA became the sites of prolonged disturbance of blood supply. The circulatory abnormality observed immediately following release of the ligature did not result from intraluminal vascular obstruction. Even after a temporary ischemia lasting 120 minutes it was still possible, immediately after removal of the ligature, to perfuse an intravitally ischemic portion of the RSCA. The significance of these results for the determination of the so-called "revival-time" of the cardiac muscle is pointed out.

ADDITIONAL KEY WORDS temporary cardiac ischemia intravital staining acridine orange coronary perfusion intracellular and interstitial edema revival-time of cardiac muscle anesthetized cats

After temporary interruption of the coronary circulation, survival of the myocardium depends primarily on whether a sufficient blood supply is established before irreversible injury has occurred. Detailed studies of the resumption of circulation in the myocardium after temporary occlusion of coronary vessels have not been done, so far as we know. Therefore, we examined the circulation in involved myocardium after a coronary artery had been occluded temporarily for 30, 60 or 120 minutes. Moreover, we wished to determine whether it might be possible to perfuse the myocardial vessels artificially in a region of prior ischemia.

Methods
Thirty-nine cats weighing 1.5 to 2.5 kg and unselected, were anesthetized with sodium pentobarbital (25 mg/kg). Respiration was maintained through an endotracheal tube attached to a respirator pump. Arterial pressures were monitored continuously by a Statham strain gauge attached to a catheter in the femoral artery. Left thoracotomy and pericardiotomy incisions remained unclosed throughout the experiment. The anterior descending branch of the left main coronary artery (together with the accompanying vein) was ligated temporarily 2 to 3 mm distal to its origin. The duration of coronary ligation was 30, 60 or 120 minutes after which the ligature was removed, allowing resumption of coronary flow for periods of five minutes to six hours before the beating heart was excised for study. Table I lists the experiments done and the number of animals in each category.

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Accepted for publication January 20, 1966.
TABLE 1

<table>
<thead>
<tr>
<th>Duration of prior coronary occlusion</th>
<th>After removal of occlusion, numbers of animals with excision of heart at</th>
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<tr>
<td></td>
<td>5 min</td>
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<tr>
<td>30 min</td>
<td>5</td>
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<tr>
<td>60 min</td>
<td>6</td>
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<td>120 min</td>
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STUDIES OF THE MYOCARDIAL BLOOD SUPPLY

Acridine orange,* a fluorescent dye, was injected intravenously (0.5 ml/kg body wt of 1% solution in phosphate buffer pH 7.4 at 37°C) four to five minutes before excision of the heart. In order to mark the “region in the myocardium supplied by the previously ligated coronary artery” (RSCA), as well as to demonstrate the possibility of postmortem arterial perfusion of this zone, the following procedures were adopted: Immediately after excision of the heart, a fine catheter was introduced via the aorta into the left coronary artery and the tip advanced to the site of previous ligation and fixed there. Light green** dye (1% in 0.2 M phosphate buffer, pH 7.4 at 37°C) was perfused through the catheter for one minute at pressures equaling the mean arterial pressure observed immediately before the excision of the heart (usually 70 to 90 mm Hg). The excised heart was frozen immediately and cross sections 10 and 30 microns thick were cut from the unfixed tissue for microscopic study and photography of fluorescence in ultraviolet light using filters BG 12 and OG 1.* As reported elsewhere¹ one could see easily the zones in the myocardium which contained either acridine orange (green-yellow fluorescence of the cytoplasm and nuclei of muscle and connective tissue) or only light green (red-brown fluorescence of the cytoplasm without nuclear fluorescence). When cells contained both acridine orange and light green, distinct yellow fluorescence was observed. Blocks were taken from base and apex of each heart, were fixed in absolute ethanol or 10% formalin, were embedded in paraffin, and sections were stained with hematoxylin eosin or by the technique of Masson-Goldner.²

* Obtainable as Acridin-orange from Merck AG, Darmstadt, Germany.
** Obtainable as Lichtgrün from Merck AG, Darmstadt, Germany.
† Leitz AG, Wetzlar, Germany.

Results

1. GENERAL OBSERVATIONS

Grossly, a dark blue-red coloration of the cardiac surface appeared immediately in the RSCA after the ligation of the vessels. The myocardial contractions in this area slowed down and stopped completely after a few seconds. After the removal of the vascular ligature, the blue-red areas changed into a light red color, indicating a prompt resumption or change of the circulation in the subepicardial tissue.

In all hearts the tissue outside the RSCA always contained acridine orange. In the RSCA the three- to four-muscle cell layers lying subendocardially and around larger subepicardial vessels also contained acridine orange.

2. OCCLUSION OF 30-MINUTE DURATION
   (ELEVEN ANIMALS)

Four to five minutes after removal of the coronary ligature in three of five animals the RSCA did not contain acridine orange, indicating that the RSCA was still ischemic, i.e., lacking blood flow interna vitam. Evidence of disturbed blood supply was found interna vitam in the fourth animal in the inner third of the RSCA (fig. 1). In the fifth cat, acridine orange was found in all parts of the myocardium, indicating that there were no areas

FIGURE 1

Heart section, five minutes after removal of a temporary coronary ligation of 30-minute duration. The dark areas, indicated by white triangles, in the inner third of the region of the myocardium supplied by the previously ligated coronary artery (RSCA) contain no acridine orange but do contain the dye, light green.
of ischemia persisting in the RSCA. Fifteen to sixty minutes after the removal of the ligature acridine orange was also found in all parts of the myocardium. In all eleven cats, the RSCA was evenly stained by light green, indicating that, at these time-intervals it is possible to perfuse an area of intravital ischemia in the isolated heart.

3. OCCLUSION OF 60-MINUTE DURATION
(TWENTY ANIMALS)

Four to five minutes after removal of the ligature the RSCA was not stained with acridine orange in all six animals, indicating that resumption of circulation had not yet occurred. In these six cats after the excision of the heart, it was easy to perfuse the RSCA with light green. Unlike the results following coronary occlusions lasting 30 minutes, in some animals small areas of the RSCA in the inner third of the ventricular wall were not stained by light green. Fifteen minutes after the removal of the ligature acridine orange appeared evenly distributed throughout the RSCA in one animal. In another cat, the blood supply to the inner half of the RSCA was disturbed (fig. 2).

Sixty to 360 minutes after the removal of the ligature, in six of twelve animals a complete recovery of circulation was suggested since acridine orange was present in all parts of the RSCA. In the six other cats, all of which had a relatively larger RSCA, areas with disturbed blood supply appeared in the inner third of the ventricular wall of the RSCA, where acridine orange was found only in nuclei of connective tissue cells (fig. 3). Perfusion of the RSCA with light green was possible in all twelve cats.

4. OCCLUSION OF 120-MINUTE DURATION
(EIGHT ANIMALS)

Four to five minutes after the removal of the ligature there was no acridine orange in the RSCA. Peripheral areas of the RSCA could be perfused with light green.

Sixty to 360 minutes after removal of the
Figure 4
Heart section, three hours after release of a temporary coronary ligation lasting for 120 minutes. The black areas in the RSCA contain neither acridine orange nor light green dye. RSCA: region supplied by previously ligated coronary artery.

ligature, zones with disturbed blood supply were recognized in every experiment. In two of six animals in the RSCA in the inner third of the ventricular wall, acridine orange was demonstrated only in the nuclei of connective tissue cells. The four other animals had a larger RSCA most of which was unstained with acridine orange, indicating ischemia (fig. 4). In these six cats, perfused light green was found only in those portions of the RSCA which also contained acridine orange.

5. MICROSCOPIC ALTERATIONS *
In routine histological examination of the hearts immediately after coronary occlusion lasting 30, 60 or 120 minutes, intracellular and interstitial edema were found in the RSCA but no degeneration or necrosis of muscle cells, nor thrombotic occlusions of the vessels were found. With survival of one to six hours after temporary coronary occlusion lasting 60 and 120 minutes the ischemic parts of the RSCA (as demonstrated by lack of intravitral acridine orange staining) were characterized microscopically by dilated and partly ruptured capillaries packed with erythrocytes, by myocardial hemorrhage and by early signs of muscle cell necrosis (fig. 5).

Discussion
1. CRITICISM OF THE METHOD
a) Intravital Perfusion With Acridine Orange
The fluorescent dye acridine orange normally passes rapidly from circulating blood into parenchymal and connective tissues. In the heart both the nuclei and the cytoplasm of normal cardiac muscle and connective tissue exhibit a characteristic brilliant green-yellow fluorescence in ultraviolet light. Degenerating cells are stained more intensely by acridine orange. Therefore, in these studies, the presence of acridine orange in normal cellular distribution proves the existence of myocardial circulation during the four to five minutes prior to cardiac excision and, conversely, lack of acridine orange indicates is-

Figure 5
Histologic section of ischemic cardiac muscle six hours after release of a temporary coronary occlusion lasting 120 minutes (Masson-Goldner-trichome stain). Myocardial capillaries are dilated, partially ruptured and packed with erythrocytes. The interstitial space is wider than normal and hemorrhages are recognizable. There are degenerated muscle cells (pyknosis of the nuclei and diffusely granular cytoplasm).

*We report the histological and histochemical results in the journal "Beitr. path. Anat." In press.
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chemia. In some animals, areas in the RSCA were observed where general staining was diminished but where connective tissue cells contained acridine orange. It is suggested that such zones represent areas of partial or relative ischemia.

b) Perfusion of the Area Supplied by the Temporarily Ligated Coronary Artery

In the postmortem perfusion studies, the placement of the catheter assured a distribution of perfusate potentially approximating that of the RSCA. At least partial perfusion of the RSCA with light green dye was possible in every experiment and was recognized grossly by the green color and microscopically by a characteristic yellow fluorescence (both acridine orange and light green content) or red-brown fluorescence (light green content only) of myocardial cytoplasm. Non-perfused areas were indicated by absence of staining.

c) Ligature of Coronary Vein and Artery

The extent of myocardial damage after the ligation of a coronary artery is reported not to be influenced by coincident ligature of the accompanying vein. Therefore, special effects resulting from ligation of both artery and vein are discounted in the present experiments. By inference, important disturbances relating to lymphatics are also discounted, tentatively.

2. RESUMPTION OF MYOCARDIAL CIRCULATION AFTER TEMPORARY ISCHEMIA LASTING 30 TO 120 MINUTES

Immediate change or resumption of blood flow was observed in subepicardial vessels after removal of the coronary ligature even after occlusions lasting 120 minutes. The subepicardial region may derive partial circulation by anastomoses with unligated coronary arteries during the occlusion, but after release of the obstruction the change of circulatory status was always obvious externally. At the same time, circulation of the deep myocardium was often markedly abnormal. Occasionally after only 30 minutes of temporary ischemia, but especially after an occlusion lasting 60 minutes or more, resumption of myocardial circulation was shown to be delayed in the deeper portions of the RSCA. That is to say, in most cats transitory ischemia was demonstrable in the RSCA immediately after release of the coronary ligature.

As to duration of effects, a 30-minute coronary occlusion caused no prolonged disturbance of myocardial blood supply. Coronary occlusions lasting 60 minutes produced evidence of prolonged disturbance in resumption of flow in six of twelve cats (these six cats had relatively larger RSCA) several hours after removal of the ligature. With still longer periods of coronary occlusion (120 minutes) prolonged disturbances of deep myocardial circulation appeared in all animals.

Shortly after release of a coronary occlusion lasting 30 to 60 minutes, all or nearly all intravitally ischemic regions of the RSCA could be perfused postmortem, whereas after 120 minutes of ischemia only peripheral portions of the ischemic region in the RSCA could be perfused. Coffman et al. observed in the dog the development of palpable firmness in the left ventricular wall after complete myocardial anoxia lasting 90 minutes. This firmness persisted even after several hours of coronary perfusion.

The mechanisms responsible for the observed disturbance of myocardial circulation after temporary ischemia are not clearly known. Although, the later abnormality of circulation in the RSCA is probably linked to degeneration of the muscle cells and capillary walls, the explanation of the circulatory disturbance observed immediately after release of the coronary ligature in the RSCA is not clear. Intramyocardial capillary thrombosis has been suggested as a cause by several investigators. In the present experiments, however, only intracellular and interstitial edema without thrombosis were present histologically at the end of ischemic periods lasting 30 to 120 minutes. Our finding that perfusions of the RSCA could be done successfully in the isolated hearts also speaks against intraluminal vascular obstruction. It has been shown that elevated intraventricular pressure modifies blood flow in noncontracting myocardium, and that ischemia of deep myocardium occurs with lowered coronary arterial
perfusion pressure combined with elevation of ventricular end diastolic pressure. All of these factors may have significance in prolonged or delayed ischemia. On the other hand, vascular spasm and edema may also play a role.

Our findings may have significance in the determination of the so-called "revival time" of the myocardium, by which is meant the length of the time from the beginning of ischemia until irreversible damage occurs. Earlier investigators have concluded that the myocardial necrosis, which they observed in dogs after 30 to 45 minutes of coronary arterial occlusion, developed during the actual period of coronary obstruction. Therefore, the revival time of the canine heart is reported to be 30 to 45 minutes. The present data suggest that some part of the irreversible cardiac damage that follows coronary occlusion may take place after removal of the obstructing ligature, a possibility mentioned also by other investigators. Further experiments are required to determine whether it is possible to prolong the cardiac revival time by such procedures as coronary perfusion after a period of ischemia.

Acknowledgment
We thank Mrs. M. Zuck and Dr. L. Zuck for assistance in the translation of the manuscript and Mr. H. Staubitz for preparation of the photographs.

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Circ Res. 1966;19:57-62
doi: 10.1161/01.RES.19.1.57

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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