The effects of vasomotor drugs and of anemia upon interarterial coronary anastomoses

By Paul M. Zoll, M.D., and Leona R. Norman, M.D., with the technical assistance of Sidney Cassin

The development of functionally significant interarterial coronary anastomoses was measured in 349 young domestic pigs by injecting the coronary arteries with the lead-agar mass of Schlesinger. By this technic well-developed anastomoses were absent in 132 normal control animals. Among the agents tested, acute coronary artery narrowing, sodium nitrite and anemia were effective in stimulating anastomoses. Anastomoses first began to appear two days after narrowing; they became larger and more frequent after seven days.

The clinical effects of coronary artery occlusion—angina pectoris, myocardial infarction and congestive failure—predominate among the cardiovascular disasters that present themselves repeatedly to the physician. It has become apparent, however, that obstruction of the coronary arteries does not always produce symptoms; in some cases significant clinical and pathologic evidence of myocardial damage may be absent despite longstanding occlusive lesions in the coronary arteries.1,2 This apparent inconsistency is explained by the demonstration of larger than normal, functionally significant interarterial coronary anastomoses in these human hearts.3

Other collateral pathways that have been described consist of intracardiac communications between the coronary arteries, capillaries or veins and the chambers of the heart and also of extracardiac anastomoses between the coronary arteries and pericardial, mediastinal, pleural and pulmonary vessels.4,5 None of these extracoronary collateral channels has as yet been clearly demonstrated in human or animal studies to be functionally significant in offsetting the effects of coronary artery occlusion.6,7

Knowledge of the speed of development of interarterial coronary anastomoses and of the conditions that affect it is of great practical importance in the immediate management of coronary artery disease. The obstruction to coronary blood flow by an occlusive process and the compensatory development of interarterial coronary anastomoses are two opposing factors the resultant of which determines the anatomic basis for coronary blood flow. The effects of coronary artery disease on coronary blood flow and, thereby, on changes in the myocardium and their clinical expressions depend in large measure on the relative magnitude and speed of development of these two processes. If interarterial anastomoses could be produced by any means so that the coronary arteries were no longer functionally endarteries, clinical manifestations of coronary disease would be much less frequent or severe.

In previous studies8 interarterial coronary anastomoses were produced experimentally in the young domestic pig by acute narrowing of a coronary artery. The coronary collateral circulation that developed after such a sudden narrowing in an otherwise normal heart was effective in protecting the myocardium from the effects of a superimposed complete occlusion. The experimental method also permitted determination of the length of time

From the Medical Research Department of the Yamin Research Laboratories and the Pathology Department of the Beth Israel Hospital, and the Departments of Medicine and Pathology, Harvard Medical School, Boston, Mass.

This investigation was supported in part by a research grant (PHS H-440) from the National Heart Institute of the National Institutes of Health, Public Health Service, the Patrons of Research and the Colonel and Mrs. Louis Rosenfield Fund of the Beth Israel Hospital, Boston, Mass.

Circulation, Volume VI, December 1952

832
necessary for the development of these inter-coronary anastomoses.

The experimental investigations reported below were designed to determine (1) the precise length of time necessary to establish a collateral coronary circulation after acute narrowing of a coronary artery, (2) the effect upon the development of anastomoses of drugs commonly used in the treatment of angina pectoris and myocardial infarction and (3) the effect of pre-existing anemia. The possible beneficial effect of anemia in stimulating coronary collateral circulation was suggested by previous clinical and pathologic studies.5, 9

Methods and Technic

The basis for the choice of animal and the methods and technic used in this study are reviewed here briefly. They have been presented in detail in the previous report.8

Choice of animal. Domestic pigs were used because their hearts ordinarily do not show interarterial coronary anastomoses when injected with lead-agar mass according to the technic developed by Schlesinger.10 Fine anastomoses of slight extent were found in only 2 of 134 normal pig hearts injected by this method. Furthermore, in 24 experiments sudden marked narrowing or complete ligation of a major coronary artery always resulted promptly in death of the pig. In dogs, however, intercoronary anastomoses were often found normally and acute coronary arterial ligation did not always produce infarction or kill the animal.11-12 Following initial narrowing of the right coronary artery to an appropriate degree, extensive functionally significant anastomoses developed in the pig. After a suitable interval of time complete ligation was usually tolerated without death or massive infarction. Upon injection of the coronary arteries the right coronary artery distal to the complete occlusion would be filled by retrograde flow with lead-agar mass from the other coronary arteries. The results with this experimental method in the young domestic pig parallel the events in the human patient with coronary disease: interarterial coronary anastomoses of functional significance are initially absent but may be demonstrated to develop within a suitable time interval after narrowing of a coronary artery.

Operative technic. In the present study, young pigs weighing from 5 to 10 Kg. were used. After premedication with 0.5 mg. atropine sulfate, anesthesia was produced with open-drop ether and maintained with endotracheal ether and oxygen. The heart was exposed by an incision through the third or fourth right intercostal space. The pericardium was opened; the right coronary artery was carefully dissected from its bed 1 to 2 cm. from its origin, and two strands of number 1 Deknatel were passed under it (fig. 1). A steel wire probe 0.8 mm. in diameter was placed alongside the vessel and one of the ligatures was tied snugly around both the wire probe and the artery. The probe was then removed, leaving the vessel narrowed approximately to the diameter of the wire. The duration of the temporary complete occlusion averaged about 20 seconds. The second thread was tucked inside the pericardium and the chest was then closed in layers, with full inflation of the lungs and aspiration of residual pneumothorax. The entire procedure was usually completed in 20 to 30 minutes, and the animals were awake and ambulatory within another 20 minutes. After a time interval of two, four, or seven days, the incision was reopened and the second ligature was located. It was then tied tightly so as to occlude completely the right coronary artery at the site of the previous narrowing. Animals that survived the complete occlusion were sacrificed four hours later. In some animals only one of these two operative steps was performed.

Administration of drugs and production of anemia. Some pharmacologic agents that are thought to have a vasodilator or vasoconstrictor action on the coro-
nary circulation were given intramuscularly to animals two or three times daily to study any possible effect in stimulating anastomoses. In no instance did the drug itself appear to have an adverse effect upon the animal. The doses of the medications were similar to those administered to animals by other investigators or larger than those used in clinical practice. They were amrinonaphylline (theophylline ethylenediamine), 15 mg. per kilogram; papaverine, 5 mg. per kilogram; quindine sulfate, 20 mg. per kilogram; khellin, 50 mg. per dose; sodium nitrite, 100 mg. per dose; vasopressin (Pitressin), 2.5 pressor units per dose; adrenocorticotropic hormone (ACTH) 5 mg. per dose.

In addition, anemia was produced in a group of 21 animals by repeated small bleedings from the jugular vein with the animal under light ether anesthesia. Depending on previous hematocrit levels and the condition of the animal, the amount of blood withdrawn varied from 50 to 150 ml. and the intervals between venesections ranged from one to six days. The level of anemia was measured by hematocrit determinations. Hematocrit levels, which averaged 41 per cent in normal pigs, were reduced after bleeding to 13 to 29 per cent, with an average of 21 per cent. These anemic levels were maintained with little variation in all animals for 7 to 14 days. The anemic animals were weak and puny and did not gain weight normally.

In one group of experiments the drugs or anemia were applied for long periods without prior coronary artery narrowing. An agent effective in this way may be regarded as having a prophylactic action in protecting the myocardium against the results of subsequent coronary artery occlusion. Sodium nitrite was given to four animals for two weeks, papaverine to eight animals for four to six weeks, and amrinonaphylline to seven animals for three to six weeks; similarly anemia was maintained in 14 animals for one to two weeks. Finally, the right coronary artery was completely occluded without preliminary narrowing in some of these animals to test possible survival. The remaining animals were sacrificed without any coronary artery surgery.

In other experiments the drugs were given only during the interval between initial coronary artery narrowing and death of the animal from final complete occlusion or sacrifice. This procedure tests the therapeutic effect of the agent in speeding the rate of development of anastomoses after the onset of coronary artery narrowing and parallels the manner in which these drugs usually are given clinically.

Pathologic methods. The coronary arteries were examined post mortem according to the injection plus dissection technic of Schlesinger. The left descending, left circumflex and right coronary arteries were cannulated and injected separately with differently colored, lead phosphate–agar mass. When the right coronary artery had not been ligated ante mortem, it was occluded during the injection so as to make all the injections comparable and to make more evident even a slight retrograde anastomotic filling of the distal vessel with mass of any color. The heart was then unrolled so that all the coronary arteries lay in one plane, a roentgenogram was taken and the arteries were dissected with the aid of the film.

The lumen of the right coronary artery was measured by calibrated probes at the narrowed area and also at adjacent normal segments. The original diameter of the right coronary artery measured between 1.8 and 2.2 mm.; the vessel was always completely occluded by the second ligature. The degree of narrowing produced was fairly uniform, being generally from 15 to 35 per cent of the original cross sectional area, the range previously found to be effective in stimulating anastomoses. The myocardium was examined for gross infarction and necrosis and representative sections were taken for microscopic study.

Criterion of anastomosis. A variety of criteria have been used as measures of the presence of functionally significant intercoronary anastomoses. They include transfer of injected materials of many varieties between coronary arteries, increased survival of animals and reduction in electrocardiographic abnormalities or area of infarcted muscle following coronary artery occlusion. The anatomic demonstration of anastomoses by a lead-agar injection mass which is confined to the arterial tree was the criterion selected in this study. In previous studies anastomoses demonstrated by this technic have been found to be functionally significant in obviating the effects of coronary artery occlusion; furthermore, they are generally absent in normal human or pig hearts.

According to criteria previously described anastomoses were determined upon careful examination of the injected vessels with the aid of the roentgenogram in three ways: (1) by finding a mixture of colors of the injected lead-agar mass; (2) by the presence of mass of any color distal to the occluded right coronary artery or (3) by demonstrating a continuous, macroscopic arterial pathway between two coronary arterial branches. Anastomoses were readily graded quantitatively in distinct groups. Slight (1+) anastomosis consisted of a few particles of mass in the peripheral portion of the right coronary artery or of a barely visible, usually incompletely injected connecting loop between two branches. Moderate (2+) or marked (3+) anastomosis consisted of extensive filling, incomplete (2+) or complete (3+), of the peripheral portion of

* The khellin used in this study was "Eskel," trademark for a product supplied for the investigation by Smith, Kline and French Laboratories through the courtesy of Mr. C. W. French.
Fig. 2. Roentgenograms of injected pig hearts showing varying grades of anastomoses distal to occlusion of right coronary artery: (a) slight (1+); (b) moderate (2+); (c) marked (3+) anastomoses.
the right coronary artery, usually with a mixture of colors. In figure 2 examples of these three degrees of anastomosis are shown. The two greater degrees of anastomosis are rarely found in normal untreated pigs. Slight (1+) anastomoses were found only in normal pigs (2 of 134 hearts). Extensive anastomotic fill of moderate (2+) or marked (3+) degree was used in this study as the most satisfactory criterion of interarterial coronary anastomoses. Use of this criterion permits determination of anastomoses in large groups of animals that need not be subjected to surgical ligation of the coronary arteries.

Although survival of the animal after complete ligation of a coronary artery depends primarily on functional interarterial anastomoses, many other unrelated factors influence it significantly. The physical vigor of the animal, the depth, duration and smoothness of anesthesia and endotracheal insufflation, the duration and difficulty of the procedure, the amount of manipulation of the heart and its fibrillation threshold, the precise level of the ligature,15 and the occurrence of various surgical accidents and complications, such as hemorrhage and atelectasis, may all affect survival and their relative importance is often difficult to assess accurately.14

In our series survival after complete occlusion did correlate roughly with the demonstration of anastomoses by the injection technique, but it was considered a much less satisfactory criterion.

The effect of drugs on coronary collateral circulation has been measured by the extent of acute myocardial infarction as observed grossly on the surfaces of the heart.17-19 Accurate measurement of infarcts is difficult, however, because of their irregular, wide borders and variable extent on the two surfaces and intramurally.20 The wide variation of size of infarcts under apparently uniform conditions also makes size of infarction a relatively unsatisfactory criterion.21

Electrocardiographic tracings of standard, bipolar limb leads I, II, and III were taken at frequent intervals during both operative procedures and particularly at the times that the coronary artery was dissected, narrowed and occluded. The observed changes in rhythm, in T waves, S-T segments and QRS complexes were those generally recognized as representing varying degrees of myocardial irritability, ischemia, injury and necrosis. The electrocardiographic abnormalities were useful in indicating the degree of coronary artery narrowing and in foretelling the likelihood of death or survival of the animal. But they were not satisfactory measures of anastomoses and could not be used as criteria of the effect of drugs upon coronary blood flow.

Statistical method. Because of the many variables involved in this study, the number of animals in any one category was necessarily small. Furthermore, anastomoses were infrequent or absent in many of the control groups. The "exact test" of Fisher22 was therefore applied to determine whether the observed frequencies of anastomoses in test groups and control groups differed significantly. It should be emphasized that this statistical method depends on the "null hypothesis," and answers the specific question: "On the hypothesis that there is no difference between two groups, could a difference as large as the one observed be the result of random variation?" A probability less than 5 percent (p < .05) may be considered as a negative answer to this question and proves the presence of a statistically significant difference. An affirmative answer (p > .05) is not evidence of a lack of difference between the two groups, but merely indicates that there is no evidence about it in the data. The statistical method can demonstrate a significant difference, but it cannot demonstrate the absence of a difference between two groups.23

Results

Table 1 presents the results obtained in this study of 349 animals. Although the experiments fall into a large number of groups, they may conveniently be regarded as consisting of three general classes. In an untreated group ("control" column) the natural incidence of anastomoses in unoperated animals and the speed of their development after initial narrowing were observed. A second group ("prophylactic group") was treated prophylactically by drugs or anemia; the production of anastomoses by these measures was noted without prior coronary artery narrowing. A third group (remainder of Table 1) was treated with drugs or anemia during the interval between initial coronary artery narrowing and death; in these animals the combined effects of narrowing plus medication on anastomoses were observed.

Control Animals

The control experiments consisted of two groups of animals that were not treated with drugs or anemia.

(1) One group was composed of 134 normal pigs that were not subjected to surgery and were not treated with any drug or other agent.

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>a + b</th>
</tr>
</thead>
<tbody>
<tr>
<td>c</td>
<td>d</td>
<td>c + d</td>
<td></td>
</tr>
<tr>
<td>a + c</td>
<td>b + d</td>
<td>n</td>
<td></td>
</tr>
</tbody>
</table>

\[ p = \frac{(a + b)!(c + d)!(a + c)!(b + d)!}{n! \cdot a! \cdot b! \cdot c! \cdot d!} \]
Extensive (2+ or 3+) anastomoses were not found in any of these animals and only two of them showed slight (1+) anastomoses, so that the natural or spontaneous incidence of anastomosis of any degree was established at 1 per cent.

Table 1—Results in 349 Pigs of Narrowing, Drugs and Anemia upon Interarterial Coronary Anastomosis

<table>
<thead>
<tr>
<th>Degree of Anastomosis</th>
<th>Controls (No Drug)</th>
<th>Sodium Nitrite</th>
<th>Papaverine</th>
<th>Aminophylline</th>
<th>Quinidine</th>
<th>Khellin</th>
<th>Vasopressin</th>
<th>ACTH</th>
<th>Anemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>132</td>
<td>3</td>
<td>8</td>
<td>7</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>1+</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2*</td>
<td></td>
</tr>
<tr>
<td>2+</td>
<td>0</td>
<td>1*</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Prophylactic Group—No Preliminary Narrowing

Preliminary Narrowing 0–7 Days Before Death

<table>
<thead>
<tr>
<th>0–1 day</th>
<th>0</th>
<th>10</th>
<th>3</th>
<th>4</th>
<th>1</th>
<th>2</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8</td>
<td>14</td>
<td>8</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>1+</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>2+</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3+</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td># survivors</td>
<td>6</td>
<td>9</td>
<td>7</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

2 days

<table>
<thead>
<tr>
<th>0</th>
<th>1</th>
<th>0</th>
<th>0</th>
<th>0</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>1+</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2+</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3+</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td># survivors</td>
<td>6</td>
<td>5</td>
<td>0</td>
<td>10</td>
<td>1</td>
</tr>
</tbody>
</table>

4 days

<table>
<thead>
<tr>
<th>0</th>
<th>4</th>
<th>9</th>
<th>2</th>
<th>8</th>
<th>7</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1+</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2+</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3+</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td># survivors</td>
<td>6</td>
<td>5</td>
<td>0</td>
<td>10</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

7 days

<table>
<thead>
<tr>
<th>0</th>
<th>5</th>
<th>4</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1+</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2+</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3+</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td># survivors</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

# = Number of survivors after complete occlusion.
* = Significantly different from control group.

(2) The second group was composed of 40 control animals in whom varying intervals up to seven days elapsed between initial narrowing of the right coronary artery and death. Eleven of them died within the first day after the initial operation from anesthetic or surgical accidents or from excessive coronary artery narrowing. Slight (1+) anastomoses were found in one of these 11 animals, an incidence which does not differ significantly from the control group (p = .23). The development of anastomoses is first apparent two days after initial narrowing in the finding of 2 of 12 hearts with extensive anastomoses (p = .0006). The natural rate of development of anastomoses in untreated animals is reflected in the increasing incidence of 2+ and 3+ anastomoses from two to seven days after narrowing. Ex-
tensive anastomoses, however, are not very frequent until after 12 days; they were found in 7 of 12 animals that survived 12 to 21 days after initial narrowing. Application of these results in young pigs to clinical situations in adult human patients is precarious. They suggest, however, that in pigs anastomoses begin to develop early but full protection from them cannot be generally expected before 12 days or more after coronary artery narrowing or occlusion.

Animals Treated Prophylactically before Coronary Artery Narrowing or Occlusion

In this group of experiments the effect of potent vasoconstrictor drugs and of anemia upon intercoronary anastomoses was tested prior to the production of a local need for anastomosis by coronary artery ligation. An agent that could stimulate the development of interarterial coronary anastomoses even before the appearance of coronary atherosclerosis would obviously be of great clinical value in the prophylactic treatment of coronary disease. Repeated dilatation of the coronary capillary bed by a potent vasodilator might, for example, lead to the enlargement of the normally present, but functionally inadequate, fine intercoronary anastomoses.

Sodium nitrite, papaverine and aminophylline were given to 18 animals over intervals of two to six weeks before acute complete coronary artery occlusion (table 1). Anastomoses were found in one of the four animals treated with sodium nitrite but in none of the others. This single instance of 2+ anastomoses in one of four pigs treated with sodium nitrite is significantly different from their complete absence in the control group of 134 pigs ($p = .03$).

A comparable effect of papaverine or aminophylline on anastomoses was not observed. No anastomoses were found in eight animals treated with papaverine and in seven treated with aminophylline. To demonstrate a significant positive effect ($p < .05$) upon anastomoses of any agent in comparison with the control group of 134 hearts, at least one instance in seven hearts of 2+ or 3+ anastomoses would be necessary. To demonstrate the absence of an effect of these agents on anastomoses, they must be contrasted with some factor, such as coronary artery narrowing, which does stimulate anastomoses. Calculations show that it would require at least 40 experiments without any 2+ or 3+ anastomoses to demonstrate that these agents differ significantly ($p < .05$) from the slight positive effect of preliminary narrowing of two days' duration.

When the data already obtained are contrasted with the marked effects of prolonged narrowing of 12 to 21 days, statistical analysis shows that papaverine and aminophylline are significantly less effective in stimulating anastomoses ($p = .01$ and .02).

In a similar manner vasopressin was administered for four days to three animals. Its powerful coronary vasoconstrictive effect might be expected to produce myocardial ischemia comparable to preliminary coronary artery narrowing and might, thereby, stimulate anastomoses. All three animals, however, died promptly after acute occlusion of the right coronary artery and none showed interarterial coronary anastomoses. The number of experiments was too small, however, to demonstrate the presence or absence of an effect of vasopressin upon intercoronary anastomoses. Here also, at least one positive result in seven experiments or a total of 40 negative experiments would be required to prove the presence or the absence, respectively, of a significant effect of vasopressin upon anastomoses.

The effect of anemia, uncomplicated by coronary artery narrowing, was tested in 14 animals. Four pigs died of excessive blood loss or anesthesia before undergoing surgery. Of the remainder, eight died immediately following acute occlusion of the right coronary artery, but two survived acute occlusion and were sacrificed four hours later. Upon injection of the coronary arteries two hearts with well-developed (2+) anastomoses were found among the 14 anemic pigs. This observation is of high statistical significance whether compared with the absence of 2+ anastomoses in the 134 control hearts ($p = .008$) or, disregarding degree of anastomosis, with two instances of 1+ anastomoses in the 134 controls ($p = .04$). No further correlation was found in these
experiments between interarterial coronary anastomoses and variations in the degree or duration of anemia.

**Therapeutic Effect of Agents Following Initial Coronary Artery Narrowing**

In this series of experiments the effect of vasomotor drugs, of adrenocorticotropic hormone and of anemia upon the rate of development of interarterial coronary anastomoses was tested at varying intervals of time following initial, adequate coronary artery narrowing. The drugs were administered immediately prior to and following the initial procedure of coronary artery narrowing and three times daily thereafter until the animal died or was sacrificed. Anemia was produced by repeated venesections for one to several weeks before the initial operation. The effectiveness of these agents was assessed by comparing animals treated with the various agents with untreated, control animals kept the same time interval between initial narrowing and final complete occlusion with respect to the incidence of extensive anastomoses. Particular emphasis was placed on the two-day interval between narrowing and occlusion because this was the shortest interval in which extensive (2+ or 3+) anastomoses were found in control experiments.

In 14 animals treated with sodium nitrite, aminophylline, quinidine, khellin or adrenocorticotropic hormone death occurred within a few minutes to 24 hours after initial narrowing. The number of injections of drugs ranged from one dose given before the operative procedure to three doses given in the first 24 hours. In only one instance were extensive (2+) anastomoses found: this animal was given one injection of sodium nitrite before operation and it died 13 minutes after narrowing of the right coronary artery to 25 per cent of its original area. These results with sodium nitrite are not significantly different, however, from the findings in the remaining treated or untreated animals that died within the first day after initial narrowing ($p = .16$).

Among the remaining experiments with time intervals from two to seven days between initial narrowing and final occlusion, sufficient time elapsed to permit a number of doses of drugs to be given. A significant difference between the untreated and control experiments in all these groups was found only in the animals treated with sodium nitrite during a two-day interval between narrowing and occlusion. The significant comparison was in the incidence of 3+ anastomoses in this group and all the other control or drug-treated animals in the two-day series ($p = .008$). No other drug showed a favorable or retarding effect upon the rate of development of anastomoses in this or any other time interval. Although anemia was found in the prophylactic study to stimulate anastomoses, a significant additive effect of the two factors of anemia and narrowing was not observed. In the presence of the positive effect of narrowing, a very large number of experiments is required to demonstrate an additional positive factor of slight or moderate effectiveness.

**Immediate Effect of Vasomotor Drugs on Coronary Arterial Spasm**

During the operative procedure it was observed that some of the drugs had an immediate effect upon coronary arterial spasm. When the right coronary artery was isolated and the threads passed under it, marked spasm of the arterial wall was usually noted in the untreated animal. In animals treated with khellin, aminophylline and sodium nitrite, vasospasm of this type in response to local manipulation was usually much less marked or entirely absent. This type of observation was not quantitated, but it appeared to be generally true and was observed repeatedly. The relation of these observations to the development of interarterial anastomoses was not investigated in this study. Such effects upon vasomotor control of the coronary arteries may well be of some clinical importance.

Electrocardiograms taken at frequent intervals during the procedures offered evidence substantiating the demonstration of anastomoses by the injection technic. In animals in whom no anastomoses were subsequently found, marked S-T and T-wave changes usually appeared following complete ligation. Slight changes occurred after complete ligation in
animals with extensive anastomoses. When anastomoses developed which protected the heart against the effects of complete final ligation, relatively little myocardial damage and little electrocardiographic changes were produced. As previously indicated, however, neither anatomic myocardial damage nor electrocardiographic change was a suitable index of the adequacy or rate of development of interarterial coronary anastomoses.

**DISCUSSION**

In these experiments the length of time necessary for the development of interarterial coronary anastomoses was determined in the young pig and the effects upon it of various commonly used drugs and of anemia were assessed. Functionally significant anastomoses began to appear very shortly after initial narrowing in otherwise untreated, control animals; they were found occasionally within two days after narrowing and with increasing frequency in seven days. These new, functionally significant collateral pathways may form by the dilatation of previously existing, finer capillary communications or by the growth of entirely new vessels. The rapidity of their appearance, within two days after narrowing, does not contravert either mechanism. Rabinovici and Fine\(^2\) found macroscopic evidence of new blood vessels crossing from adherent omentum to bowel wall within three days after occlusion of a mesenteric artery or vein. Within six hours they saw a new capillary crossing a large thrombus and a perithelial reaction about capillaries suggesting a process of conversion to arterioles.

Of all the drugs studied sodium nitrite alone was found to speed the rate of development of anastomoses. It was effective when given prophylactically to normal pigs and also when given after coronary artery narrowing. These results indicate that vasodilator drugs of this type may have clinical value in addition to their transient vasomotor action: they may change the fundamental anatomy of the coronary system by stimulating interarterial anastomoses so that the coronary arteries are no longer functionally end-arteries.

Anemia was also an effective stimulus for the occasional production of interarterial coronary anastomoses in the absence of coronary arterial narrowing. This finding confirms experimentally in the domestic pig previous clinico-pathologic observations in humans that anemia stimulates interarterial coronary anastomoses\(^3\) and increases coronary perfusion rates.\(^4\) From these observations it appears that anemia may occasionally have a beneficial effect in coronary artery disease. Anastomoses stimulated by pre-existing anemia (either spontaneous or purposefully induced anemia) may protect the myocardium from the effects of coronary artery occlusion and diminish or obviate entirely clinical symptoms of coronary disease. It is well recognized, however, that anemia of marked degree or rapid development may aggravate clinical symptoms of coronary artery disease and even precipitate myocardial infarction.\(^25,26\) Delterious effects of anemia on the myocardium have been reported by us and by others as well.\(^5\) Our previous observations indicate that the potentiality of anemia to stimulate intercoronary anastomoses is intimately related to its capacity to produce myocardial damage. Anemia may stimulate anastomoses by producing tissue anoxia and vasodilatation; if the anemia is of marked degree, however, irreversible tissue damage may occur. Although these two abnormalities are produced by varying degrees of cardiac anoxia from any cause, nevertheless they do not always appear together. In some instances anastomoses produced by anemia are sufficiently protective to forestall or diminish the development of myocardial damage even in the presence of severe coronary artery disease. A critical degree of anemia may be found which will stimulate anastomoses and yet will not result in significant myocardial lesions. Anemia may then conceivably have some therapeutic application in the treatment of patients with coronary artery disease.

Unfortunately, none of the agents studied exhibited a striking or constant effect in stimulating interarterial coronary anastomoses. Extensive anastomoses were not produced uniformly or even in the majority of animals in
any of the experiments. Anemia, drugs and even previous coronary artery narrowing cannot be relied upon with assurance to prevent the dire effects of coronary artery occlusion or to hasten the time of re-establishment of adequate coronary blood flow. The inconstancy of success in producing anastomoses suggests that unrecognized factors may be of critical importance in this process. The degree and duration of narrowing have been established as important factors. Whether the degree and duration of anemia are of major or great importance appears questionable from the preliminary observations made in this study and in the clinicopathologic study of human material.

It must be emphasized that only one very special attribute was tested of the various agents examined, namely, their effect upon the speed of development of interarterial coronary anastomoses as measured by the special methods of this study. Many other characteristics more or less unrelated to this attribute might be more important in determining their applicability to clinical situations. Such factors include effect on blood flow in the coronary bed distal to a narrowing or occlusion, relative effect on coronary and systemic vasodilatation and effect on cardiac output. For example, their lack of effect on anastomoses notwithstanding, several of the drugs did reduce local coronary arterial spasm following local irritation. These drugs may thus be of clinical value to patients with coronary artery disease by mechanisms unrelated to their effect on interarterial anastomoses.

These experiments reaffirm the efficacy of coronary artery narrowing, or of relative cardiac anoxia produced by any means, in stimulating anastomoses. They do not, however, bear on the basic mechanisms by which this is accomplished. The length of time necessary to establish collateral coronary circulation after acute coronary arterial narrowing in young pigs has been established more precisely. This technic may therefore be used as a tool to investigate many of the problems concerning interarterial coronary anastomoses that are still unsolved.

Summary

The length of time necessary for the development of interarterial coronary anastomoses and the effects upon it of some vasomotor drugs and anemia were determined in 349 pigs by an injection plus dissection study of the coronary arteries. Functionally significant anastomoses first appeared two days after narrowing of a coronary artery and became more frequent after seven days.

Sodium nitrite showed significant effect in stimulating anastomoses when given prophylactically to normal pigs and when given therapeutically after coronary artery narrowing. Anemia also stimulated anastomoses significantly in normal pigs and occasionally protected animals against subsequent acute coronary artery occlusion.

REFERENCES


INTERARTERIAL CORONARY ANASTOMOSES

The Effects of Vasomotor Drugs and of Anemia upon Interarterial Coronary Anastomoses

PAUL M. ZOLL, LEONA R. NORMAN and SIDNEY CASSIN

Circulation. 1952;6:832-842
doi: 10.1161/01.CIR.6.6.832

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1952 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on
the World Wide Web at:
http://circ.ahajournals.org/content/6/6/832

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

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

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