Studies in Peripheral Arterial Occlusive Disease

I. Methods and Pathologic Findings in Amputated Limbs

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Despite the clinical importance and the intense interest in arterial disease of the lower extremities, knowledge of the extent of the lesions and of the anastomotic circulation in the normal and in various disease states is incomplete. Although it is generally recognized that anastomoses exist, their extent, their size, their normal functional significance and the derangements that may occur in disease have not been adequately studied. The situation is seemingly analogous to that which existed regarding the coronary circulation before studies by various investigators were undertaken in the last decade. It has been the purpose of this investigation to bridge some of these gaps in our knowledge by clarifying the relationship of the pathologic changes in the arteries, and other tissues of the extremities, to the clinical manifestations of arterial insufficiency.

This initial report describes a method for the arterial injection and dissection of human legs that were obtained subsequent to surgical amputation. It includes data relative to arterial narrowing, occlusion, and calcification, and their relation to interarterial anastomosis and gangrene. In addition, some of the clinical implications of these observations are discussed.

Literature

Shortly after the introduction of the roentgen ray, vascular structures of the human body were injected with radiopaque substances and visualized on x-ray film. Before the turn of the century, calcification of the peripheral arteries in man had been noted on the roentgenogram. Despite these early observations, there have been relatively few post-mortem studies of the peripheral arteries utilizing radiopaque media. The paucity of these observations is in sharp contrast to the extensive literature relating to arteriography of the vessels of the lower extremities during life.

In 1924 McKittrick and Root injected the arteries of 15 amputated extremities with a fine suspension of barium sulfate and x-rayed and dissected the specimens. They noted the extent of the col-
lateral circulation as visualized on the roentgenogram and suggested that there was a correlation between collateral circulation and the extent of gangrene. Horton in 1900, using metallic mercury, injected the arteries of 42 amputated extremities and dissected the vessels with the roentgenogram as a guide. He concluded that "the older the person, the less adequate the collateral circulation," and emphasized that there was no way clinically to distinguish between patients who at amputation were found to have widely differing amounts of arterial obstruction. In 1934, Belou injected more than 400 human legs with a variety of radiopaque substances. He was, however, primarily interested in normal anatomic relationships. In 1950, Lindfors injected, x-rayed, and dissected the lower extremities in 186 cadavers. He used a gelatin-aceacia-barium sulfate mixture. The principal object of this investigation was to study the roentgen localization of arterial thrombosis in the large arteries of the leg.

In all the papers mentioned, interest was focused principally on the main vessels and to a lesser degree on the collateral circulation. Little information was reported concerning pathology in the branches of the major arteries. The extent of the collateral circulation was estimated from the roentgenogram but scant data on the size or location of anastomotic channels was recorded.

**Methods**

**A. Technic of Injection and Dissection**

The development in this laboratory of a technic for injecting and dissecting the coronary arteries, and its use in the study of coronary heart disease, suggested that a similar method might be profitably employed in the study of the arteries of the legs. Contributing significantly to the success of the coronary artery injections were the nature of the standard injection mixture and the method of unrolling the heart so that the coronary arteries lay in one plane.

The lead-agar mass employed so effectively for injection of the coronary arteries required that the heart be immersed in a water bath until the temperature of the entire specimen was at 44 C. Such a procedure was attempted and a small series of amputated extremities were injected with this mass. The method was, however, technically awkward, and the results less reliable than those obtained in the heart. During the course of this preliminary work a technic was devised for unrolling the leg so that its main arteries all lay in one plane. It remained to develop an injection mass having the desirable properties of the lead-agar mixture without the necessity of warming the extremity to 44 C.

1. **Injection Mass.** (a) **Properties of Mass.** It was felt that, in addition to being fluid at temperatures ranging from 0 C. to 20 C. so that the specimen need not be warmed, the ideal injection mass should satisfy the following criteria: (1) it must not be injurious to tissues; (2) it must have sufficient radiopacity to allow visualization of small vessels; (3) it should penetrate to all the smallest arterioles but not enter the capillary bed or beyond; (4) it should remain entirely within the vascular tree; (5) it should be of such a nature that the injection may be completed rapidly; (6) it should be possible to harden the mass permanently and rapidly after injecting it, so that none could escape during the unrolling process; (7) it should be flexible enough, after hardening, so that the leg could be unrolled without distortion; (8) it should contain no large particles, to obviate the danger of producing factitious occlusions; (9) it should be readily removable during the dissection of the arteries; and finally, (10) it should not interfere with the preparation of microscopic sections if left in the injected specimen. Several masses employing latex or various plastics as a base were considered but none was found to be suitable. A mass utilizing lead and gelatin in appropriate proportions met all of the criteria just listed except that it required two and one-half hours to harden and on rare occasions went across to the veins.

The lead-gelatin mass is a creamy, white suspension containing 15 per cent of lead and 10 per cent of gelatin. The insoluble lead phosphate falls out of suspension so slowly as not to interfere with the injection procedure. The pH of the mass after paraformaldehyde has been added is 6.3. Relative to distilled water at 25.5 C. and 400 mm Hg pressure, and tested on the same viscosimeter, the mass after the setting agent, paraformaldehyde, has been added has an initial viscosity of 11. The viscosity of the mixture gradually increases after the setting agent is added. The mass remains relatively fluid for 30 minutes, doubles its viscosity in 90 minutes and becomes solid in two and one-half hours.

Although the viscosity is definitely a function of time and of temperature, neither of these factors has affected the setting time sufficient to interfere with the injection of amputated extremities. Mass that has been stored at room temperature for as long as two months before adding the setting agent has been entirely satisfactory.

The level of penetration of the lead-gelatin mixture evidently depends on many properties in addition to the size of the individual particles. Most of the particles of lead phosphate are less than 3 micra in diameter, yet the mass does not penetrate vessels many times larger. Small variations in the herein described method of preparation of the mass or of the technic of injection apparently cause wide differences in the extent of penetration. These differences range from "stumpy" injections of only the larger arterioles to very "fine" injections with filling of many unusually small arterioles. Over a 12-
month period, however, only two preparations of mass penetrated to the venous circulation.

The mass was regularly found in vessels 60 micra in internal diameter, irregularly penetrated as far as vessels from 60 to 12 micra in diameter, but was not found in vessels under 12 micra in diameter. These measurements of vessel size were made on stained microscopic sections prepared from tissue fixed in formalin and embedded in paraffin; they are, therefore, subject to a correction of about 50 to 100 per cent for shrinkage from the fresh unfixed state.

b) Preparation of Mass

Stock Solutions

1. 80 per cent phenol (acid carbolic, liquified).
2. 0.5 per cent phenol red (water soluble).
3. 1 molar lead acetate (Pb(CH3COO)2.3H2O) C.P.

Caution: Do not allow lead acetate crystals or solution to remain exposed to air.

4. 1 molar potassium acid phosphate (KH2PO4) C.P.
5. 1 molar potassium alkaline phosphate (K2HPO4) C.P.

Caution: Add 2 cc. 88 per cent phenol (solution 1) per liter potassium alkaline phosphate to prevent mold growth.

6. 6 per cent paraformaldehyde (trioxymethylene).

140 cc. distilled water
0.7 cc. 0.3 per cent phenol red (solution 2)
0.6 cc. 1 molar potassium alkaline phosphate (solution 5)
12 grams paraformaldehyde

Heat to vigorous boil in 500 cc. Erlenmeyer flask with vented stopper; when color turns yellow, allow to cool, filter, bring volume to 200 cc. and store.

(7) 1 per cent watery solution fast green

Lead Phosphate Precipitate (Mixture A)
Prepare eight 2500 cc. reagent bottles in the following manner:

100 cc. 1 molar lead acetate (solution 3), 75 cc.
1 molar potassium alkaline phosphate (solution 5). Shake well, bring to 2400 cc. with tap water, shake well; after precipitate settles, suction off supernatant to 400 cc. mark. Repeat procedure of filling with tap water to 2400 cc. mark and suction off to 400 cc. mark six times in each bottle.

Divide contents of eight bottles equally between two 2000 cc. graduates. After precipitates settle, suction off supernatant to 800 cc. mark in each graduate.

Gelatin-Potassium Iodide Mixture (Mixture B)
Thoroughly mix 200 Gm. Difco Bacto gelatin and 300 Gm. granular potassium iodide (KI) C.P.

100 cc. 1 molar potassium alkaline phosphate (solution 5); 75 cc. 1 molar potassium acid phosphate (solution 4). Dilute to 1000 cc. with distilled water; heat 750 cc. of above mixture to 70 to 80 C. in pyrex dish; remove from heat and stir with power mixer; add 8 cc. 88 per cent phenol (solution 1); add gelatin-potassium iodide mixture (500 Gm.); continue stirring (for at least two hours) until all particles dissolve.

Lead-Gelatin Mixture

800 cc. mixture A; 800 cc. mixture B; mix well; allow to settle; suction off supernatant to 1000 cc. mark, filter through 44 micro sieve and store.

2. Injection Procedure. Lateral and anteroposterior roentgenograms of the amputated leg were made before injection. The extremity was suspended in the upright position by inserting No. 22 copper wire through a hole drilled through the most proximal cut end of bone. A flanged glass cannula was secured within the popliteal artery with size 0 surgical silk.*

Just prior to injection 2 cc. of 1 per cent fast green (solution 7) and 10 cc. of 6 per cent paraformaldehyde (solution 6) were added to 100 cc. of the lead-gelatin mass and shaken vigorously in a glass stoppered graduate. To this mixture 3 drops of caprylic alcohol were added to remove air bubbles from the mixture. The graduate containing the mass was immediately attached both to the arterial cannula and a mercury manometer which was so connected that a source of constant pressure of 400 mm. Hg could be imparted to the system.

On completion of the injection, lateral and anteroposterior roentgenograms were again obtained. The specimen was then placed in the refrigerator for two hours by which time the mass in the arteries was solid. The limb was unrolled according to the procedure described below and additional roentgenograms taken. The x-ray factors employed were as follows: for intact extremities 15 ma., 55KV, 1.25 to 1.50 second, 48 inches (tube to film); for unrolled specimens 15 ma., 48KV, 0.50 to 0.75 second, 48 inches (tube to film).

The lead-gelatin mass was injected at a pressure of 400 mm. Hg. This pressure was chosen because at 200 mm. Hg, injection of the toes often was incomplete. Pressures higher than 600 mm. Hg often caused rupture of arteries and extravasation of mass.** Furthermore, 400 mm. Hg pressure did not result in intimal tears, dislocation of thrombi, vascular rupture, or extravasation.

During injection, mass invariably leaked at the cut edges from vessels severed at the amputation.

*S In several of the lower leg amputations, two or three of the principal arteries were cannulated separately. This permitted multiple color injections.
level. These leaks could be partially controlled with hemostats. The injection was permitted to continue until no more mass entered the leg or leaked from vessels at the cut edges of the leg. This required about 30 minutes by which time the mass in the graduate had become very viscous although not so solid that it would not flow.

3. Method of Unrolling the Leg. A method of unrolling the leg was devised so that the major vessels would lay in as close to one plane as possible and so that the dorsal and plantar arches of the foot could be separated. In addition, some of the bones in the leg had to be removed so that they would not unduly obscure the arterial shadows. The steps of this unrolling procedure follow.

Step 1. A single incision was made through the skin and carried down to the underlying bones. This incision coursed along the anterior aspect of the leg from the cut end of the femur to the ankle. There it turned medially, anterior to the medial malleolus, and passed along the medial aspect of the foot to the first metatarsal phalangeal joint. At this point it turned dorsally crossing the dorsum of the foot at the metatarsal-phalangeal joints. The incision ended at the lateral aspect of the fifth metatarsal phalangeal joint (fig. 1A).

Step 2. The femur was cleared of surrounding soft tissues, disarticulated from the tibia, fibula and patella, and removed from the specimen. The patella was left in situ. The tibia was then cleared of surrounding soft tissues, disarticulated from the fibula at the knee and the fibula and talus at the ankle, and removed from the specimen (fig. 1B). The fibula was left in situ.

Step 3. To facilitate the separation of the dorsal and plantar arches, the talus was disarticulated and removed from the specimen (fig. 1C).

Step 4. The five metatarsal phalangeal joints were disarticulated, and then dissected from the soft tissues of the sole of the foot. Using the soft tissues lateral to the fifth metatarsal phalangeal joint as a pivot, the metatarsal bones were extended laterally with the soft tissues of the dorsal aspect of the foot (fig. 1D).

The fibula was left undisturbed in order to provide a rigid support to the specimen and preserve the constancy of the anatomic relationships. Removal of bones in the foot, other than the talus, added little to the roentgenologic visualization of the arteries and caused additional fracture of important vessels.

4. Dissection Techniques. Using the film of the unrolled leg as a guide, almost all vessels 1 mm. or larger were dissected. Selected labeled sections of artery, vein, nerve, muscle, and skin were taken for microscopic examination. Arteries containing calcium were decalcified by the method of Foord after being fixed in formalin.

During dissection the nature and location of the arterial pathology as well as the extent of the dissecible and nondissectible interarterial anastomoses and of necrosis were carefully determined and compared with the roentgenogram. This data was preserved for subsequent study by transfer, at the time of the dissection, to tracing paper overlying the roentgenogram. Fluid blood or postmortem clots were occasionally found in the arteries. Although these artefacts could simulate arterial occlusion or narrowing on the film of the injected leg, they were easily recognized at the time of dissection.

B. Criteria for Occlusion, Narrowing, Calcification, and Anastomosis

The technic employed disclosed the occlusions, narrowings, vascular calcifications and interarterial anastomoses in the visualized arteries in each leg.

1. Occlusion. Although areas of occlusion and narrowing stood out on the film as irregularities in the shadow of the injected mass, the presence, degree, and age of the arterial occlusions and narrowings were decided finally during the arterial dissection. Except where segments were taken for microscopy, all injected arteries were routinely opened down to small branches. Solidified mass was found in them as a cast of the size and shape of the lumen. This facilitated determination of the presence of complete occlusion or the degree of narrowing. The staining of the intima by dye diffused from the mass also aided dissection of small or narrowed vessels. At sites of occlusion, a complete break in the continuity of the lumen was demonstrated when neither mass nor stained intima was observed at the time of the dissection. Occlusions were further classified as fresh or old on the basis of their gross appearance. Microscopic studies of many areas of occlusion were made for more accurate estimation of the duration and nature of the occlusive process. The term “occlusion” as used in this communication always denotes complete occlusion.

2. Narrowing. The degree of narrowing for each narrowed segment in the arteries of each leg was classified as slight, moderate, or marked. Small, intimal atheromas without any grossly distinct narrowing of the lumen were placed in the normal group without narrowing. “Slight narrowing” referred to vessel segments with slight but definite constriction of the lumen; “marked narrowing” signified unequivocal, extreme reduction in the arterial lumen; “moderate narrowing” included all vessel segments with intermediate degrees of obstruction. In several extremities the diameters of the major vessels were measured from the roentgenograms, so that the percentage reduction in diameter at areas of narrowing in comparison with immediately adjacent zones was quantitatively determined. Qualitative estimates of slight narrowing were found to correspond approximately with a reduction in diameter of 25 per cent or less; marked narrowing was equivalent to 75 per cent reduction.
or more. Narrowings were further classified as fresh or old on the basis of their gross appearance.

3. Calcification. The extent of arterial calcification could in large part be determined from the roentgenograms taken before and after injection and from the arterial dissection. The relation of such calcification to the various elements composing the arterial wall was often difficult to determine from the films or at the time of arterial dissection. This was particularly true when, as so often hap-
pened, both intimal and medial calcification were present in the same zone. Indeed, such a distinction in some of these sites was often difficult in the microscopic sections.

4. Interarterial Anastomosis. It was found that the roentgenogram alone, even of the unrolled leg, could not be relied upon to prove the existence of anastomotic communications. Overlapping vessels often misleadingly appeared on the film to intercommunicate. Conversely, anastomoses later clearly demonstrated on careful dissection were not traceable on the roentgenogram because of extensive overlapping of injected vessels. On the basis of careful dissection three types of proof were accepted: (1) demonstration, after opening, of a continuous, intact, endothelial-lined small channel filled with mass connecting two larger arteries, or demonstration of an absolutely continuous, intact, distinct vessel with a diameter too small to be opened in its entirety but which was shown at both ends to connect with larger opened endothelial-lined channels; (2) presence of injection mass distal to a complete occlusion; (3) visualization of a mixture of color in the injection mass. In the first instance the pathway was dissectable, in the latter two instances the demonstration of a dissectible pathway was not essential.

Grossly dissectible anastomoses were demonstrated only when the vessel exceeded 0.2 mm. in diameter, which also was the smallest vessel regularly visualized on the roentgenogram. Although some anastomoses 60 to 70 microns in diameter could be traced along their entire course with the aid of the dissecting microscope, they were not routinely looked for in this study.

The second criterion for interarterial anastomosis depended on the presence of a complete occlusion. Since such a lesion prevented direct filling of the vascular tree peripheral to it and since the mass did not ordinarily reach capillaries or veins, or escape from the vascular tree, mass found beyond an occlusion must have arrived there via collateral arterial pathways.

The third criterion for interarterial anastomoses depended on a mixture of color in the injected mass. In those legs in which the popliteal artery was completely occluded, or in which a lower leg amputation had been performed, more than one artery was cannulated and differently colored masses were used in each cannula. Since the color of the injected mass identified its source, a mixture of colors necessarily indicated a connecting pathway between the two separately injected arteries.

**Material**

A total of 72 amputated lower extremities were studied; 37 of these were amputated at the Beth Israel Hospital, and 35 at the Boston City Hospital.*

The etiologic factors responsible for amputation are indicated in table 1.† These included arteriosclerosis (66 cases), embolism (3 cases), osteomyelitis (2 cases) and sarcoma (1 case). There were no cases of thromboangiitis obliterans. In 11 of the 72 legs the occlusive process in the large arteries near the amputation site was so extensive that no vessels suitable for injection could be found. These extremities were dissected without injection and although no data in them was available concerning interarterial anastomoses, information on occlusions in the main stems was utilized. In five of the legs the major vessels after being injected and x-rayed were dissected from the remainder of the extremity. These vessels were fixed in toto, decaled, again x-rayed and selected sections taken for microscopic study. By this special study certain details concerning the branches of the main vessels and some information relating to collateral circulation were sacrificed. However, such data as was available from these five legs was also used.

**Results**

A. Arterial Anomalies

The configuration of the major arteries of the lower leg was relatively constant although minor anomalies were present in 15 per cent of the 72 extremities. Four distinct variations were noted. In six legs the peroneal artery was absent; in three limbs the anterior tibial artery did not extend to the ankle, the dorsalis pedis being formed in each instance from the peroneal artery; in one extremity the posterior tibial artery was absent, and in another leg it arose from the popliteal, above the origin of the anterior tibial. In no extremity did more than one of these anomalies occur. All of the variations could be readily seen on the films of the unrolled leg. The relative constancy of the arterial pattern of the major arteries of the lower leg facilitated the use of the roentgenograms in this study.

B. Arterial Occlusion

For the study of arteriosclerosis the legs amputated for arterial embolism (cases 21, 35, 64), for osteomyelitis (cases 28, 70), and for sarcoma (case 16) were not used. This left 66

* The legs from the Boston City Hospital were made available through the courtesy of Dr. Frederick Parker, Jr. and his associates at the Mallory Institute of Pathology.

† At the request of the Editor all tables are being omitted. These will be furnished on request.
legs in which arteriosclerosis and gangrene were sufficiently severe to necessitate amputation. Fifty-two of these were low thigh amputations and 14 were removed below the knee (table 2).* Because of congenital anomalies and the varying level of amputation there were 244 major arteries (popliteal, anterior tibial, posterior tibial and peroneal) available for study (table 3).*

1. Incidence of Occlusions. (a) Main Vessels. Extensive occlusive disease was present in the major arteries. In each amputated limb at least one point of complete occlusion was found; in 91 per cent of the extremities two or more of the four major vessels were completely occluded, usually at more than one site. The frequency of occlusions of each of the four major vessels is indicated in table 3.* Among the 46 injected low thigh amputations in which complete observations were possible there were 398 old and fresh occlusions—an average of more than eight sites of complete occlusion per leg (figs. 2 and 3).

The extent of the arterial occlusive disease was also evaluated by determining relative lengths of the vessels occluded rather than the number of occlusions per leg or the number of major vessels involved. In 51 amputations in which injections of the major arteries were successfully carried out, accurate measurements of the entire vessel lengths of the arteries of the lower leg and of the lengths occluded were made from the roentgenograms at the time of dissection. Twenty-six per cent of the total length of these four major arteries were completely occluded (table 4).*

(b) Branches. Occlusions in primary branches of the four main arteries 1 mm. or more in diameter were found in 56 per cent of the extremities. The incidence of occlusions in the branches was appreciably less than in the main stems and they were, on the average, shorter than those in the major arteries. Although fewer occlusions were present in the main popliteal artery than in the other main vessels, the incidence of occlusions in its branches was significantly greater than in all the branches of the other three major vessels combined (table 5).*

With the method used, the terminal branches of the anterior and posterior tibial arteries in the foot were readily visualized on the x-ray film but very difficult to follow and dissect. Only a few such detailed dissections in comparison with the accompanying roentgenograms were carried out. These dissections together with careful study of the roentgenograms in the other legs showed very few occlusions in the smaller arterial branches in the nongangrenous portions of the foot and toes, although various degrees of narrowing did occur.

2. Localization of Occlusive Disease. In five legs in which the entire lengths of the tibial arteries were available, only one of the three main arteries in the lower leg was occluded (cases 39, 54, 65, 68, 69). In all five instances the occlusions were in the posterior tibial artery. The length of the occlusions varied from 0.4 cm. (case 54) to 21 cm. (case 39).

There were 12 legs (cases 5, 19, 32, 44, 45, 46, 51, 52, 53, 57, 60, 72) in which only two of the three main arteries to the lower leg were occluded. In all 12 of these extremities the posterior tibial was one of the two vessels occluded; the occlusions ranged from 0.5 cm. to 34 cm. in length. It would appear from these observations that the posterior tibial artery is truly the “artery of occlusion” in the leg. Not only is it the most frequently (table 3)* and most extensively (table 4)* occluded of the tibial vessels, but it is also the first to become completely obstructed by the arteriosclerotic process.

In seven patients both legs were available for injection as a result of bilateral low thigh amputations. Extensive occlusive disease was present in each pair of extremities. In only one patient, however, was the extent of the occlusive disease markedly different in the right and left extremities (table 6).*

Although 91 per cent of the legs had two or all three major arteries of the lower leg occluded by either fresh or old occlusion, there were a few extremities in which one or two of the four major vessels showed absolutely no arteriosclerosis. In one leg (case 50) with multiple occlusions in both the posterior tibial and popliteal arteries and several sites of narrowing in the peroneal artery, the anterior
tibial artery throughout its length was without an atheromatous plaque. In another leg (case 67) there was extensive occlusive disease of the anterior tibial, posterior tibial, and popliteal arteries, with the peroneal artery entirely free of atherosclerosis.

There was an equal amount of occlusive disease in the major arteries in the proximal, middle and distal thirds of the lower legs whether the amount of occlusive disease was small or large.

3. *Fresh Occlusions.* Fresh, completely occluding thrombi were present in 46 per cent of the extremities. The distribution among the major vessels is indicated in table 7*. Fresh occlusions were present in 17 per cent of 52 popliteal arteries examined, in 17 per cent of 66 anterior tibial arteries, in 22 per cent of 65 posterior tibial arteries and in 15 per cent of 61 peroneal arteries examined. Similar fresh occlusions were present in four legs in branches of the popliteal artery and in another leg in a branch of the peroneal artery. Although most of the fresh occlusions were about 1 cm. long, they varied from 0.2 cm. to 13 cm.

Fresh occlusions frequently were multiple in the same artery or present in more than one vessel. Half of the extremities with fresh occlusions had two or more such occlusions. All but four of the 71 fresh occlusions were in vessels in which old occlusions were already present; three-fourths of them were distal and one-fourth proximal to the old occlusions. Each fresh occlusion was attached to the adjacent arterial wall at a site of previous narrowing.

In one-third of the extremities there were one or more recent narrowings caused by mural thrombi. These nonoccluding thrombi were always superimposed on sites of old arteriosclerotic narrowing. The injection procedure itself apparently did not dislodge any of these mural thrombi, for free unattached thrombi were never found during the dissection.

C. *Arterial Narrowing*

In the presence of such extensive old and fresh occlusive disease the functional role of arterial narrowing was difficult to assess. It was clear that as the amount of occlusive disease increased not only the extent but also the degree of narrowing increased. Thus, in legs with occlusions limited to one artery the amount of slight narrowing exceeded the amount of marked narrowing; in legs with occlusion of all three lower leg arteries, the relative amounts of slight and marked narrowing were reversed. Each extremity amputated because of arteriosclerotic gangrene showed occlusion of at least one major leg artery. It appeared, therefore, that narrowing, in the absence of arterial occlusion, may not sufficiently compromise the circulation to produce gangrene.

Areas of narrowing often served as a nidus for the development of fresh thrombi, many of which went on to complete occlusion. On the other hand grossly dissectible anastomotic channels were frequently found distal to sites of old narrowing. These partial obstructions apparently were a stimulus to the development of collateral channels which protected the leg against the sequelae of complete arterial obstruction.

D. *Arterial Calcification*

In 19 amputated legs preinjection roentgenograms showed arterial calcification in one to four major arteries. The lengths in centimeters of calcification of the popliteal, anterior tibial, posterior tibial, and peroneal arteries and their branches visualized on the preinjection roentgenogram were determined with a map measurer. Because we wished to compare it with the calcification often demonstrated during life, only calcification visible before injection and unrolling was utilized for the present analysis. In these 19 legs there were thus visualized 797 cm. of calcified major arteries. Of these 797 cm. only 19 per cent were found after injection and dissection to be completely occluded. The remaining 618 cm. showed either varying degrees of narrowing or normal lumens. An additional 88 cm. of calcified branches were visible before injection. Of these only 1 cm. was demonstrated to be completely occluded after injection and unrolling.

In these same 19 legs, the combined lengths of the remainder of the anterior tibial, posterior

* See footnote\(^*\) on p. 646.
tibial, peroneal, and popliteal arteries totaled 1339 cm. of which 23 per cent were occluded (table 8).* Thus, in these amputated limbs with advanced arteriosclerotic disease and gangrene, the incidence of occlusion was about

E. Interarterial Anastomosis

As stated earlier interarterial anastomoses can be demonstrated in one of three ways: (1) tracing of a grossly dissectible pathway, (2) finding of mass distal to a complete occlusion, and (3) mixing of differently colored masses injected into separate arteries. The unrolling procedure made visible on the roentgenogram many anastomoses that otherwise would not have been suspected. However, the procedure itself destroyed some anastomotic pathways particularly in the foot where the dorsal and ventral arches were separated.

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* See footnote† on p. 646.
Other factors interfering with the dissection and tracing of possible anastomoses included the richness and extensive overlapping of small vessels and presumably collateral blood supply and the devious paths of the small vessels deep within the muscle bundles.

In the arteries of the 56 amputated arteriosclerotic legs successfully cannulated and injected, there were 438 complete occlusions, some old and some fresh. Except in areas of extensive gangrene, or as a result of technical errors, anastomoses were proved by the finding of injection mass in the occluded vessel distal to all but two of these 438 occlusions. One of these two occlusions was in a branch and the other was a fresh occlusion in a major artery. Injection mass could reach these zones distal to complete occlusions only by anastomotic interarterial pathways most of which were not dissectible.

In 39 extremities grossly dissectible anastomoses were searched for with meticulous care. In 38 of this specially studied group such communications varying in number from 1 to 12 per leg were found (fig. 5A–B). In all, 147 such dissectible anastomoses were found in these 38 legs.

Other evidence of collateral circulation was furnished by the leaks from the cut edges at the amputation level. These leaks were multiple, usually could not be completely controlled with hemostats until the mass had become semisolid, and were subsequently shown by roentgenogram and dissection to have come from severed arteries at the amputation site. In the low thigh amputations these leaks signified an anastomotic circulation around the knee joint connecting major vessels of the lower leg with branches of the femoral artery.

In 2 of the 10 injected lower leg amputations it was possible to cannulate more than one major artery and inject each with a differently colored mass. In one lower leg (case 13) two arteries, and in the other (case 16) three arteries were thus injected. In both legs appearance of a mixture of colors distal to several occlusions in all three arteries to the lower leg indicated the richness of the collateral blood supply.

The evidence concerning interarterial anastomoses was amplified in some extremities by additional data of a less conclusive nature than that just presented. In the regions of occluded arterial segments there was frequently on the roentgenogram a diffuse, increased density of negative shadows over what would be found in similar areas with nonoccluded arteries. This increase in density was presumably due to an increased number of small nondissectible arterial shadows. It was not due to grossly extravasated mass. Many of these anastomotic vessels, dissectible and nondissectible, had on the roentgenogram a characteristic cork-screw shape. This has previously been noted during clinical arteriography.12

Even in the unrolled leg the richness of the overlapping small vessel injection made it difficult to determine in what plane the anastomoses occurred. In a few legs the skin and subcutaneous tissues were dissected off from the muscles and x-rayed separately. This
FIG. 4. Roentgenograms of amputated leg showing inverse relation between arterial calcification and occlusion (case 66).

A: Lateral roentgenogram prior to injection. Visible calcification of entire popliteal and anterior tibial arteries and upper fourth of posterior tibial artery. B: Lateral roentgenogram after injection. Popliteal, anterior tibial and upper fourth of posterior tibial arteries patent. Occlusion of remainder of posterior tibial and all of peroneal arteries. C: Roentgenogram of unrolled leg showing sites of patency and occlusion more clearly than in B.

FIG. 5. Roentgenograms of amputated legs after unrolling illustrating size of interarterial anastomoses. A indicates anastomotic vessel.

A: Interarterial anastomosis between posterior tibial and peroneal arteries (case 61). B: Interarterial anastomosis between peroneal and anterior tibial arteries (case 69).

examination showed that the small arteries in the skin and subcutaneous tissues of patients with extensive occlusions of all three lower leg arteries were very plentiful while the arterial skin circulation in patients with no occlusions or only short occlusions of one tibial artery
was considerably less abundant. Similar results were consistently found in other legs studied in this way.

F. Gangrene

In the total series there was marked variation in the amount of gangrene present and the amount of occlusive disease found in the arteries. The necrosis in each extremity was roughly graded on a scale of one to four, and the amount of occlusive disease similarly graded. Correlation between the amount of gangrene and the extent of occlusion was poor (table 9). Very minimal gangrene was found with extensive occlusive disease and vice versa.

Discussion

The injection and dissection technic described in this paper has disclosed the number and proportionate lengths of occlusions in the arterial pathways of extremities amputated for arteriosclerotic gangrene. The radiopaque injection mass has such desirable properties that it is being used in this laboratory to examine the arterial circulation of the brain, kidney, and bowel. It holds promise of general use as an adjunct in the study of disease in hollow viscera as well as in the investigation of the blood supply of various organs.

Unpublished injection studies of nongangrenous legs of cadavers indicate that occlusions in the femoral artery are much less frequent than in the main arteries of the lower leg. Lindbom came to similar conclusions. In the arteriosclerotic limb, therefore, the main arteries from the knee to the ankle become the principal bottleneck in the flow of blood from the aorta to the toes. This bottleneck area is most difficult to visualize completely even with a good contrast media. It is much easier both during life and at necropsy to demonstrate the femoral artery, the popliteal artery, or the terminations of the anterior and posterior tibial arteries in the foot than to visualize the tibial arteries in the lower leg. These observations indicate some of the limitations of arteriography of the limb in the living patient. Knowledge of the localization of the occlusive process and the limits of arteriography should temper any surgical plan to relieve the symptoms of vascular insufficiency in the arteriosclerotic limb by restoring the integrity of the arterial lumen in the thigh.

Sappington and others, using unaided dissection of the main arterial stems, have demonstrated that occlusive disease in the leg must be extensive before amputation is necessary. However, there are few published reports estimating the degree of this obstruction in quantitative terms. We have found, in general, that the extent of arterial occlusive disease in the gangrenous leg was of an entirely greater order of magnitude than that found in infarcted hearts studied by a similar technic. Almost no data by a comparable technic is available concerning the degree of arterial obstruction in other viscera. Unaided dissection studies, however, suggest that the extent of occlusive disease in the leg arteries may exceed that of any other segment of the human arterial tree.

An extremely rich interarterial collateral circulation was associated with the extensive arterial obstructive disease found in the leg. Anastomotic channels of sufficient caliber to be grossly dissectible were almost invariably present in these gangrenous extremities. Both the frequency and size of these large channels was of a greater order of magnitude than that found in the heart. Occasionally the internal diameters of these anastomotic leg vessels approached those of the original major arteries they connected (fig. 5A–B). This observation may explain the return of pulsatile flow to portions of arteries distal to sites of complete occlusion.

Although in almost every case it was quite clear that the dissectible anastomoses were serving as by-passes, circumventing occluded arterial segments, the question of preformed anastomoses was raised by occasional dissectible anastomoses which did not appear to by-pass specific arterial obstructions. Among the specimens examined in this study were two legs with nonvascular pathology: one of these was a lower leg amputation (case 16) for a sarcoma of the heel in a 41 year old man; the other a lower leg amputation (case 70) for a chronic osteomyelitis of the foot in a 27 year

* See footnote† on p. 646.
old woman. Both specimens exhibited entirely normal arteries and, in both, dissecible anastomoses were demonstrated proximal to the sites of pathology. Moreover, three color injections were possible in both of these extremities, and the extensive mixing of colors also indicated the richness of the anastomotic circulation. It must, of course, be recognized that although these two cases approached normal material from the viewpoint of arteriosclerosis, they were not true controls.

Further support for the existence of preformed interarterial anastomoses in the leg was afforded by the injection of the femoral arteries of cadavers free of arterial disease. Injection of these vessels resulted routinely in extensive filling with mass of the arteries of the abdominal wall.

Is this rich anastomotic circulation in the legs preformed or does it develop in response to arterial obstruction? This question can be answered definitively only by subjecting a series of normal extremities to a similar examination. Although final evaluation awaits such a study, if it can be undertaken, we suspect that interarterial communications may exist normally in the human leg. Preformed collateral channels in the normal leg may, for example, explain why arterial embolization to the limbs of young patients with rheumatic heart disease so rarely leads to gangrene. In the lower leg, anastomotic arteries do not, however, present a uniform pattern comparable to the circle of Willis at the base of the brain or the arcuate arteries of the intestine. Although interarterial communications may be present in the normal human leg, arterial occlusion leads to an increase in their number and size: this was demonstrated by the richness of injected vessels as seen on the roentgenogram near sites of obstruction and the increase in the number and the change in the configuration of the skin arteries with increased obstruction. The localization or "tailoring" of anastomoses to circumvent specific occlusions also supports the hypothesis that anastomoses are stimulated by the occlusive process. Most of the occlusive disease in these gangrenous limbs was limited to the major vessels between the knee and the ankle. The commonest site for large grossly dissecible communications was in the region of the ankle joint. This is precisely where they would be most effective.

From these studies it should be clear that arterial obstruction is primarily responsible for the development of gangrene and that the rich collateral circulation compensates in part for the obstruction to arterial flow, thereby permitting the occlusive disease to become extensive before gangrene supervenes. The pathologic substrate of occlusion and anastomosis which in part determines the fate of a limb, should not, however, be thought of as a constant and invariably picture. It is a shifting background in which changes frequently occur quite rapidly. Although there was a parallel increase of narrowing and occlusion, such narrowing did not necessarily or commonly go on progressively and gradually to occlusion. Instead the process was periodically accelerated by the sudden development of complete thrombotic occlusions. Indeed, fresh thrombotic occlusions were found in about half of these legs. Such thrombi were frequently several centimeters in length and often multiple. Although most of them were distal to sites of old occlusions, they still played an important role in diminishing the blood supply to the foot. They often plugged arterial segments which previously functioned as conduits by which blood was passed from one collateral vessel to another. This sudden impairment of an already precarious circulation surely had a role in the appearance of gangrene, the extension of gangrene, or the failure of an amputated stump to heal. Anticoagulant therapy may have its main value in preventing or retarding the development or the propagation of this type of lesion in segments of a complicated anastomotic circulation.

Arterial calcification in the lower extremity has been noted frequently in young men free of any symptoms or signs of arterial insufficiency. It is found more often in the male than the female, and is common in the diabetic. Arterial calcification is said to be of some diagnostic value in distinguishing between arteriosclerosis and thromboangiitis obliterans in which it is absent or minimal. Possibly it is only an indication of early
arteriosclerosis. Intimal and medial calcification can perhaps be distinguished on the roentgenogram. Some of our cases with relatively little arterial obstruction showed only medial calcification. In the majority, however, both intimal and medial calcification were present together in the same zone and, when extreme, even microscopic study could not always distinguish between them.

Others previously have reported a lack of correlation between arterial calcification and occlusion, and even an inverse relationship between the two. It has been claimed that calcified vessels when associated with hypertension show little tendency to thrombosis. Our own data clearly demonstrate this lack of correlation between calcification and occlusion in patients in whom the clinical picture of arteriosclerosis and gangrene was sufficiently severe to necessitate amputation.

Vagaries in the distribution of atherosclerosis throughout the arterial system were exemplified by the findings in the arteries of the lower leg. Any theory of the pathogenesis of atherosclerosis must take cognizance of the fact that the posterior tibial artery is usually the most extensively involved and the first in the lower leg to become occluded, that there are more occlusions in the popliteal branches than in the tibial artery branches and that, on occasion, two of the arteries in the lower leg may become completely obstructed and the third remain free even of an atheromatous plaque.

In a few extremities extensive gangrene occurred in the presence of relatively little occlusive disease and a demonstrable collateral circulation. The development of gangrene in such instances may, in part, be determined by superimposed factors such as shock, trauma, infection, and nutritional states which increase the discrepancy between supply and demand for blood beyond the limits which can be tolerated by tissues with a compromised circulation. A study of such clinical factors will be subsequently reported.

**Summary**

1. A new technic is described for the study of the arterial circulation of amputated extremities. The method is based on the use of a radiopaque injection mass containing lead and gelatin in appropriate proportions, and on a procedure for unrolling the extremity so that the major arteries lay in one plane. The properties of the mass and its value for studies of various organs are indicated.

2. The extent and location of arterial occlusion, narrowing, calcification and interarterial anastomosis were studied in 66 legs amputated because of arteriosclerotic gangrene.

3. One or more occlusions were found in each extremity examined; in 91 per cent of the legs two or more of the four major arteries were occluded usually at more than one point with an average of 11 sites of occlusion per leg. More than one-fourth of the lengths of these arteries was occluded by the time amputation was necessary.

4. In these arteriosclerotic limbs, the three lower leg arteries were the principal bottleneck in the flow of blood from the aorta to the toes.

5. The posterior tibial artery was invariably the first artery to be occluded and occlusions in this vessel were more extensive than in the other lower leg arteries.

6. Fresh completely occluding thrombi were present in about half of these extremities and in 25 per cent these fresh occlusions were multiple.

7. The roentgenologic demonstration of arterial calcification bore no relation to the presence or location of arterial occlusion. Examples were presented of markedly calcified arteries with little or no obstruction of the lumen as well as of arteries free of grossly visible calcification in which complete obstruction was found.

8. An extensive interarterial collateral circulation was clearly demonstrated. This rich anastomotic circulation permitted the occlusive disease to become extensive before gangrene supervened.

9. The clinical implications of these observations are discussed.

**Sumario Español**

Una nueva técnica se describe para el estudio de la circulación arterial de extremidades amputadas. El método se basa en el uso de inyección de substancia radiopaca conteniendo
plomo y gelatina y de un procedimiento de exponer la extremidad de manera que las arterias principales estén en un solo plano. Datos se presentan concernientes a la extensión y localización de la oclusión, estrechez, calcificación y anastomosis intrarteriales en 66 piernas amputadas debido a gangrena arterioslerótica.

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9 Unpublished data.
10 Unpublished data.
Studies in Peripheral Arterial Occlusive Disease: I. Methods and Pathologic Findings in Amputated Limbs

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