A Simplified Technique for Postmortem Evaluation of Coronary Arteries

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The growth of complex diagnostic and therapeutic technologies in the clinical management of cardiovascular diseases has mandated a more comprehensive and detailed analysis of cases which reach the pathology laboratory. This report describes in detail the relatively simple techniques and protocol which we have employed for postmortem evaluation of the coronary vascular bed and myocardium. The key elements include the use of a pigmented gelatin mass containing radiopaque material (Barosperse), proper injection technique with simultaneous filling of the main coronary vessels at identical pressures, postmortem arteriography, cardiac dissection, and histologic confirmation of coronary and myocardial lesions. Three cases with sharply differing cardiac diseases are presented to illustrate the kind of information which may be obtained with this approach. Our experience in terms of frequency and distribution of occlusive coronary vascular disease and the relationship to age and sex has been summarized. Significant disease (>75% lumenal obstruction) was identified angiographically and confirmed by dissection in 46 of 57 cases of clinically suspected disease. None of six hearts from patients without clinical evidence for cardiovascular disease demonstrated actual or angiographically false-positive occlusive coronary disease. It is suggested that a more detailed analysis of the coronary vascular bed can be accomplished in the pathology laboratory with this relatively simple approach and that important information bearing on clinical management can be reliably obtained.

Postmortem angiography has been used for many years to study the anatomic pattern and pathologic changes of human coronary arteries. A variety of injection methods have been developed (1). However, these techniques have not been widely employed for routine use in the autopsy pathology laboratory. This probably reflects the relative complexity of preparing the injection medium, cumbersome injection procedures, and the time delay for dissection. Recently, Hales and Carrington (2) described a modification of Schlesinger’s buffered iodinated gelatin injection mass (3) that greatly simplified the injection techniques. Multicolored injection of the coronary system is now feasible for routine use by an autopsy service for radiographic visualization and gross examination of anatomic and pathologic details of the coronary arteries. Moreover, the material is readily identified in intramyocardial vessels in routine histologic sections and the color easily recognized. This provides some indication of the major coronary vessel which likely supplied a given segment of myocardium and permits better correlation between myocardial ischemic changes and coronary occlusive disease.

MATERIALS AND METHODS

Injection Mass and Perfusion System

The physical characteristics and specific formulas for the preparation of the modified Schlesinger’s gelatin injection medium have been described in detail by Hales

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and Carrington elsewhere (2). The basic materials are listed in Table 1A, and the procedures for making the coronary injection medium are presented in Table 2. The coronary perfusion system is illustrated in Fig. 1, and the necessary materials for the injection apparatus are listed in Table 1B.

**Coronary Artery Injection Procedures**

(i) After removal from the body, the heart is washed and clots are removed. It is then weighed, photographed, and placed in the warm (37°C) saline bath for 15 min.

(ii) The main coronary arteries are cleared as close to the aorta as possible with minimal dissection. Ligatures are placed loosely around the vessels.

(iii) Right and left main coronary arteries are cannulated with saline-primed polyethylene catheters of appropriate size and with flared ends. They are then slowly flushed with 5 ml of saline to disperse residual air bubbles that may be trapped near the ostia. It is important that the external diameter of catheters be only slightly less than the coronary artery ostia. The catheters are then tied in place and again slowly flushed with a small amount of saline to check the catheter–artery system for leakage.

(iv) Catheters are connected to the perfusion system via three-way stopcocks, and the perfusion bottles are primed with 20 ml of saline. The coronary arteries are then perfused with 15 ml of saline at 40 mm Hg of pressure. The perfusion pressure is controlled by a pressure regulator and monitored with a gauge.

(v) The perfusion bottles are primed with 20 ml of working pigmented gelatin injection medium. The working injection medium is made within 2 to 3 min before injection by mixing 18 ml of pigmented gelatin mass with 2 ml of optimal concentration of formalin (see Table 2). Usually, the red-pigmented gelatin is used for left coronary injection and the blue-pigmented gelatin for right coronary injection.

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**TABLE 1**

| Materials for Injection Masses and Perfusion System |
|----------------------------------------------------|
| **A. For pigmented gelatin mass**                  |
| 1. Gelatin, Knox type 2136 (Kind and Knox Gelatin Co., Camden, N.J.) |
| 2. Monastral red pigment, RW-768-P (DuPont Co., Wilmington, Del.) |
| 3. Monastral blue pigment, BW-372-P (DuPont Co., Wilmington, Del.) |
| 4. Potassium iodide (crystalline)                  |
| 5. Monobasic sodium phosphate                      |
| 6. Diabasic sodium phosphate (Na₂HPO₄·7H₂O)         |
| 7. 2-Octanol (capryl alcohol)                      |
| 8. Phenol, U.S.P., liquified                        |
| 9. Barosperse, barium sulfate U.S.P. formulation (Mallinckrodt) |
| 10. H₂O                                           |

| **B. For perfusion system**                        |
|---------------------------------------------------|
| 1. Perfusion bottles                              |
| 2. Pressure gauge                                 |
| 3. Tubing clamps                                  |
| 4. Needle valve (gas pressure regulator, Nutro Co., Cleveland, Ohio) |
| 5. Polyethylene tubing (PE 100-320)               |
| 6. Tygon and rubber tubing                        |
| 7. Three-way stopcocks (K-75, Pharmaseal, Inc.)   |
| 8. Luer stub adapters (15–20 gauge, Clay Adams)   |
| 9. Plastic syringes (10 ml)                       |
| 10. Beakers (50 ml)                               |
| 11. Silk (2–0)                                    |
| 12. Tongue depressors                             |
TABLE 2

| Part A | Part B |
|---|---|
| **Materials** | **Quantity** | **Blending time** | **Materials** | **Quantity** | **Blending time** |
| | **Red** | **Blue** | (high speed) (min) | | (low speed) (min) |
| 1. H₂O | 160 ml | 170 ml | | 1. H₂O | 200 ml | 10 |
| 2. Potassium iodide | 130 g | 130 g | | 2. Barosperse | 600 g | |
| 3. Monobasic sodium phosphate (1 mmole/ml) (NaH₂PO₄) | 30 ml | 8 ml | | | |
| 4. Diabasic sodium phosphate (1 mmole/ml) (Na₂HPO₄) | 15 ml | 37 ml | | | |
| 5. Octanol-phenol mixture (40/60, w/v) | 3 ml | 3 ml | | | |
| 6. Gelatin | 130 g | 130 g | 2 | | |
| 7. Pigment | 110 g | 100 g | 2 | | |

*Parts A and B are deaerated overnight in covered beakers at room temperature. The two parts are then mixed by manual stirring until the gelatin mass appears homogeneous. It may be stored in a bottle under refrigeration. The optimal concentration of formalin for the desired solidification time (about 30 min at room temperature) is determined (see Ref. 3). This is generally about 7.5% for the blue and 10% for the red mass.*

FIG. 1. Coronary artery injection apparatus. Dual reservoirs permit simultaneous injection of gelatin mass with identical pressure heads. Heart immersed in saline bath at 37°C. Standard procedure is to use red mass in left coronary system and blue in right. Color of mass is readily identified in vessels in histologic sections routinely stained with H & E. Source of blood supply for a given segment of cardiac tissue can thus be identified.
(vi) The injection medium is introduced into each coronary artery at the same pressure, and the pressure is gradually increased over a period of 3 min to a maximum of 140 mm Hg and maintained at that level for 15 min.

(vii) After the injection has been completed, the tubing between the perfusion bottles and catheters is clamped while the injection medium is still under pressure. The three-way stopcocks are turned off and disconnected from the tubing.

(viii) Solidification of the injection medium is facilitated by immersing the heart with catheters and stopcocks in iced water for 10 min.

(ix) Radiographs of the heart are taken in the anteroposterior and lateral planes with a portable X-ray unit (Picker X-ray Corp., Model 59). For normal hearts the radiographic factors are 100 mA, 70 kV for 2.5-sec exposures, with a columnator to film distance of 38.5 in. Appropriate variations in factors are made for enlarged hearts.

(x) After completion of the angiography, the major coronary arteries are removed and the heart is bread-loafed or subjected to standard dissection to evaluate the coronary vasculature and myocardial lesions.

RESULTS AND COMMENTS

Postmortem angiograms from three selected cases will be presented to illustrate the information which may be gained with this method in widely disparate cardiac disorders. The example shown in Fig. 2 is from a 47-year-old male with rheumatic valvular heart disease since childhood. Clinically, he was found to have mitral stenosis and aortic valvular insufficiency. Because of severe and intractable heart failure a Starr–Edwards prosthetic aortic valve was inserted. At operation significant tricuspid insufficiency was also present, presumably on the basis of right heart failure, and a Hancock porcine valve was inserted in the tricuspid ring. The patient died shortly after the surgical procedures and at autopsy was found to have a massively enlarged and hypertrophied heart which weighed 1070 g (normal weight is about 350 g). The prosthetic valves are seen in Fig. 2, and it is evident that the seat of the Starr–Edwards valve is well below the origin of the coronary arteries and did not obstruct their orifices. It can also be seen that the coronaries are enlarged, consistent with the massive size of the heart. No sites of lumenal narrowing or occlusion are present which might have contributed to heart failure on an ischemic basis. Moreover, the penetrating intramural vessels are readily visualized and free of significant obstructive disease. It is clear then that far more detailed and complete information can be obtained from this simple procedure than with the traditional examination done by removal of the epicardial vessels and subsequent sectioning.

The second example, shown in Fig. 3, is from a 48-year-old male with polycystic kidney disease, renal failure, and hemodialysis for 4 years. He had undergone subtotal parathyroidectomy for hypercalcemia but nonetheless had significant osteoporosis, pulmonary microlithiasis, and calcific deposits in many sites, including the aorta, circle of Willis, and coronary vessels. Much of the right coronary artery was completely occluded and extensively calcified as is clearly evident in the postmortem angiogram shown in Fig. 3. It was not possible to cannulate this vessel, and the injection was made only into the left system. It is evident that this supplied the entire left ventricular free wall and septum. Posterior descending branches of the right coronary artery have been filled via large collateral communications. Moreover, a right marginal branch supplying the right ventricular free wall is filled retrogradely by apical communications from the left anterior descending artery. Hence, collateral
communications and the source of blood supply to portions of myocardium normally perfused by the obstructed coronary system can be readily identified with this technique, information which could not be obtained by standard autopsy methods.

The third example is from a 58-year-old male with severe atherosclerotic coronary disease and angina who underwent coronary bypass surgery. At autopsy the heart weighed 320 g and was found to have complete old occlusion of the right coronary
FIG. 3. Heart from patient with adult polycystic disease of kidneys, renal failure, hypercalcemia, and calcification of many tissues. Right coronary vessel completely obstructed from origin. Heavy calcification readily apparent in radiograph extending from aorta to posterior descending branches. Latter are filled retrogradely through collaterals from injected left coronary system. Right marginal branch filled from apical collaterals. Nodal artery (seen at atrial level) filled from left system.

(RCA) 4 cm from its origin. A corresponding old posterior (inferior) organized infarct was present. The left anterior descending (LAD) vessel was about 75% occluded. A left internal mammary artery anastomosis was made to the distal LAD and a saphenous vein bypass graft was applied posteriorly to the distal RCA.

Postmortem injections of the left coronary system and the two grafted vessels were made and the arteriogram is shown in Fig. 4. Several important features are quickly apparent. Filling of the LAD ceases abruptly near the anastomosis site. There is no filling of the distal LAD from the internal mammary graft. This was subsequently found due to a technical error in the suturing procedure which occluded the lumen. There is filling of narrowed distal right coronary and posterior descending branches from the vein graft. However, substantial extravasation of contrast is
FIG. 4. Heart from patient with atherosclerotic coronary disease and severe angina. Coronary bypass surgery performed with anastomosis of left internal mammary artery to left anterior descending (LAD) coronary artery. Opaque bars are surgical clips on branches. LAD obstructed at site of anastomosis and there is no filling distally. Right coronary (RCA) completely occluded 4 cm from origin. Vein graft to distal RCA posteriorly. Mass of dye indicates leakage at anastomosis site. Caliber of injected vessels markedly reduced in size. Irregularity of left cardiac margin due to surgical resection of portion of LV free wall for suspected intraoperative infarction.
present and this correlated with widespread subepicardial fresh hemorrhage over the posterior surface of the heart originating from a leaking anastomosis. There is filling of some branches of the right coronary system via collaterals from the left coronary vessels. The irregular contour of the left margin of the heart is consequent to resection of a portion of the LV free wall at surgery for suspected acute infarction during operation.

In the 11-month period between September 1974 and August 1975 a total of 57 hearts with clinically suspected coronary artery disease and 6 presumably normal hearts was studied as described above. The latter demonstrated no significant occlusive disease angiographically, and this was confirmed by subsequent gross and microscopic examination. Thus, "false positives" due to air bubbles or other filling defects were not observed. Of the group with clinically suspected disease, 46 of the 57 cases demonstrated significant occlusive disease (>75% lumenal obstruction) arteriographically in the major coronary vessels, and the lesions were subsequently identified and confirmed by dissection. The frequency and distribution are summarized in Fig. 5, and the extent and localization in each sex are tabulated in Table 3. In 27 of the 46 hearts complete occlusions were identified (Table 3) and these were far more frequent in the LAD or right coronary system. Only one was found in the left circumflex artery. In 61% of cases, occlusive disease was found in both coronary systems; the left only in 24% and the right only in 15% (Fig. 5). The distribution was independent of age or sex, consistent with other observations (4). However, the average age of these patients indicated that the males were significantly younger (61.6 yr) than females (70.2 yr) (P < 0.01). Also, the mean total cardiac weight was less in females (448 g) than in males (526 g) (P < 0.05), although substantially in excess of normal values of 300 and 350 g, respectively.

The injection mass of Hales and Carrington offers a number of advantages when applied to postmortem examination of the coronary vascular bed. For example, the mass can be adjusted to achieve a uniform degree of vascular penetration to the desired level (2). In this study filling of vessels extended to small arteries, including collaterals, but not to the capillary level which would have obscured the vessels of

![FIG. 5. Distribution of significant coronary occlusive lesions (>75% lumenal obstruction) in 46 postmortem cases studied by gel mass injection and arteriography and confirmed by subsequent dissection. In most cases there was involvement of both coronary systems.](image-url)
interest. The colors are the result of a homogenous dispersion of pigments of small particle size and therefore do not diffuse from the vascular compartment or stain surrounding tissue. This was a significant problem with the original Schlesinger method. Moreover, the pigments are not soluble in organic solvents used in the histologic preparation of tissues. The colors are readily identified in both gross and microscopic sections. This is of value in suggesting the probable blood supply of a given segment of myocardium, indicating whether it was derived from the right or left coronary system or both. Histologic preservation is excellent and not detectibly altered by the injection mass. Fresh occlusive thrombi have been identified in some of our injected specimens which from microscopic examination were clearly in their original antemortem location. It is of course possible that small, nonadherent mural thrombi or fragments of thrombi may be dislodged during the injection procedure and swept to a more distal site. None have thus far been identified, however.

The striking advances in diagnosis and management of coronary artery disease and the advent of revascularization surgery, in particular the now widely used coronary artery bypass graft procedure, demands more sophisticated and accurate postmortem analysis by the pathology laboratory. Application of the methods described in this report has proven of significant value in verifying antemortem diagnosis as well as identifying diagnostic and technical errors. The development of a cardiovascular pathology laboratory is of great importance for an effective cardiovascular program directed to sustained high quality of current diagnostic and therapeutic modalities and for the development of new methods for improved patient care.

**SUMMARY**

The advent of complex diagnostic procedures in cardiovascular medicine and the application of advanced technology in treating atherosclerotic vascular disease mandates the introduction of more sophisticated and accurate methods for postmortem analysis in the pathology laboratory. Prosthetic cardiac valve replacement, coronary artery bypass surgery, and the use of balloon counter-pulsation apparatus, for example, are now relatively routine procedures in any major medical center. To be an effective member of the team, the pathologist must be prepared to support this effort by using methods which are sufficient to confirm diagnostic accuracy and the technical success of surgical procedures and to identify errors which may be pertinent in the interpretation of an unsuccessful result. From a practical standpoint the introduction of adequate postmortem diagnostic methodology, while important, will not be widely adopted unless the techniques are relatively easily applied, rapid, and inexpensive. The objective of this report was to detail certain techniques which have proven to meet these criteria during approximately 1 year of testing in an active autopsy service dealing with a large number of primary cardiovascular cases. A multicolored injection mass containing contrast material for radiographic visualiza-

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**TABLE 3**

Incidence of Coronary Occlusions in 57 Clinical Cases of Myocardial Infarction (1974–1975)

| Group          | Complete occlusion (major artery only) | Partial occlusions | Total  |
|----------------|----------------------------------------|--------------------|--------|
|                | LAD * | LCA | RCA |                |        |
| Male (N = 35)  | 5     | 1   | 9   | 12              | 27     |
| Female (N = 22)| 8     | 0   | 4   | 7               | 19     |

*LAD, left anterior descending coronary artery; LCA, left circumflex coronary artery; RCA, right coronary artery.*
tion as described by Hales and Carrington was used for detailed analysis of the coronary tree in 57 human hearts. The techniques for preparation of the mass, cannulation, use of the injection apparatus, preparation of the coronary tree and heart have been presented in detail. Modifications for special circumstances (e.g., coronary bypass graft) have been described. Examples from three selected cases with widely divergent cardiac diagnoses have been shown and problems which may be identified with these methods are illustrated. It is concluded that the accuracy and relative simplicity of this approach will permit general use in comparable institutions.

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