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A PIGMENTED GELATIN MASS FOR VASCULAR INJECTION†

Almost any study of fine or coarse vascular anatomy or pathology can be assisted at some phase by the use of one or more of the various vascular injection techniques. Gelatin, which is superior for demonstration of vessels of microscopic and near-microscopic dimensions, is so rarely used now that few workers have had any practical experience with it. This lack of popularity is understandable to those who have attempted to use the gelatin injection techniques that are described in the texts on anatomical methods. These require melting the gelatin and warming the specimen, or, if "cold" or iodized gelatin is used, immersing the entire specimen in formalin to solidify the injection mass.

Over a decade ago, Schlesinger[1] described an ingenious modification of Tandler's cold or iodized gelatin mass that greatly simplified and expanded its use. Schlesinger demonstrated that if the gelatin solution were iodized sufficiently to be fluid at room temperature, and were buffered properly, formalin could be added directly to the mass just before injection; only after sufficient time had elapsed for good vascular penetration would the mass gel. Designed primarily for roentgenographic visualization of arteriosclerotic and thrombotic lesions of the coronary arteries, however, Schlesinger's mass has distinct limitations for other use. The extent of the injection is restricted to vessels larger than 50-100μ by the use of a large amount of barium sulfate for radiologic opacification. The water-soluble dyes he recommended for multicolored injections stain the tissues, and lose their color during bleaching, clearing, or preparation of the injected tissue as routine histological sections. The bacteriological gelatin which he recommended was designed for another purpose and has been found to vary greatly in the characteristics which are important in its use as an injection mass. In order to overcome these limitations we undertook analysis of certain properties of Schlesinger's basic mass, employing a new gelatin of constant character and using inert pigments of fine particle size instead of soluble dyes. Through this study we have evolved several simple formulae which yield injection masses suitable for a variety of purposes. In addition

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to presenting these formulae and the methods for their utilization, this report includes data from some of the analysis which may be of value to those who wish to modify the system further for applications other than those we have made.

MATERIALS AND METHODS

I. *Materials*

A. Gelatin, Knox type 2136, for injection purposes*

B. Pigments

Monastral Red B, RW-768-P (aqueous dispersion), DuPont**
Monastral Blue B, BW-372-P (aqueous dispersion), DuPont **
Monastral Green BW, GP-511-D (dry), DuPont**
Chrome Yellow Medium, Y-469-D (dry), DuPont**
Green-Gold, YW-613-P (aqueous dispersion), DuPont**
Sterling R. carbon black, Cabot†
Barosperse, barium sulfate U.S.P. formulation, Mallinckrodt‡
Barium sulfate, U.S.P., radiographic, Mallinckrodt‡

C. Buffer

Monobasic sodium phosphate (NaH_2PO_4)
Dibasic sodium phosphate (Na_2HPO_4)

D. 2-octanol (secondary n-octyl alcohol, secondary caprylic alcohol)

E. Phenol, U.S.P., liquefied

F. Potassium iodide, U.S.P., granular

G. Formaldehyde solution, 40%, U.S.P. (formalin):

In preparing the various dilutions to be used, this stock concentration is taken as 100% formalin. Thus a 20% formalin solution is prepared by mixing two volumes of this stock solution with eight volumes of water.

II. *Equipment*

A. Blender, Waring or similar type, 1 quart size, 2-speed.

B. Pressure injection chambers. Wide-mouth polyethylene bottles, 500 ml. size, with thick screw caps, are very satisfactory. They are air tight, and are easily cleaned even if the residual mass has solidified. With a cork borer, small holes can be cut through the side near the top and bottom. Tygon tubing forced through these holes provides an air-tight inlet at the top for air pressure, and an outlet at the bottom for the gelatin (Fig. 1).

C. Pressure source and gauge. A pressure bulb with an air release valve, and a small aneroid pressure gauge, such as used in clinical sphygmomanometry, are very compact and convenient. When connected by a Y-tube with a long lead to the injection chamber, they can be kept well out of the field of dissection (Fig. 1).

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* Kind and Knox Gelatin Co., North 5th St., Camden, New Jersey.

** E. I. du Pont deNemours & Co., Inc., Pigments Department, Wilmington, Delaware 19898. For experimental purposes the aqueous dispersed pigments are available in one-gallon containers. The order should read: "Material to be used for investigative purposes for post mortem studies."

† Godfrey L. Cabot, Inc., 77 Franklin Street, Boston, Massachusetts.

‡ Available only through distributors of radiologic supplies.

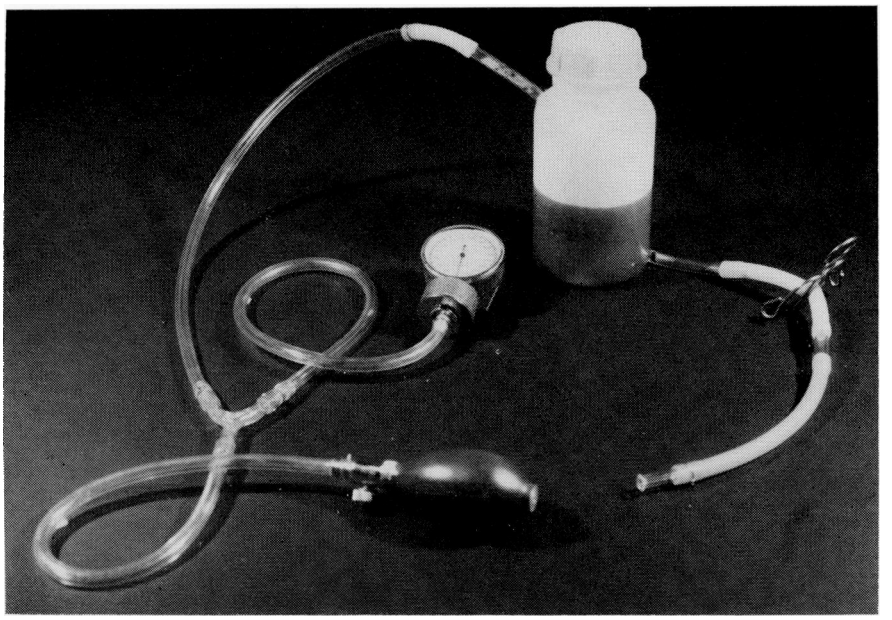

FIG. 1. Simple pressure injection system for gelatin. The wide-mouth polyethylene bottle with thick screw cap is air tight at 200 mm Hg pressure. The air inlet at the top and the gelatin outlet at the bottom are made by forcing short segments of Tygon tubing through small holes cut through the wall of the bottle with a cork borer. The bottle is easily cleaned even after the gelatin has solidified within it. Generous lengths of Tygon tubing between the bottle and the aneroid manometer and pressure bulb remove the latter from the injection field.

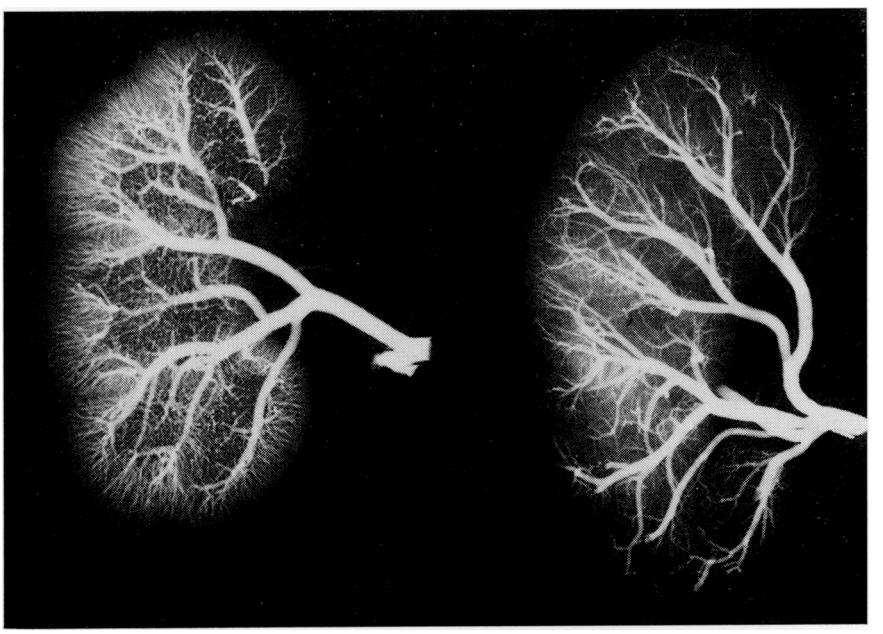

FIG. 2. X-rays of two hemisectioned kidneys which were injected arterially with different Barosperse gelatin masses, the one on the left with the Monastral Blue-Barosperse mass, and the one on the right with the Monastral Red-Barosperse mass. As demonstrated, the extent of vascular penetration can be regulated by choice of the proper mass; on the left, most of the capillaries have been filled, whereas on the right the injection has stopped at the level of small arteries and arterioles. These Barosperse masses have high radiographic contrast and homogeneity.

D. Cannulas, of varying sizes appropriate for the particular vessels being injected. Polyethylene tubing can be drawn out and "lipped" over a tiny flame to make small cannulas. The ends of rimmed test tubes are the most convenient source for larger cannulas.

E. pH meter. Necessary only if it is desired to modify the formulae which have been given.

F. Viscometer. Necessary only if it is desired to study the effects of further modifications on the basic behavior of the gelatin masses. We found timed gravity delivery from the cylinder of a 50 ml. syringe encased in a water jacket to be sufficiently accurate and reproducible for our studies. Calibration in millipoise was accomplished using standard viscosity oils from the National Bureau of Standards.

III. *Preparation of Injection Masses*

A. Specific formulae:

Table 1 presents the formulae for nine basic masses, any three, or at the most four of which should be sufficient for almost any contemplated use. The selection of the most suitable masses is explained in Section V and Table 2.

B. General mixing procedure

1. Masses not containing Barosperse
   a. Into the blender, pour the water, potassium iodide, buffer, and octanol-phenol mixture (40% octanol and 60% phenol by volume). Blend one minute at high speed.
   b. Add gelatin. Blend one minute at high speed.
   c. Add pigment. Blend one minute at high speed.
   d. Deaerate by allowing to stand overnight at room temperature in a covered beaker.
2. Masses containing Barosperse. These require careful dispersion of the Barosperse in water before combining with the other components. When prepared in this manner they provide excellent grain-free radiopaque injection media (Fig. 2).
   a. Add Barosperse very slowly to 200 ml. water in Waring blender running at slow speed. The addition should extend over at least a five minute period. Set aside in covered beaker overnight.
   b. Using the remainder of the water called for in the formula, mix other ingredients as described above for masses not containing Barosperse.
   c. On following day, mix the two (a & b above), stirring manually until final mass appears completely homogeneous. Further deaeration is not usually necessary.

C. Determination of optimal concentration of formalin for desired solidification time

1. After the mass has been deaerated, place 27 ml. in each of three 50 ml. beakers.
2. While stirring actively, add 3 ml. of appropriately diluted formalin to each beaker. For the first beaker use the formalin concentration given in Table 1, for the second beaker use formalin 5% more concentrated (i.e., 20% instead of 15%), and for the third beaker use formalin 5% more dilute (i.e., 10% instead of 15%). Note the time of each addition.

TABLE 1. SPECIFIC FORMULAE

	Monastral Red	*Monastral Blue*	*Monastral Green*	*Chrome Yellow*	*Green-Gold*	*Sterling R Black*	*Barium Sulfate (f)*	*Monastral Red + Barosperse*	*Monastral Blue + Barosperse*
Water, gm. or ml. (*a*)	270	290	450	450	288	450	325	360	370
Potassium iodide, gm.	130	130	120	130	130	130	70	130	130
Monobasic sodium phosphate (NaH_2PO_4), mM (*a*)	30	20	27	30	40	30	20	30	20
Dibasic sodium phosphate (Na_2HPO_4), mM (*a*)	15	25	18	15	5	15	15	15	25
Octanol-phenol mixture, ml. (*b*)	3	3	3	3	3	3	3	3	3
Gelatin, gm.	130	130	80	130	130	130	50	130	130
Pigment, gm.	220(*c*)	200(*d*)	15	40	262(*e*)	70	200	110 Red + 600 Barosp.	100 Blue + 600 Barosp.
Approx. final vol., ml.	550	550	550	550	600	600	400	550	550
Approx. conc. of formalin to be used	15%	10%	30%	15%	15%	15%	40%	15%	10%

(*a*) Buffer is most easily added as IM solutions, 1 ml. representing 1 mM. (Reduce water accordingly.)
(*b*) 40% octanol and 60% phenol, by volume.
(*c*) 220 gm. of aqueous dispersed Monastral Red pigment is equivalent to 40 gm. dry pigment and 180 gm. water.
(*d*) 200 gm. of aqueous dispersed Monastral Blue pigment is equivalent to 40 gm. dry pigment and 160 gm. water.
(*e*) 262 gm. of aqueous dispersed Green-Gold pigment is equivalent to 100 gm. dry pigment and 162 gm. water.
(*f*) For coarse injections, this may be colored by hand-mixing four parts of the mass with one part of any colored mass. There will be no significant change in solidification time or extent of vascular penetration.

TABLE 2. EXTENT OF VASCULAR PENETRATION*

Injection mass	*Systemic arteries*	*Systemic veins*	*Pulmonary arteries*	*Portal, hepatic, pulmonary veins*
Monastral Red	Arterioles & few capillaries	Capillaries	Capillaries	Capillaries sinusoids
Monastral Blue	Capillaries	Capillaries	Capillaries	Capillaries & sinusoids
Monastral Green	Capillaries	Capillaries	Capillaries	Capillaries & sinusoids
Chrome Yellow	Large arterioles	Venules & few capillaries	Large arterioles	Venules, few capillaries & sinusoids
Green-Gold	Arterioles & some capillaries	Venules & some capillaries	Capillaries	Capillaries & some sinusoids
Sterling R Black	Capillaries	Capillaries	Capillaries	Capillaries & sinusoids
Barium Sulfate	Large arterioles	Large venules	Large arterioles	Large venules
Monastral Red + Barosperse	Most arterioles	Venules & few capillaries	Arterioles & few capillaries	Capillaries & some sinusoids
Monastral Blue + Barosperse	Arterioles & few capillaries	Venules & few capillaries	Arterioles & few capillaries	Capillaries & some sinusoids

* Anticipated at 20-25°C. (Penetration may be significantly greater at 30-35°C. and significantly less at 10-15°C.)

3. Follow the progress of solidification by withdrawing the stirring rod (split tongue blade or applicator stick) from the mass every few minutes. Note the time each becomes 1) definitely stringy, and 2) rigid. We consider the mass definitely stringy when it flows slowly from the stirring rod in a continuous string, rather than dropwise. We consider it rigid when the stirring rod is held sufficiently firmly by the gelatin that beaker and all may be lifted by the stirring rod. Record the concentration of formalin that causes the mass to become definitely stringy in 15-30 minutes, and rigid in 45-70 minuutes. Use of this concentration for the actual injection provides sufficiently long working time for good filling of the vascular bed, yet a sufficiently short setting time for dissection without unreasonable delay (30-60 minutes).

D. Storage

Each batch may be stored for weeks or even months in a stoppered bottle in the refrigerator. A warm water bath will liquefy the gelatin and bring it to room temperature in a few minutes just before use. If left uncovered for more than a few hours, sufficient evaporation may occur to alter the viscosity significantly.

IV. *Injection Procedure*

Just prior to injection, and after the vessel has been cannulated and connected by rubber tubing to the pressure chamber, the properly diluted formalin is added to the gelatin in a beaker while stirring briskly, one volume of the diluted formalin being added to nine volumes of the gelatin mass. The mixture is transferred quickly to the chamber, and the pressure within the chamber raised to, and maintained at the desired level until the gelatin has solidified. We use pressures of 140-160 mm Hg for injecting systemic arteries, and 40 mm Hg for injecting veins and pulmonary arteries. The progress of the solidification can be observed in a small amount of the mass left in the beaker. Using a gelatin-formalin mixture that becomes definitely stringy in 15-30 minutes, the specimen can usually be dissected at the end of an hour without fear of significant leakage. If a solid injected organ, such as liver or kidney, is to be sliced on the day of injection, prior chilling for an hour or two in the refrigerator may be desirable in order to eliminate stickiness or smearing of the gelatin. If the organ is sliced eight or more hours after injection, prior chilling is not necessary. Injected lungs are most advantageously cut after they have been fixed in the inflated state by intrabronchial formalin or glutaraldehyde; they may be dissected fresh, however, if desired.

V. *Selection of Proper Gelatin Mass*

Which of the masses is most suitable for a particular study depends on the system being injected and the extent of vascular penetration which is desired. The formulae given in Table 1 have been evolved in order to provide masses in a range of colors that can be relied upon: 1) to penetrate vessels no smaller than 50-100μ, 2) to penetrate most small arterioles and venules but fill only occasional capillaries, 3) to fill most of the capillary or sinusoidal bed. The behavior that can be expected for each mass is given in Table 2. As indicated by the Table, some masses which will not penetrate capillaries from systemic arterioles will penetrate capillaries from pulmonary arterioles; some masses which will not penetrate capillaries from arterioles will penetrate them from venules; and some masses which will not penetrate capillaries from systemic venules will penetrate capillaries from pulmonary venules, and will penetrate hepatic sinusoids from portal and hepatic venules. Also, masses which will not penetrate capillaries from arterioles or venules at room temperature (20-25°C.) may fill the capillary bed at 30-35°C., and masses which will fill a capillary bed at 20-25°C. may not penetrate beyond arterioles or venules in a recently refrigerated, 10-15°C. specimen. The predicted behaviors given in Table 2 are for injections in which the masses and the specimen are near room temperature (20-25°C.).

FACTORS AFFECTING THE BEHAVIOR OF A GELATIN INJECTION MASS

Although the formulae which have been given in Table 1 and the behavior indicated in Table 2 have been determined empirically, our studies have indicated some of the factors that control the behavior and usefulness of gelatin injection masses. These may be of some interest to the sophisticated injector, who will wish to modify the technique and masses for specific usages which we have not investigated.

I. *The extent of vascular penetration* is controlled in part by the viscosity of the mass, in part by the nature and amount of the particular pigment, and to a slight degree by the injection pressure.

Viscosity: The final viscosity of the injection mass is determined by 1) the type and concentration of the gelatin, 2) the type and concentration of the pigment, 3) the concentration of potassium iodide, and 4) the temperature. Gelatins with high bloom numbers, or in high concentration, have high visocity. Increased amounts of pigment also increase the viscosity, but different pigments in the same concentration may yield solutions of rather different viscosity. The latter is probably related primarily to the size of the pigment particles, and to their tendency to flocculate or to remain dispersed in the mass; but these do not appear to be the only factors, since different, evenly dispersed pigments of essentially the same particle size may have measurably different effects on viscosity. The concentration of potassium iodide and the temperature exert reciprocal effects on viscosity. It is the ability of potassium iodide to lower the temperature of reversible gelation that makes possible gelatin injection masses such as these, which can be used at room or even refrigerator temperatures. Following Schlesinger's example, we use sufficient potassium iodide to lower the gelling temperature to 5-8°C. This results in a mass which is quite viscid in the 10-15°C. range, however, and if recently refrigerated specimens are being routinely injected, it may prove necessary to increase the amount of potassium iodide, or to change to a thinner mass, in order to obtain sufficient vascular penetration.

Originally it was our hope to regulate the extent of vascular penetration by simply changing the viscosity of the mass by increasing or decreasing the concentration of gelatin and/or pigment. This was not successful, yielding injections which were highly variable from specimen to specimen, and even from region to region in the same specimen. Vascular filling was often too extensive in tissues near the site of vascular cannulation, while distally it was spotty and restricted. We accordingly regulate the extent of vascular penetration by other means. Although still a factor to be considered, especially when injecting very cold or very warm specimens, viscosity really controls the extent of injection within quite narrow limits.

Pigment: The concentration and especially the type of pigment in the mass are the most important factors regulating the extent of vascular penetration. This is an independent effect, quite apart from the effects of pigment on viscosity. The behavior of the different pigments in this regard has had to be determined empirically and is not solely a factor of particle size or presence or absence of flocculation. Actually, all of the pigments described previously, except radiographic barium sulfate, have particle sizes well below 1μ, and with the exception of Chrome Yellow, which flocculates slightly,

all remain uniformly dispersed in the gelatin mass. Yet two very similar, well-dispersed pigments in the same concentration may affect vascular penetration quite differently. With some pigments, such as Monastral Red and barium sulfate the extent of vascular penetration can be reduced by increasing the concentration of the pigment, but with others such as Monastral Blue or Green, this is not the case within any practical limits. Since the extent of penetration of barium-containing masses seems to depend primarily on the concentration of this material, we have found the supplemental addition of barium sulfate or Barosperse to be the most convenient and reliable way of reducing the extent of vascular penetration of the colored masses when this is necessary. Fortunately, the presence of barium does not increase the tendency to flocculation of any of the pigments, although this is not true of some other pigment mixtures. For preparations which are to be studied only grossly or macroscopically, particle size and flocculation of the pigment are of little importance, but if it is desired to label small vessels for identification in 5μ, routinely stained histological sections it is essential that the pigment be of very small particle size and completely and uniformly dispersed. When microscopic flocculation does occur, some vessels in the section will contain only gelatin, and it will be impossible to tell if they were really injected, or by what route.

Pressure of injection: Within limits, the injection pressure is only a minor factor in determining the extent of vascular filling, although it is important in the time needed for penetration. With a formalin-gelatin mixture that does not become visibly thickened before 20-30 minutes, we use pressures of 140-160 mm Hg for injecting systemic arteries, and 40 mm Hg for injecting veins and pulmonary arteries. This allows a reasonably complete injection of sizable vascular beds, without excessive expenditure of time. The pressures may slightly overdistend some of the larger, more proximal vessels but this does not seem to be the case at the arteriole, venule, or capillary level. Pressures higher than physiologic levels are necessary in order to overcome the surface tension of air bubbles in the small vessels.

II. *Solidification of mass and quality of the resultant gel.* The behavior of an iodinated pigment-gelatin solution after adding formalin depends on a number of factors: the pH of the solution, the amount of formalin added, the temperature, the concentration of potassium iodide, the concentration of the gelatin, and the character of the gelatin.

pH: Exposure to formalin will produce irreversible solidification of almost any gelatin solution, and the speed with which this occurs is closely related to the initial pH of the solution. As noted by Schlesinger, the pH range over which satisfactory solidification will occur, but be sufficiently delayed to allow vascular injection, is surprisingly narrow. Differences of

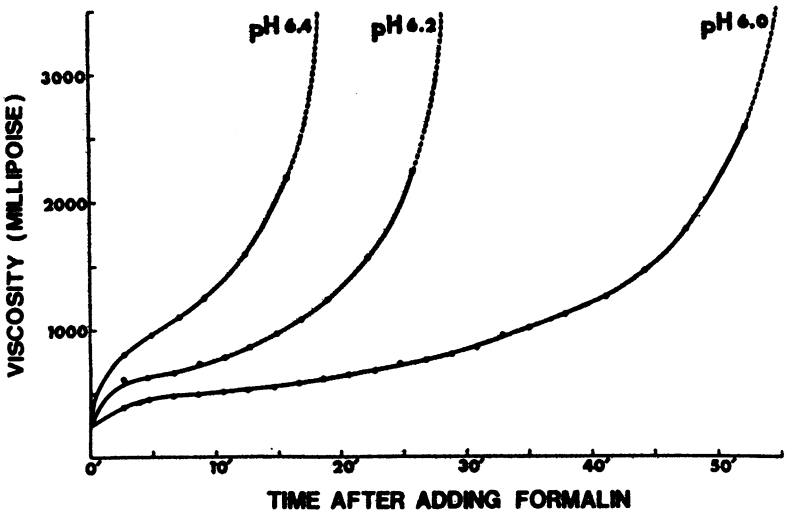

FIG. 3. Effect of pH on gelatin solidification time. Three equal volumes of a Monastral Green mass were titrated with acid to slightly different pH's, and repeated viscosities were determined as each solidified after the addition of the same amount of formalin. Very slight differences in pH effect the rate of gelation significantly.

only a few tenths of a pH unit may change the solidification time from almost an hour to just a few minutes (Fig. 3). Only within certain limits can this be compensated by use of less concentrated or more concentrated formalin. Below a pH of 6.0, for instance, 70-90% formalin may be required to solidify a mass in 30 minutes, and then the resulting gel may be too friable for satisfactory cutting. Above pH 6.4, the formalin may have to be diluted to concentrations of less than 1% in order to increase the time of solidification to as long as 15-20 minutes; such extreme dilutions of formalin are unreliable and the solidification times are correspondingly variable. As suggested by Schlesinger, a pH of approximately 6.2 seems most satisfactory. The addition of monobasic and dibasic phosphate is the most convenient way of obtaining this desired pH, compared with titrating the gelatin solution with acid or base, using a pH meter. Since the natural pH and the natural buffering action varies greatly between different gelatins and different pigments, the needed ratio of monobasic to dibasic phosphate has had to be determined empirically for each formula.

Amount of formalin: The time required for solidification varies inversely with the amount of formalin added (Fig. 4). In the range of solidification times which are convenient for injection purposes (30-45 minutes), increasing the amount of formalin by 50% will reduce the solidification time by approximately half; doubling the amount of formalin will reduce the

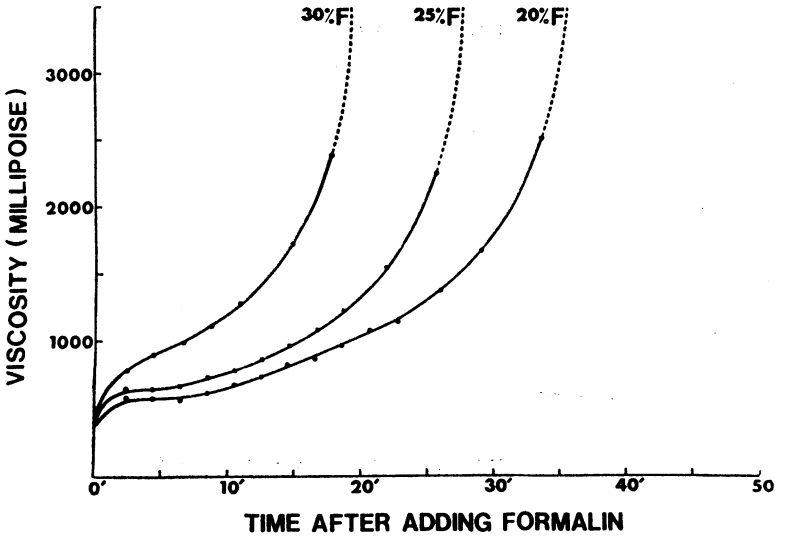

FIG. 4. Effect of amount of added formalin on gelatin solidification time. Formalin solutions of different concentrations were added to samples from the same Monastral Green mass, and repeated viscosities were determined as each solidified. Decreasing the amount of added formalin by 50% approximately doubles the solidification time.

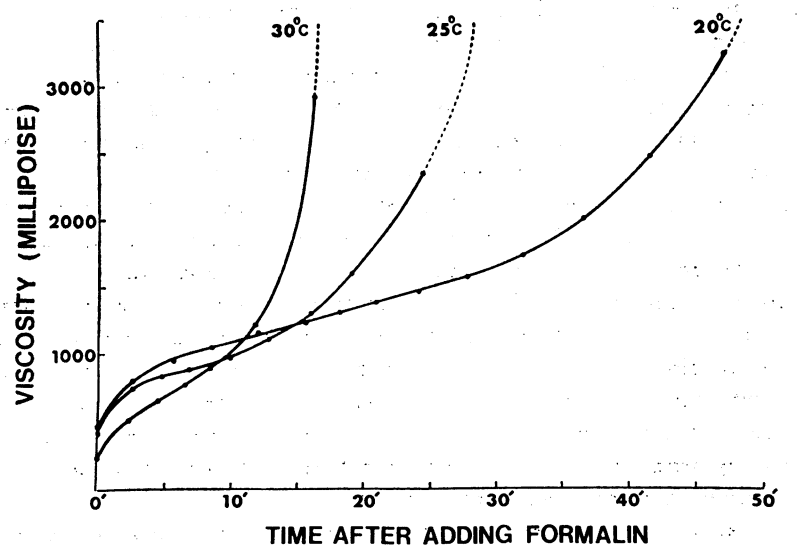

FIG. 5. Effect of temperature on gelatin solidification time. The same amount of formalin was added to each of three equal volumes of a Monastral Green mass held at different but constant temperatures. Repeated viscosities were determined as each solidified. A 10°C. difference in temperature has a significant effect, the solidification time at 30°C. being less than one third that at 20°C.

solidification time to approximately one fourth of what it was before. Because the optimal concentration of formalin is so critical, it is worth the effort to determine this concentration for each separate batch of the mass. Adding 20% instead of 30% formalin to one batch, for instance, may needlessly double the time required for injection; and using 30% instead of 15% formalin with another batch may cause solidification before adequate vascular penetration has occurred.

Temperature: The time for solidification after adding formalin is significantly increased by high temperature (Fig. 5). Using a constant concentration of formalin to produce gelation, a mass which solidifies in 25 minutes at 25°C. may gel in only 15 minutes at 30°C., but not for 50 minutes or more at 20°C. In actual practice, if the optimal formalin concentration has been determined at room temperature and the gelatin is near this temperature at the time of injection, the working time will still be within practical limits, even if the specimen is reasonably cool (15°C.) or fairly warm (30°C.). If the specimen is cooler than 15°C. it will be time-saving to increase the concentration of formalin, and if the specimen temperature is much above 30°C., the concentration of formalin may have to be reduced to allow adequate time for vascular penetration.

Concentration of potassium iodide: Increasing the concentration of potassium iodide slows the speed of solidification slightly (Fig. 6). An increase of threefold in the potassium iodide concentration will approximately double the solidification time unless the concentration of the added formalin is also increased.

Concentration of gelatin: Solutions with higher concentrations of gelatin solidify significantly more rapidly than do more dilute solutions of the same gelatin, and hence require less formalin to obtain solidification times in the same range (Fig. 7). A mass with 20% gelatin may solidify in less than 10 minutes, whereas a mass with 12% gelatin may require five times as long to become solid, using the same strength formalin.

Character of the gelatin: Since any gelatin solution of sufficient concentration can be made to gel by the addition of sufficient formalin, it would seem that any commercially available gelatin could be used in an injection mass. Actually, finding a completely suitable gelatin has been one of the major problems faced in this study. Gelatins prepared for different commercial uses vary greatly from each other in physico-chemical characteristics such as natural pH, isoelectric point, gel strength, viscosity and ash. Any one or several of these characteristics may be of little importance for a particular commercial use, and hence may be allowed to vary appreciably in the gelatins sold for that purpose, even under the same label. For this reason, the readily available commercial gelatins, such as those sold for bacterio-

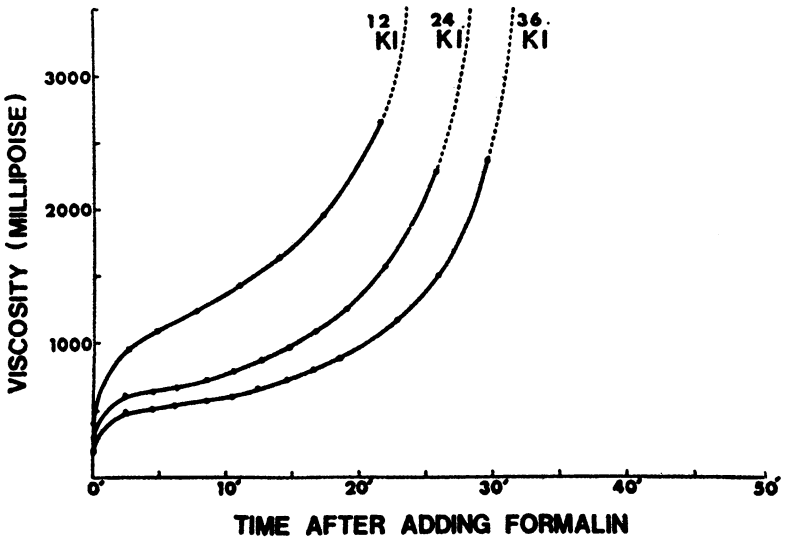

FIG. 6. Effect of potassium iodide concentration on gelatin solidification time. Three Monastral Green masses were prepared identically except for different concentrations of KI. Repeated viscosities were determined as they solidified after the addition of the same amount of formalin to each one. The effect of KI on the speed of irreversible gelatin after formalin is relatively minor; doubling the concentration of KI prolongs the solidification time only by about one third.

logic or kitchen use, have been found to be either totally unsuitable for Schlesinger's injection mass, or too variable from package to package to allow reproducible results using standard formulae. Because of this problem, the assistance of the research department of a large gelatin manufacturer was solicited. For testing in our laboratory, a number of gelatins were made available which were of suitable viscosity and gel strength, and which the manufacturer could closely reproduce should the present supply be exhausted. The type selected on the basis of our testing has been set aside and made available for injection purposes only. Should another batch of this type ever be needed, it will be submitted to the authors for prior testing so that any necessary modifications in the formulae can be noted on the label.

COMMENT

For many purposes we have found that this modified Schlesinger's mass has significant advantages over the other injection techniques. Since the vat time is brief but controllable, it can be used as conveniently for injections of tissues *in situ* as for injection of organs which have been removed from the body. Even multicolored, *in situ* injection of several different vascular beds need delay the dissection no more than an hour. By selection of the proper

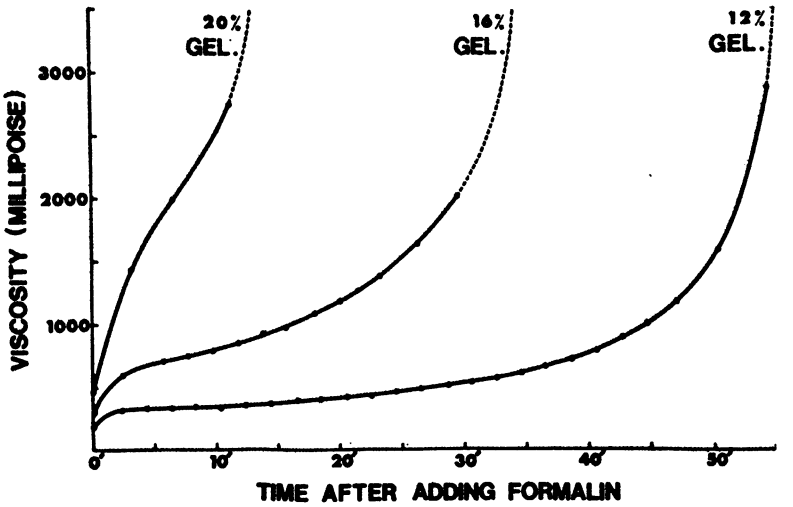

FIG. 7. Effect of gelatin concentration on solidification time. Three Monastral Green masses were prepared identically except for the concentration of gelatin in each. Repeated viscosities were determined after adding the same amount of formalin to each one. In this range, decreasing the gelatin concentration by only 25% (i.e., 16% to 12% concentration) almost doubles the time before solidification, and increasing the gelatin concentration by 25% (i.e., 16% to 20% concentration) shortens the working time to less than one third the former interval.

mass, the extent of the vascular penetration can be regulated fairly closely—from large arterioles and venules, to small arterioles and venules, to nearly complete capillary filling. The technique is adaptable for gross and/or roentgenographic studies (Fig. 2), for macroscopic studies of small vessels in cleared blocks or 300μ sections, and for microscopic studies of labelled vessels in routinely prepared and stained microscopic sections. Derived from pigments of small particle size, the colors do not diffuse from the vessels or stain surrounding tissues, as do soluble dyes. The pigments are all sufficiently inert that the tissues can be bleached with concentrated hydrogen peroxide before clearing, without loss of color from the injection masses. The pigments are not soluble in water or in any of the organic solvents used in dehydrating and clearing tissues. The particles are sufficiently small and evenly dispersed that the colors remain strong and brilliant even when viewed in routinely stained histologic sections cut at 5μ or less. All of the ingredients are readily obtainable at modest cost, and the injection masses are quickly and easily prepared in the laboratory without requiring long experience or complicated and expensive equipment.

We have used this modified Schlesinger's mass for *in situ* post mortem injection to aid in dissection, to demonstrate varices and other collateral

channels which collapse after death, and to localize small sources and sites of gastrointestinal bleeding. Macroscopic study of cleared blocks or large Gough-type sections of multicolored injection specimens is the most satisfactory method we have found to establish or exclude the presence of very small vascular shunts, and to evaluate the source and relative size of small vessels and their capillary beds, as in primary and metastatic tumors of the lungs or liver, or such complex structures as the plexiform or angiomatoid lesions associated with pulmonary hypertension. Lesions located by macroscopic examination can be photographed and then prepared as routine serial histologic sections, in which the vessels remain labelled by their contained pigment.

We have been impressed by the number of workers who seek assistance from our laboratory for vascular studies with the vinylite injection corrosion technique who actually have problems that can be much more satisfactorily resolved with gelatin. Although for many uses the vinylite corrosion technique is superb, it has distinct limitations for study of vessels smaller than 50-100μ diameter, and for vessels that need the presence of the surrounding tissue to clarify their anatomical relationships. Latex will penetrate very tiny vessels but also has many disadvantages; the extent of vascular penetration cannot be controlled, and *in situ* injections are not practical since solidification of the latex requires immersion of the entire specimen in formalin or some other acid solution. Although gelatin is one of the oldest injection media, it still deserves a respected position among the tools available to the experimental anatomist and pathologist.

SUMMARY

A modification of Schlesinger's buffered iodinated gelatin injection mass is described, with presentation of specific formulae and techniques for obtaining multicolored vascular injections of controllable extent. Some of the factors important to the behavior of the mass are discussed, and some of its advantages and uses are enumerated.

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