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Glutaraldehyde Cross-Linked Collagen (GAX): A New Material for Therapeutic Embolization

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In a search for a better agent for use in therapeutic embolization, a newly available bovine collagen product, glutaraldehyde cross-linked collagen (GAX), was evaluated to determine its effectiveness in causing arterial obstruction, its persistence after embolization, and the acute and chronic pulmonary toxicity resulting from direct pulmonary embolization. GAX is an effective agent for causing arterial obstruction: 3–4 ml caused prompt flow arrest when injected into the internal iliac artery of six dogs. In this canine model, the material persisted within embolized tissue for as long as 2 months, and at follow-up intervals of 2 days, 2 weeks, and 2 months, its presence did not produce any cellular response. Studies of both acute and chronic pulmonary toxicity reveal that when GAX is embolized directly into the pulmonary circulation it causes adverse effects only by mechanical blockage of pulmonary arteries. GAX offers several advantages over other currently available agents and is of sufficient safety that clinical trials in humans can be undertaken.

Since therapeutic embolization has evolved from a rarely used procedure of unproved benefit into one of demonstrated value and a routine part of the management of numerous diseases, the inadequacies of available embolic materials have become apparent. In an effort to identify materials that offer advantages over existing agents, we have evaluated, through animal studies, a variety of potential compounds. One of these, glutaraldehyde cross-linked collagen (GAX), was found to be especially promising and was studied further in experiments designed to determine its effectiveness, persistence, host-tissue compatibility, and pulmonary toxicity. This experience forms the subject of this report.

Materials and Methods

GAX was provided in a concentration of 15 mg GAX per ml in sterile 1 ml syringes by the Collagen Corp. (2500 Faber Place, Palo Alto, CA). All studies were carried out in adult mongrel dogs that weighed 40–70 pounds. Only male animals were used for the studies of effectiveness and persistence. Anesthesia was induced with Nembutal and, after endotracheal intubation, was maintained with 1.0–1.5% Halothane and 100% O_2. For the experiments directed at understanding the mechanism of the acute pulmonary toxicity of GAX, all animals were paralyzed and ventilated at a respiratory rate of 14–16 per min with a tidal volume of 400–500 ml.

Effectiveness, Persistence, and Host-Tissue Tolerance

To assess the effectiveness of GAX as an embolic agent, its persistence, and the host-tissue tolerance to the presence of the material, the following experiments were done. Six male mongrel dogs were divided into three equal groups according to whether they would be reexamined at 1 week, 2 weeks, or 2 months after embolization. One of the internal iliac arteries of each dog was then embolized with GAX in the following manner. A 5-French catheter was inserted percutaneously into one of the femoral arteries and positioned just

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above the aortic bifurcation. After a control angiogram, one of the internal iliac arteries was selectively catheterized, and then, using fluoroscopic control, 3–4 ml of a mixture of GAX and undiluted ionic contrast medium was injected slowly into this vascular bed. After embolization, the catheter was repositioned just above the aortic bifurcation and a repeat angiogram was done without delay. Upon recovery from anesthesia, the animals were returned to the animal care facility where they were housed during the interval between embolization and the follow-up examination.

At the appropriate interval, follow-up examinations were carried out as follows. The abdominal aorta was catheterized and an angiogram was performed using the same technique as that used for the control and immediate postembolization studies. The animals were euthanized and tissue was taken from the prostate and bladder base ipsilateral to the embolized internal iliac artery. Tissue from the ipsilateral testicle served as a control. After fixation in 4% formaldehyde for 2 weeks, sections were cut and stained.

Acute and Chronic Pulmonary Toxicity

The pulmonary toxicity of GAX was assessed in two sets of experiments. To establish an estimate of the lethal dose of GAX in acute embolization of the pulmonary circulation, and to define the mechanism of action of this toxicity, the following studies were performed in four adult mongrel dogs. Catheters were introduced percutaneously and employed as follows: (1) a triple lumen Edwards Swan Gans thermodilution catheter was introduced into the right femoral vein and was then positioned in either the left or right main pulmonary artery. Measurements of pulmonary artery pressure could be obtained from this catheter and, in conjunction with an Edwards thermodilution cardiac output computer (model 9520A), the catheter was also used for calculation of cardiac output. (2) A 5-French single-end-hole catheter was introduced into the right femoral artery and was positioned within the abdominal aorta where it was used to record systemic arterial pressure and to obtain samples for arterial blood gas measurements. (3) A 5-French end-hole catheter was inserted into the left femoral artery and was advanced into the left ventricle; it was used to obtain left ventricular pressures. (4) A 3-French end-hole catheter was introduced into the left femoral vein and was advanced to the inferior vena cava just above the level of the diaphragm; the GAX was embolized into the pulmonary circulation through this catheter. (5) In one animal, a 4-French end-hole catheter was introduced into an upper extremity vein and advanced into the right ventricle where it was used to obtain right ventricular pressure levels.

After establishing baseline values of cardiac output; pulmonary artery, left ventricular, and systemic arterial pressures; and arterial pO_2, pCO_2, and pH, a 30 mg bolus of GAX was injected into the inferior vena cava. At 5-min intervals arterial blood gas determinations and hemodynamic measurements were again made and then another 30 mg bolus of GAX was injected into the inferior vena cava. The experiment was continued in this manner until the animal died. Tissue was obtained from the upper, middle, and lower portions of both lungs. After fixation in 4% formaldehyde for 2 to 3 weeks, sections were cut and stained for histologic examination.

Another series of experiments designed to evaluate the chronic effects of GAX embolized directly into the pulmonary circulation was performed as follows. Twelve adult mongrel dogs were divided into three groups according to whether they would be reexamined 1 week, 1 month, or 2 months after sublethal pulmonary embolization with GAX. Percutaneously, a catheter was inserted into one of the femoral veins and was then positioned in the main pulmonary artery. When baseline measurements of respiratory rate and pulmonary artery pressure had been established, GAX was infused through this catheter at a rate of 0.01 ml/min/lb. The total dose administered was one-half the average lethal dose as established by the acute pulmonary toxicity experiments. During, and for 60 min after, the start of embolization, pulmonary artery pressure and respiratory rate were monitored and recorded. At the time of reexamination a catheter was again placed into the main pulmonary artery, and pulmonary artery pressure and respiratory rate were recorded. After euthanasia, tissue samples were taken from the upper, middle, and lower portions of both lungs and were placed into 4% formalin for fixation. After 2 weeks of fixation these were cut and sectioned for microscopic examination.

Results

Effectiveness, Persistence, and Host-Tissue Tolerance

In all animals studied, GAX was shown to be an effective agent for producing arterial occlusions. Embolization with 3–4 ml of a mixture of GAX and iodinated contrast medium uniformly resulted in complete arrest of flow within the internal iliac arterial bed.

The control, immediate postembolization, and follow-up angiograms of each animal were evaluated qualitatively for evidence of arterial obstruction, change in the pattern of vascular supply, symmetry of the circulation time, and alterations of vascular caliber. All of the immediate postembolization angiograms showed striking evidence of arterial occlusion, there was almost no flow in the internal iliac artery or its branches in five of the six dogs (Fig. 1). Evidence of persistent arterial obstruction was also present in all of the delayed follow-up examinations; however, the extent of the arterial blockage was always less than that present on the immediate postembolization studies (Figs. 2 and 3). There was no clear evidence of neovascularity in any of the follow-up studies. On all of the delayed postembolization angiograms, the internal iliac artery and its major branches were patent but were of smaller caliber than on the control studies.

GAX was found within arteries in all of the tissue samples taken from the territory of the embolized internal iliac artery (i.e., prostate and bladder), and this was true at all follow-up intervals. In the sections taken 2 days after embolization, the GAX appeared as a compact amorphous mass filling, to a variable degree, the lumen of small arteries and arterioles. The endothelial cells adjacent to GAX appeared swollen. In the sections taken at both 2 weeks and 2 months after embolization, the GAX also appeared as a featureless intraluminal collection that seemed to be adherent to the endothelium of small arteries and arterioles. Unlike their appearance in the immediate postembolization sections, however, the endothelial cells from these tissues appeared normal. No cellular response was present at any of the follow-up intervals either within the walls of these vessels or in the adjacent extravascular tissue (Fig. 4). Frequently, cellular debris was present, trapped within the interstices of the GAX. In sections taken at follow-up intervals of both 2 weeks and 2 months, small C- and U-shaped clefts were frequently seen along the luminal surface of some arteries that otherwise were filled with GAX. These channels were lined by normal-looking endo-

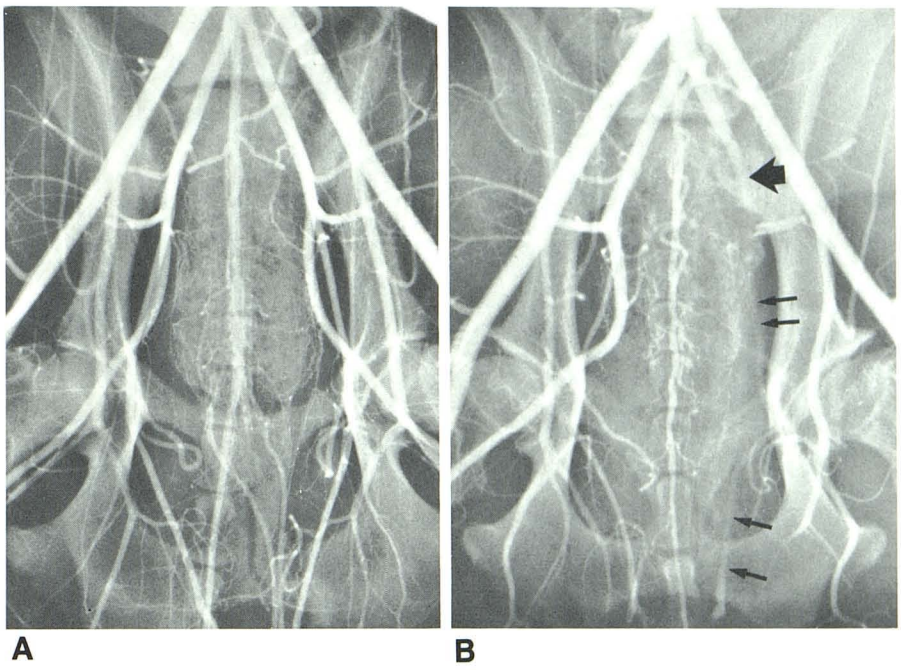

Fig. 1.—Control (*A*) and immediate postembolization (*B*) pelvic angiograms.

***A*, Control pelvic arteriogram done just before embolization of 3 ml mixture of GAX and iodinated contrast medium into left internal iliac artery.**

***B*, Pelvic arteriogram of same animal done immediately after embolization. Internal iliac artery is obstructed just distal to its origin (*large arrow*). Contrast stasis is present within several branches of this artery (*small arrows*).**

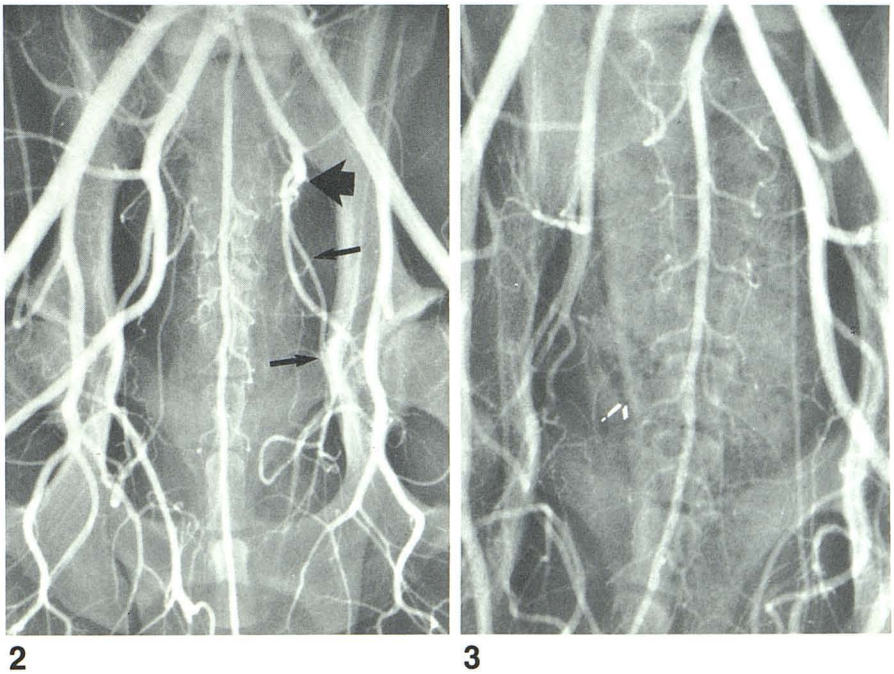

Fig. 2.—Pelvic arteriogram of same animal as in Fig. 1 done 2 weeks after embolization of left internal iliac artery with 3 ml mixture of GAX and iodinated contrast medium. Caudal gluteal artery remains occluded at its origin (*large arrow*). Internal iliac and internal pudendal arteries are much smaller than on preembolization study (*small arrows*). Flow is slow within distal distribution of internal pudendal artery.

Fig. 3.—Pelvic angiogram done 2 months after embolization of right internal iliac artery with 3 ml mixture of GAX and iodinated contrast medium. There is persistent reduction in size of embolized internal iliac artery. Flow through distal territory of this artery is slow.

thelial cells, and in some instances they contained viable-appearing red blood cells (Figs. 5 and 6). GAX was not found within veins in any of the histologic material.

Acute and Chronic Pulmonary Toxicity

Acute Pulmonary Toxicity. During pulmonary embolization with GAX, all animals experienced a rapid and dramatic increase in both their pulmonary artery pressure and pulmonary vascular resistance. Concomitant changes in systemic blood pressure, cardiac output, and systemic vascular resistance were, until the terminal stages of the experiments, minimal and compensatory in nature. The values for mean systemic arterial pressure (Ao), mean pulmonary artery pressure (PA), cardiac output (CO), systemic vascular resistance (SVR), and pulmonary vascular resistance (PVR) as measured at baseline, one-third, two-thirds, and the full dose of GAX required to cause death are listed in Table 1. Table 2 provides

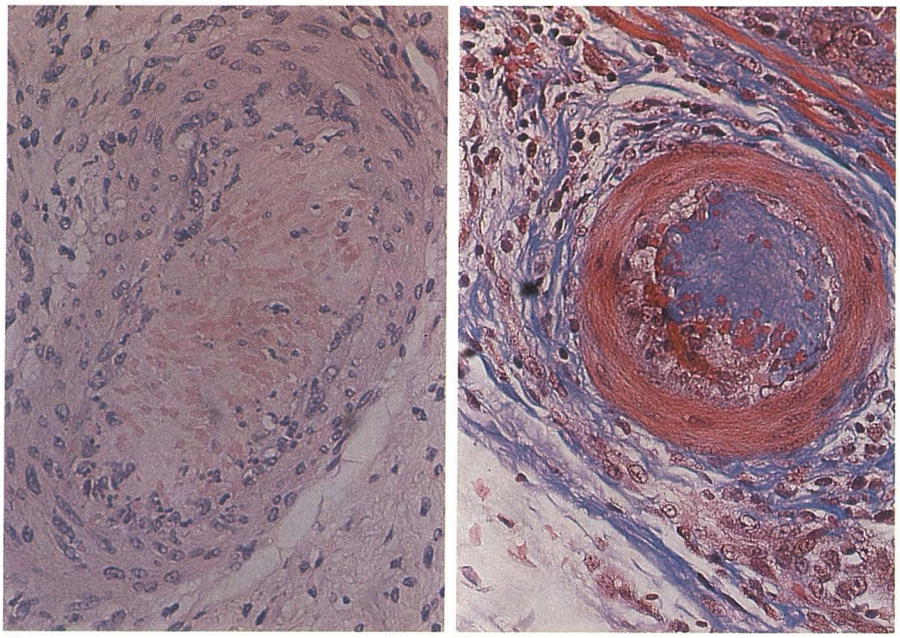

Fig. 4.—*A*, Section from prostate showing small artery filled with GAX 2 days after embolization. Embolic material is adherent to endothelium, which is swollen. No cellular reaction is present either in or around this artery. Red and white blood cell debris is trapped within GAX. (H and E)

***B*, Section from bladder base. Endothelium of this small artery filled with GAX is swollen. No cellular reaction is present. (Trichrome)**

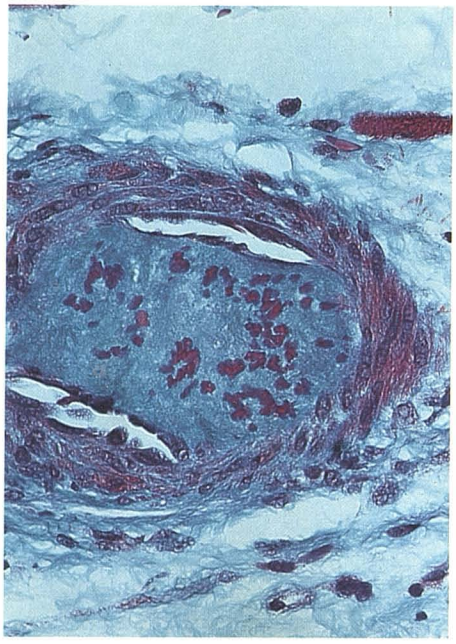

Fig. 5.—Section from bladder base 2 weeks after embolization. Small artery is filled with GAX. Two small clefts are seen along intimal margin of artery. GAX appears tightly adherent to endothelium. There is no evidence of inflammatory response. (Trichrome)

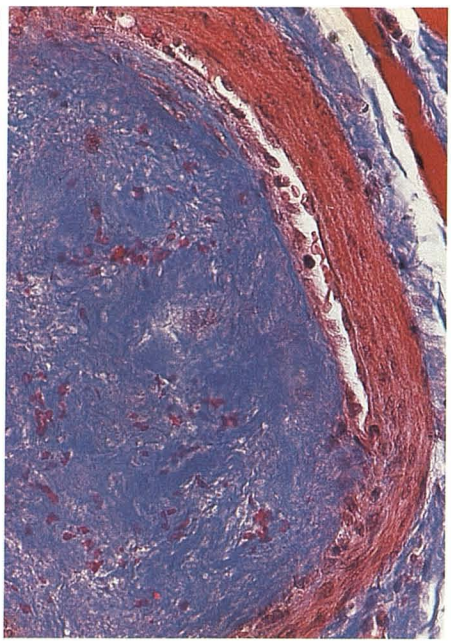

Fig. 6.—Section from prostate 2 months after embolization. GAX remains as compact mass attached to endothelium of this artery. A small new lumen has formed along intimal surface and viable-appearing red blood cells can be seen within it. There is no evidence of inflammatory response or of cellular ingrowth into collagen embolus. (Trichrome)

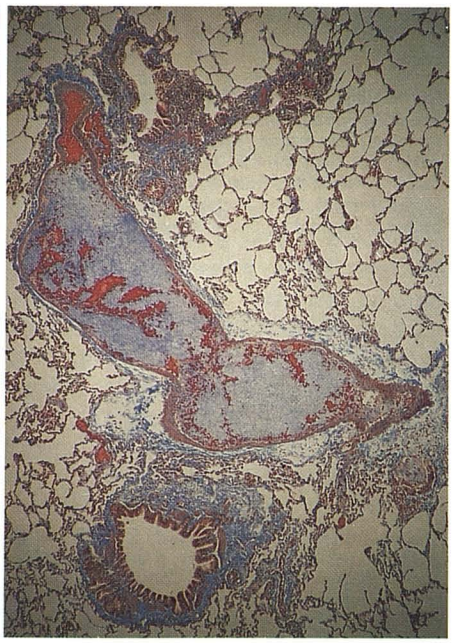

Fig. 7.—Section from lung of animal undergoing lethal pulmonary embolization with GAX. This large pulmonary artery is blocked with GAX, which contains red blood cell debris. Adjacent airways are normal.

the average percent change from baseline of these same hemodynamic values (n = 4). No significant alteration of arterial blood gas values occurred until the animals were agonal.

Microscopically, the lungs from all of these animals were similar; widespread blockage of the lumen of both medium-sized and small pulmonary arteries with GAX was present throughout all portions of both lungs (Fig. 7). The appearance of GAX within the lumen of these pulmonary arteries was identical to that seen in tissue from the animals described

TABLE 1: Hemodynamic Values at Baseline, One-Third, Two-Thirds, and Full Lethal Dose of GAX Embolized Directly into the Pulmonary Circulation

	Mg. GAX	Ao	PA	CO	SVR	PVR
Animal 1	0	130	12	3.7	3473	160
	90	95	7	3.4	2730	143
	150	85	7	1.8	4716	388
	240	35	12	1.0	3290	1410
Animal 2	0	121	14	5.5	1701	73
	120	115	23	6.4	1371	187
	270	116	39	4.8	1850	521
	390	83	50	1.5	3942	1892
Animal 3	0	146	9	4.6	2449	34
	60	134	11	4.6	2325	87
	120	138	26	4.1	2644	405
	180	134	38	3.0	3503	902
Animal 4	0	96	8	7.6	997	63
	90	95	15	6.6	1145	181
	210	93	24	7.2	1039	235
	330	34	38	4.0	1184	743

Note.—Ao = systemic arterial pressure, mm Hg; PA = pulmonary arterial pressure, mm Hg; CO = cardiac output L/min; SVR = systemic vascular resistance, dynes cm^{-5} sec; PVR = pulmonary vascular resistance, dynes cm^{-5} sec.

TABLE 2: Average Percent Change from Baseline of the Hemodynamic Values Shown in Table 1 ($n = 4$)

% Lethal Dose	Ao	PA	CO	SVR	PVR
1/3	−11	+43	−8	−8	+131
2/3	−12	+142	−20	+13	+511
3/3	−37	+249	−56	+47	+1726

Note.—Ao = systemic arterial pressure, mm Hg; PA = pulmonary arterial pressure, mm Hg; CO = cardiac output L/min; SVR = systemic vascular resistance, dynes cm^{-5} sec; PVR = pulmonary vascular resistance, dynes cm^{-5} sec.

TABLE 3: Dose of GAX That Was Fatal with Direct Pulmonary Embolization

Animal	Weight	Total Dose	Dose per kg
1	20.0 kg	240 mg	12.0 mg
2	25.0 kg	360 mg	14.4 mg
3	16.0 kg	210 mg	13.1 mg
4	28.5 kg	330 mg	11.6 mg

previously. In some of the sections, taken adjacent to a plural surface, small areas of acute hemorrhages were present. These tended to dissect along bronchovascular planes. The alveoli and air spaces in all specimens looked normal. The doses of GAX that caused death when embolized directly into the pulmonary circulation are given in Table 3.

Chronic Pulmonary Toxicity. During sublethal pulmonary embolization with GAX, all of the 12 animals studied experienced a rapid elevation of their main pulmonary artery pressure, and all but two of them had an associated dramatic increase in their respiratory rate. The time required for embolization varied between 6 and 7 min. Maximum elevations in respiratory rate and pulmonary artery pressure occurred between 10 and 15 min after the start of the embolization. By 60 min after the start of embolization, pulmonary artery pressure had returned to baseline levels in five animals. In the other four who survived the experiments, pulmonary artery pressure remained elevated to values ranging between 25% and 75% of baseline levels. Three animals did not survive this "sublethal" embolization on account of acute pulmonary distress. At the time of follow-up examination all measurements had returned to baseline. None of the animals exhibited any signs of respiratory distress during the interval between embolization and follow-up examination.

Microscopic examination of lung tissue from these animals revealed changes identical to those seen in the systemic arteries of the animals used to study the effectiveness and persistence of GAX. There was no evidence of any cellular response either in or around arteries that contained GAX. The GAX always appeared as a compact, amorphous mass, often with cellular debris trapped within it. It seemed to be attached to the endothelium, which looked normal. In sections taken 2 months after embolization, small new vascular channels that contained viable-looking red blood cells were often present. These were directed along the luminal margin of arteries containing GAX (see Figs. 6 and 7).

Discussion

An ideal embolic agent should have the following characteristics: (1) be effective in producing vascular obstruction, (2) be safe for long-term implantation, (3) be persistent, (4) pass easily through small, flow-directed catheters, and (5) be suitable for use regardless of the degree of arteriovenous shunting that exists within a particular vascular bed.

Over the last decade, therapeutic embolization by means of percutaneous catheter techniques has become established as an important technique in treating a variety of vascular and neoplastic conditions. In spite of its demonstrated value, however, full utilization of the technique has been retarded, at least in part because of the unavailability of an embolic agent that meets the above criteria. Because of this deficiency, we evaluated a variety of potential embolic agents. These included GAX, non-cross-linked bovine collagen, bovine bone collagen, and irradiated bovine bone collagen. Of these, GAX was found to have the most promise.

Collagen is a major structural constituent of normal tissues. It is the most abundant protein found in animals and exists in numerous genetically distinct types, the structure and tissue distribution of which have been the subject of numerous investigations over the last decade. As the result of these efforts, several collagen products have become available and have been shown to be useful for such diverse applications as topical hemostasis, suture material, vascular grafts, heart valves, and soft-tissue augmentation [1, 2].

Through the use of purification and solubilization processes employing pepsin digestion, ultrafiltration, ion exchange chromatography, and centrifugation it is possible to obtain from

bovine dermis a variety of highly purified, injectable collagen compositions suitable for implantation in humans. These products developed by the Collagen Corp. are composed of a population of collagen fibrils that, depending upon the conditions of reconstitution, can be a variety of sizes. The average fibril size of the material used in this study was 5×75 μm. In vitro and in vivo studies in both laboratory animals and humans have shown that the host compatibility and persistence of injectable fibrillar suspensions of collagen are improved by cross linking of the fibrils through a process that exposes them to low concentrations of glutaraldehyde. This technique reduces the immunogenicity of the collagen, and by making it less susceptible to removal by collagenase also increases its persistence [1].

GAX is one of these collagen materials; it is made up principally of type 1 collagen. It can be prepared in mixture with standard radiographic contrast medium. Concentrations ranging from 3 to 65 mg of GAX per ml of solution have been tested. Those in concentrations up to 15 mg per ml pass easily through a 2-French polyethylene tubing or a 0.035 open-end guidewire.

In experiments that tested the capability of GAX to produce arterial occlusion, the material was shown to be an effective embolic agent. In all instances, 3–4 ml of a mixture of GAX (15 mg per ml) and radiographic contrast medium injected slowly into the internal iliac artery brought about the prompt arrest of blood flow. Persistent angiographic evidence of vascular obstruction was seen in all animals when follow-up examinations were done at intervals of 2 days, 2 weeks, or 2 months. The mechanism by which arterial obstruction is produced after embolization with GAX appears to be mechanical; there is no evidence that GAX has any direct effect on either blood coagulation or thrombus formation. The method by which mechanical obstruction takes place, however, is unproved. In our tissue samples, the frequent observation of GAX attaching to the endothelium of incompletely obstructed arteries at a postembolization interval before arterial recanalization could have occurred, suggests that in some way the irregular surface of the collagen fibrils fastens directly to the vessel wall. Perhaps at sites of turbulence or branching GAX adheres to the endothelium and then, as additional embolic material is introduced, a larger embolic plug accumulates. Similar phenomena occur with other embolic agents [3, 4].

In all histologic sections examined, no evidence of inflammatory response was found either in the walls of arteries or in tissue around the arteries filled with GAX. This was true regardless of the interval between embolization and reexamination or of the tissue from which the sections were taken. This observation differs markedly from the intense cellular reaction uniformly observed in response to the intravascular presence of microfibrillar collagen (Avitene) or bucrylate (isobutyl-2-cyanoacrylate) [4, 5]. The lack of inflammatory response is one indication of acceptable immunogenicity of GAX. Additional evidence is provided from the results of studies carried out by the Collagen Corp. No anticollagen antibodies were detected in 246 human volunteers evaluated 30 days after an intradermal injection of GAX. Evaluation of these same subjects at 6 months ($n = 132$) and 12 months ($n = 131$) has not demonstrated the presence of anticollagen antibodies (personal communication, Pharriss BB, Collagen Corp.).

Although many of the risks of therapeutic embolization are related more to the nature of the procedure itself than to the material used, some complications may occur or be aggravated by the poor host-tissue tolerance of the embolic materials. For example, both pain and temporary cranial nerve palsies after embolization of a variety of extracranial lesions have been reported and have been ascribed to ischemia. Although an intense inflammatory reaction is known to occur as the result of ischemia alone, the degree to which it may be aggravated or modified by an embolic agent with intrinsic inflammatory properties is unknown. The use of materials without such effects, however, would seem prudent, especially in circumstances in which the tissue to be embolized exists in a tightly confined space where swelling alone may compromise tissue integrity. Likewise, the angionecrosis that occurs after embolization with either microfibrillar collagen or Bucrylate is of particular concern when these agents are used in treatment of arteriovenous malformations, since as the result of these changes, arterial thinning is known to occur [4, 5]. Such an alteration occurring in vessels with inadequate or abnormal repair mechanisms may well be detrimental [4].

In terms of the amount of material remaining in place within an embolized vascular territory, it is not feasible to assess quantitatively the persistence of an embolic agent. So long as the embolized tissue remains viable, however, all emboli of a particular kind within an arterial territory should be acted upon equally by those forces that operate to remove them. Evidence, therefore, either of the persistence or removal of material from within an arterial lumen would seem to serve as one indicator of the permanence of the agent.

In all histologic sections examined, GAX was found to persist within the embolized tissue. In those instances where reexamination was carried out at 2 days (prostate, bladder, and lung), GAX was often seen as an amorphous mass filling the lumen of small arteries, of which the endothelial cells appeared somewhat swollen. In all tissues examined at intervals of 2 weeks (prostate and bladder), 1 month (lung), and 2 months (lung, prostate, and bladder base), there was evidence of formation of new vascular channels. These were oriented along the intimal surfaces of some of the arteries that contained GAX; in some of these new vessels, viable-appearing red blood cells could be seen.

It is emphasized that so long as a tissue remains viable it will have a blood supply. Tissue that is viable but ischemic as the result of therapeutic embolization will, under ordinary circumstances, reestablish, to some degree, its blood supply regardless of the nature of the material used for the embolization.

On the basis of the results of these experiments, we contend that the persistence of GAX is such that it should be considered an acceptable embolic material for all types of embolization. Since, however, the ability of endothelial cells to remove intraluminal material varies in different tissues, and as there is no information concerning the fate of intraluminal GAX in humans, final judgment on this matter must await

additional experience.

Should an embolic agent inadvertently reach the pulmonary circulation, it may cause an adverse effect either because of mechanical obstruction of parts of the pulmonary circulation or because of direct injury to the lung itself. In experiments directed at understanding the nature of acute toxicity after pulmonary embolization with GAX, the mechanism of action was shown to be a pure mechanical effect; all animals showed only an increase in their pulmonary artery pressure and pulmonary vascular resistance until an agonal state was reached. Microscopic examination of the lungs from these animals revealed only widespread arterial obstructions with no evidence of chemical injury. In the chronic studies of the effects of pulmonary embolization, follow-up examinations revealed that all abnormal measurements returned to baseline levels. When the lungs of these animals were examined with light microscopy, there was no cellular response either in or around the walls of arteries containing GAX. None of the animals in these chronic pulmonary embolization studies had any evidence of impaired pulmonary function. No attempt was made, however, to assess this quantitatively; and the possibility of permanent significant functional sequelae cannot be excluded. The fact that three of the 12 animals undergoing "sublethal" pulmonary embolization did not survive emphasizes the variability in the ability to tolerate an insult of this nature.

It is not possible to be precise as to the level at which vascular obstruction may occur with this material. It will pass into quite small arteries and arterioles but does not appear to enter the capillary bed. Obstructions at a site more proximal than would be expected on the basis of the size of the collagen fibrils may occur, since the material in some way attaches to the endothelial surface. Because factors such as the geometry of vascular branching, arterial dilatation and spasm, and the characteristics of blood flow have such influence on the level at which occlusion occurs, one cannot be certain that the agent will pass into vessels of a size similar to that of the individual collagen fibrils.

In patients embolized under the conditions of the acute pulmonary toxicity experiments—i.e., controlled respiration—inadvertent pulmonary embolization would likely be occult. Therefore, great care must be taken to assure that this is not occurring to a significant degree. This phenomenon, however, is probably not unique to GAX, and a similar risk likely exists for all materials that cause vascular obstruction in a purely mechanical manner. Inadvertent pulmonary embolization that occurs when respiration is not controlled would likely become apparent because of an associated increase in respiratory rate. The presence of this change appears to be a sensitive indicator of GAX reaching the pulmonary circulation.

On the basis of these observations we conclude that GAX as an embolic material offers several advantages over other currently available agents, and that its safety is such that clinical trials in humans can be safely undertaken.

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