Experimental Evaluation of a Cross-Linked Gelatin Adhesive in Gastrointestinal Surgery

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AN acceptable tissue adhesive would be useful in a variety of surgical applications, but experiences with the most promising agents currently available, the cyanoacrylates, have revealed a number of disadvantages which limit their effectiveness.[1, 2, 4-6, 8] A new tissue adhesive system, developed in collaboration with members of the Polymer Research Division of Battelle Memorial Institute, is being investigated at the National Heart Institute, and experiences with its early development and application as a hemostatic agent have been previously described.[3, 7] This report reviews briefly the development of the adhesive system and describes experimental applications of the current system in gastro-intestinal surgery.

Adhesive System

As initially developed, the adhesive system consisted of gelatin-resorcinol (G/R) in a ratio of 5:1 dissolved in water. This mixture, viscid at room temperature, was liquefied prior to use by warming to 40° C. The addition of formaldehyde to the solution initiated a reaction which cross-linked the G/R, and resulted in a bond with acceptable strength in less than 1 minute.[7] The speed of this reaction necessitated the addition of formaldehyde directly to the tissue to be bonded, with a resultant moderate degree of surface tissue injury. Although this degree of tissue damage was acceptable in certain applications, as on large parenchymal organs such as liver and kidney, it was more critical in others, such as blood vessels, bronchi, and hollow viscera. Attempts to perform gastro-intestinal anastomoses with the G/R/F system as described above resulted in dehiscences of the anastomoses secondary to tissue necrosis, not to failure of the adhesive bond. Since the noxious effect of the G/R/F system resulted almost entirely from the direct application to tissue of substantial amounts of aldehyde, a variety of changes in the G/R/F system were investigated in which the aldehyde was added directly to the adhesive, and only in amounts sufficient to initiate cross-linking. The rapidity of the subsequent reaction, which had been considered initially an obstacle to this approach, was found to be pH dependent. Acidification of the G/R mixture to a pH of approximately 5 with hydrochloric acid delayed the onset of cross-linking until the G/R/F was applied to the tissue, at which time the addition of a suitable base, such as sodium bicarbonate, initiated cross-linking. This system, although initially promising, required an additional step and had somewhat less bond strength than the original method because of the carbon dioxide bubbles generated by the reaction. It was found subsequently that regulation of the amount of acid added to the G/R/F allowed precise control of the speed of cross-linking and eliminated the need for the addition of a base.

In a different approach to the problem of reducing toxicity, formaldehyde was replaced with glutaraldehyde and the resulting adhesive was assessed on the spleen. The bond achieved with glutaraldehyde appeared to have less initial strength, but greater durability, *in vivo*. A mixture of formaldehyde and glutaraldehyde was then

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assessed in an attempt to combine the high initial strength of the formaldehyde bond with the increased durability of the glutaraldehyde bond. Suitability of these modified systems for use on the intestine and spleen was then evaluated in the manner described below.

Methods

Group I. Intestine. Twenty adult mongrel dogs weighing 15 to 30 Kg. were allowed free access to water and a regular kennel diet until operation. No intestinal antibiotics were administered. Under light pentobarbital anesthesia and through a midline abdominal incision, the distal ileum was exposed, and a 1.5 cm. transverse incision was made 10 cm. proximal to the ileocecal valve. In 12 animals traction sutures were placed which inverted the edges of the incision, but the sutures were not tied. A small amount of acidified G/R/F was applied to the incision, and after 1 to 2 minutes the traction sutures were released. The intestine was replaced in the abdomen, and no attempt was made to cover the incision with omentum. In eight control animals identical incisions were made in the ileum, but traction sutures were not placed and no adhesive was applied.

All animals were permitted free access to food and water immediately upon awakening. Intramuscular penicillin and streptomycin were administered postoperatively for 3 days. All animals in the adhesive group survived and were sacrificed at intervals of a week to 3 months. Those animals in the control group which survived were sacrificed at intervals of 1 to 4 weeks. Thorough abdominal exploration was carried out at the time of sacrifice and the distal ileum was preserved for gross and microscopic examination.

Group II. Spleen. Twelve additional adult mongrel dogs were utilized to assess the effectiveness of the adhesive systems described above in controlling hemorrhage from the spleen. Preoperative and postoperative care was identical with that described for Group I animals.

Under light pentobarbital anesthesia, the spleen was exposed through a midline upper abdominal incision and bisected transversely. Bleeding from the larger vessels was temporarily controlled by manual compression of the spleen and adhesive was applied to the cut surface. Occasionally, 2 or 3 applications were necessary to control persistent oozing. In 6 dogs, glutaraldehyde alone was used to replace formaldehyde in the system. In 6 additional dogs, a mixture of formaldehyde and glutaraldehyde, in a ratio of 3:2, was employed. All 12 animals survived and were sacrificed at intervals of 3 days to 3 months postoperatively.

Results

Group I. Intestine. Of 8 animals in the control group, 4 died within 48 to 72 hours of operation with generalized peritonitis, and of these 1 also had massive pneumoperitoneum. In 2 of the remaining animals, sacrificed at 1 and 2 weeks, respectively, there was no healing of the incision site, but fatal leakage of intestinal contents had been prevented by loosely adherent loops of small intestine. In a third animal, sacrificed 2 weeks postoperatively, a similar event had occurred but a large abscess was present. In 1 of the 8 animals, complete, spontaneous healing of the incision without adhesions was found at the time of sacrifice 4 weeks after operation.

All 12 animals in the adhesive group survived, and appeared healthy at the time of sacrifice. With the exception of 2 animals which were sacrificed 2 weeks postoperatively, and in which small, localized abscesses were found, all incisions in this group were healing or had healed at the time of sacrifice (depending upon the interval since operation), and no leakage was evident (Fig. 1). No tissue injury was grossly visible either at the time of opera-

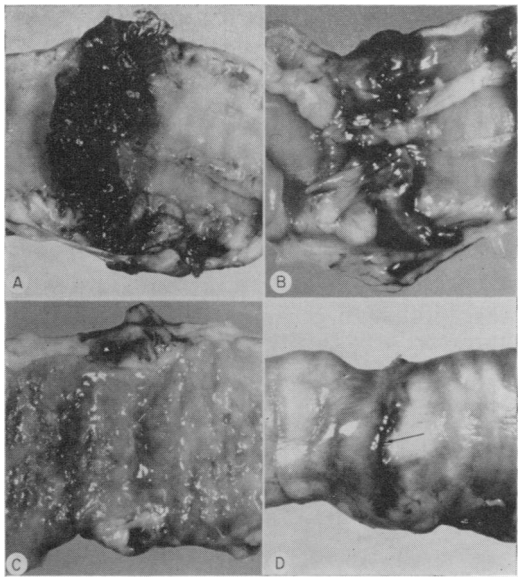

FIG. 1. Gross appearance of transverse ileal incisions, sealed with adhesive, at various intervals after operation. A) Three days postoperatively, the incision is completely sealed by firmly adherent adhesive. B) One month postoperatively, the adhesive remaining on the incision is reduced in amount but firmly adherent. C) The luminal surface of the same specimen as in B, shows complete healing of the incision. D) Two months postoperatively, no residual adhesive is visible grossly. The arrow indicates fibrous tissue at the site of the healed incision.

tion or at later sacrifice. The glue remained adherent to the incision and the amount grossly apparent decreased gradually after the second week, disappearing completely by the second to third month after operation (Fig. 1D). Significant adhesion of omentum or adjacent loops of intestine did not occur.

Microscopically, the bowel wall revealed a mild inflammatory reaction from the first to the third weeks, but the reaction around the adhesive was no greater than that seen around the traction sutures, which had not been tied, but which had been left in place for purposes of comparison (Fig. 2). Macrophages in great numbers appeared to contain adhesive after the first week, but no foreign-body giant cells were seen (Fig. 2B). After 2 months, adhesive was found only in the macrophages and was no longer seen in clumps (Fig. 2C).

Group II. Spleen. Hemorrhage was effectively controlled in all 12 animals. At the time of sacrifice, from 3 days to 3 months postoperatively, there was no evidence of hemorrhage, and all animals were in apparent good health.

Both types of adhesive (glutaraldehyde and glutaraldehyde-formaldehyde) persisted grossly for 4 months, which was substantially longer than on intestine, but the glutaraldehyde-formaldehyde mixture appeared to be more adherent (Fig. 3). Microscopic findings resembled those described above, but were more difficult to interpret because of the architecture of the spleen.

Discussion

Experiments with surgical adhesives have indicated certain properties which an acceptable adhesive should possess. Most important of these are prompt establishment of a bond on living tissue in the presence of moisture, maintenance of bond strength during immersion in body fluids until tissue healing occurs, ease of handling, lack of systemic toxicity, histotoxicity no greater than that caused by conventional sutures, and satisfactory shelf life. The cyanoacrylates, of which Eastman 910 has been most extensively investigated, have been disappointing in a number of these qualities. The bond, although rapidly established, is difficult to achieve in the presence of moisture [1, 2] and bond strength *in vivo* is often inadequate. Lack of viscosity, brittleness when polymerized, and short shelf life are additional undesirable properties of Eastman 910.

Gelatin-resorcinol-formaldehyde/glutaraldehyde systems utilized in these studies have excellent initial viscosity and prolonged shelf lives. Bond is rapidly established in the presence of normal amounts of moisture without the application of pressure, and bond strength is maintained until tissue healing occurs. Histotoxicity of the adhesive system as originally formu-

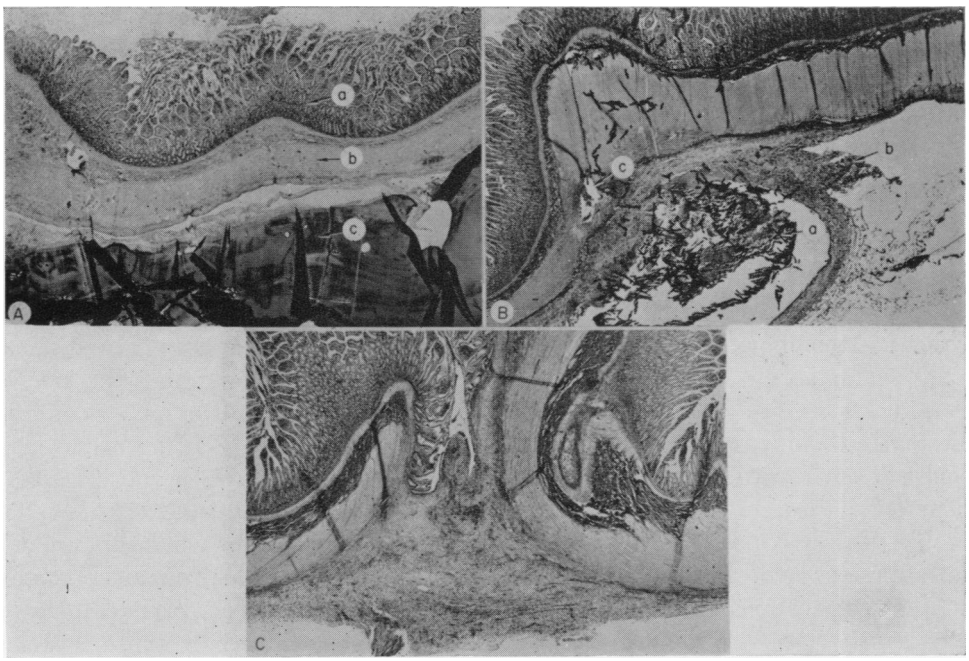

FIG. 2. Microscopic appearance of ileal wall at the site of adhesive applications. A) Three days postoperatively, the mucosa (a), and muscularis (b), adjacent to the adhesive (c), have a normal appearance. The separation of adhesive from bowel wall is an artifact of sectioning (11.5×). B) One month postoperatively, a section of the same specimen as in Figure 1B shows residual clumps of adhesive (a) and numerous macrophages containing large amounts of adhesive (b). A silk suture which was used for traction is also seen (c) (11.5×). C) Two months postoperatively, a section of the specimen in Figure 1D shows no residual clumps of adhesive, which can now be found only in macrophages. Abundant mature fibrous tissue has bridged the incision (15×).

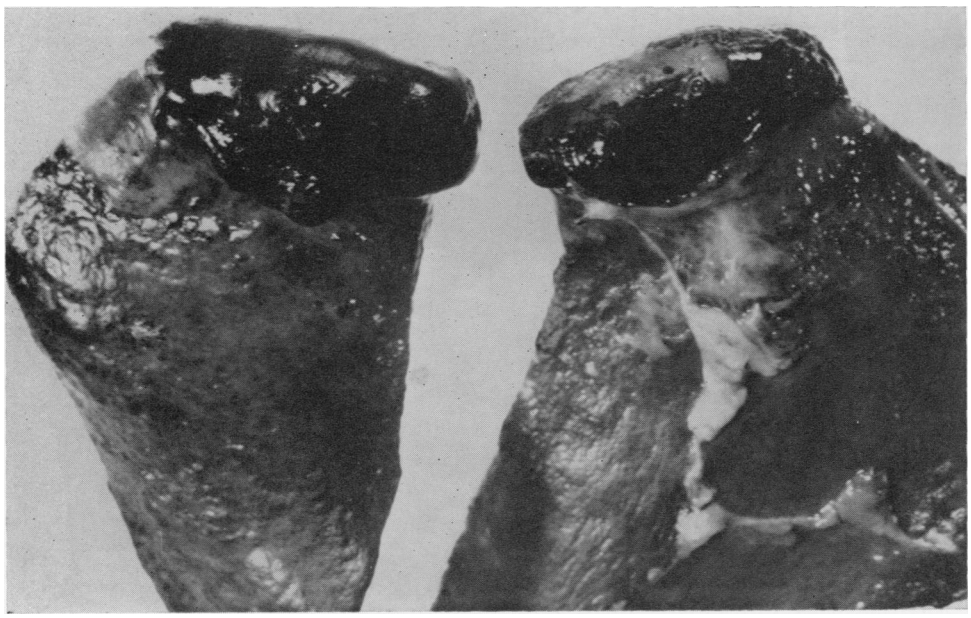

FIG. 3. Appearance of glutaraldehyde-formaldehyde adhesive on the bisected spleen 2 weeks postoperatively.

lated was due almost entirely to the large amounts of formaldehyde which, of necessity, were applied directly to the tissue. Recent alterations in the G/R/F system have decreased the degree of histotoxicity to an acceptable level, not only on large parenchymal organs, but on intestine as well. Additional methods to reduce further the toxicity of this promising adhesive system are under study. In particular, other aldehydes and combinations of aldehydes are being evaluated.

Experimental studies in this laboratory suggest that although the development of an ideal adhesive with properties which would permit its use on all body tissues is a desirable goal, it is possible that a spectrum of adhesives with different properties for different applications would more successfully satisfy the surgeon's needs. In the control of bleeding from a large liver laceration, for example, the establishment of a rapid, strong bond would be of primary importance, and surface injury of even 2 to 3 mm. of the tissue would be a desirable alternative to partial resection. Similarly, necrosis of a few grams of splenic tissue would be acceptable if the technical difficulties and the postoperative morbidity of splenectomy (e.g., increased incidence of infection in children) could be avoided. In intestinal surgery, however, when the adequacy of a suture line is in doubt, as in difficult closure of a duodenal stump, a bond achieved more slowly (3–5 minutes) would be acceptable, if the absence of histotoxicity were assured.

Although the results of the present experiments indicate potential clinical usefulness of the G/R/F system, the material cannot yet be recommended for use in man, because knowledge of all of its properties and effects remains incomplete.

Summary

Experiences with the development and experimental application of a new tissue adhesive system consisting of gelatin-resorcinol-formaldehyde are reviewed. Certain advantages over the most widely used surgical adhesive, Eastman 910, are the ability to establish a rapid bond in the presence of moisture without the application of pressure, high initial viscosity, and prolonged shelf life. The histotoxicity of the original adhesive system has been markedly reduced.

Experimental evaluation of the adhesive for intestinal surgery was carried out in 20 dogs. Transverse incisions in the distal ileum failed to heal in 7 of 8 control animals, but complete healing occurred in 10 of 12 animals in which the adhesive system was used to seal the incisions. Similar adhesive systems, in which glutaraldehyde or a glutaraldehyde-formaldehyde mixture was substituted for formaldehyde, were also evaluated. Both modified systems effectively controlled hemorrhage from the spleen in 12 dogs, but the glutaraldehyde-formaldehyde mixture appeared to maintain its bond strength *in vivo* more satisfactorily.

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