THE PREVENTION OF PERITONEAL ADHESIONS WITH HEPARIN*

AN EXPERIMENTAL STUDY

EDWIN P. LEHMAN, M.D.,

AND

FLOYD BOYS, M.D.

CHARLOTTESVILLE, VA.,

FROM THE DEPARTMENT OF SURGERY AND GYNECOLOGY, UNIVERSITY OF VIRGINIA, UNIVERSITY, VA.

THE PREVENTION of the formation of peritoneal adhesions has been essentially an unsolved problem. The conservative nature of the early adhesive process is recognized when the peritoneum has been contaminated with bacteria. After the acute phase is past, however, the often resulting peritoneal scar constitutes an independent pathologic entity, resulting frequently in intestinal obstruction. Following the surgical relief of obstruction, the reformation of many adhesions may be expected; in fact, the total amount of scar after such intervention may be greater than before, with correspondingly greater chance of further obstruction. The experience of every surgeon includes many examples of this unfortunate sequence.

The formation of peritoneal adhesions is the final result of an exudate on the peritoneal surface. This exudate, at first serous or seropurulent, becomes fibrinous, and the fibrin, in turn, is organized by the connective tissue and blood vessel elements of the subserosa. The process of organization always takes place in fibrin, and cannot take place without fibrin. It seems logical, therefore, to expect that the prevention of the formation of fibrin will prevent the organization that is the essential element of a permanent pathologic nature. One cannot prevent exudation, but it may be possible to prevent the coagulation of the exudate.

It is known that heparin will prevent the formation of fibrin in the blood. The possibility suggests itself that heparin might also prove effective in preventing this reaction in an exudate, and thereby might constitute a physiologic preventative of peritoneal adhesions. The present preliminary report records a series of animal experiments testing this hypothesis.

It is not necessary to review the general literature relative to the prevention of peritoneal adhesions. Except for the generally accepted influence of surgical cleanliness and gentleness as prime factors in preventing adhesions, no ancillary measures have been uniformly successful. The most significant attacks on the problem have been attempts (1) to destroy fibrin; and (2) to prevent its formation. Under the first heading, the use of amniotic fluid,[1-5]

* This study, presented before the Southern Surgical Association, December 6, 1939, was illustrated by motion pictures. No illustrations are here presented, inasmuch as the number of observed adhesions cannot be indicated by single exposures. Submitted for publication, December 6, 1939.

presumably acting through the resulting hyperleukocytosis, and the use of papain,[6-15] as a direct digestant of fibrin, have received the most attention. In both instances, later experimental work has not confirmed earlier reports.

After the initiation of this study, it was found that Miki and Satani,[16] in 1935, had experimented with heparin in the peritoneum of rabbits with some success, employing the old, crude product. They reported that the introduction of heparin-saline solution into the abdominal cavities of rabbits, the gastric, duodenal and omental serosae of which had been scarified with dry gauze, "considerably precluded" intraperitoneal agglutination. They found heparin-saline solution much superior to sodium citrate, and did not observe any toxic effects of heparin in their animals. In 1936, Widstrom and Wilander[17] proposed the same principle for the prevention of pleural adhesions. After producing an initial slight unilateral pneumothorax in rabbits, they created a chemical (iodine) pleuritis on the same side, which was uniformly followed by copious exudation with many fibrinous adhesions. Heparin solution (non-toxic Jorpes product[18]) was injected into the pleural cavities at 6, 24 and 48 hours, respectively, after the induction of iodine pleuritis. Sacrifice of the experimental animals at 48 hours showed uniformly no fibrinous coatings or adhesions. The control animals at this time showed fibrinous adhesions, and these were observed to organize within a few weeks.

It should be noted that other anticoagulants have been unsuccessfully tried in the past, for the prevention of the coagulation of exudates in serous cavities, such as hirudin,[19] citrate and oxalate solutions,[20-25] and others.[25]

Heparin, first purified by Charles and Scott,[27, 28] in 1933, is believed by Mellanby and other recent investigators,[29-33] to be an antithrombin. In proper dosage it will completely prevent any formation of fibrin in blood. Recently, it has been applied extensively to vascular surgery, both in the laboratory and the clinic.[34-37] Its application to prevent the formation of vegetations in endocarditis has been proposed.[38, 39] Except as noted above, no record of the employment of heparin in the prevention of the coagulation of other exudates has been found.

Heparinization of animals and man, in vascular surgery, to an extent that is effective in preventing thrombosis, has not been found to be hazardous from the point of view of wound hemorrhage if complete hemostasis is obtained at the time of closure.[35, 36]

Methods Employed.—Two sets of experiments, with corresponding controls, have been carried out, one with rabbits and the other with dogs. In the former, the formation of adhesions caused by mechanical damage and by bacterial contamination was studied. In the latter, to simulate the usual surgical problem, adhesions were first formed as the result of peritoneal contamination; they were then divided and the degree of their reformation was observed. A total of 70 rabbits and 82 dogs were employed. On account of deaths from peritonitis, in attempting to create adhesions, and experimental variations, the protocols of only 56 rabbits and 30 dogs are included in the statistics reported.

All surgical operations were carried out under anesthesia (in the rabbits,

local infiltration with 1% novocain; in the dogs, intratracheal ether) and the most scrupulous aseptic technic and extreme gentleness were employed. Fine silk was used for intraperitoneal ligature. Whenever a repeated survival celiotomy was indicated, the incision was made through an untouched area of the abdominal wall. Closure of the incision was effected with particular care to prevent exposed cut surfaces and suture material from presenting within the abdominal cavity.

RABBITS: (A) *Adhesions Caused by Mechanical Damage.*

Adhesions were produced by a modification of Donaldson's[15] pledget method. A pledget of folded dry gauze of constant dimensions was introduced close to the angle between the mesial surface of the cecum and the lateral surface of the ileum after light dry gauze scarification of the underlying serosa. It was anchored in this position by two fine silk serosal sutures. From three to five days later the abdomen was reopened and the pledget and sutures were removed. At this time the animals were divided into three control groups and one experimental group. In the first control group, the abdomen was closed after separation of adhesions without intraperitoneal administration of any solution. In the second control group, 25 cc. of normal saline solution were left in the peritoneal cavity. In the third control group, 25 cc. of amniotic fluid* were administered intraperitoneally before closure. In the experimental animals, 25 cc. of normal saline, containing 750 units of heparin (30 mg. per cent) were injected into the peritoneum.† The injections were repeated in both control and experimental animals by paracentesis on the first and second postoperative days. One week later the animals were examined for the final determination of the presence or absence of adhesions.

(B) *Adhesions Caused by Peritoneal Contamination.*

At the first operation, the appendix was perforated near the tip and its contents smeared over the adjacent serosal surfaces.[40, 41, 42] At this time the various solutions were injected intraperitoneally, in spite of an unclosed opening in the intestine. The rest of the experiment was carried out exactly as above, except that no amniotic fluid control group was established.

DOGS: *Reformation of Adhesions Caused by Peritoneal Contamination.*

At the first operation, the appendix was perforated near its tip and the contents smeared over the adjacent serosa. A large number of dogs (40 per cent) died from peritonitis following this procedure, and a few (11 per cent) developed localized abscesses. These animals are not reported. Six weeks after the perforation of the appendix, the abdomen was opened and the resulting adhesions were counted and carefully and completely divided, largely by sharp dissection. Bleeding was controlled by hot packs or by fine point ligation with silk. At this time the same three control groups and the heparin experimental group were established as in the rabbit experiments described

* The preparation used was Amfetin, furnished by the courtesy of Eli Lilly and Company, Indianapolis.

† The heparin employed was purchased from the Connaught Laboratories at the University of Toronto. It contained 110 units per milligram.

above. One hundred cubic centimeters of normal saline, 100 cc. of amniotic fluid and 100 cc. of the same heparin solution (representing 3,000 units) were introduced into the peritoneal cavity in the proper groups, respectively. The injections were repeated by paracentesis on the first and second postoperative days. Two weeks later, the reformation of adhesions was observed at a final celiotomy, and any reformed adhesions were counted.

In counting adhesions in the dog experiments, any continuous adhesive band or sheet was considered as a single adhesion. In most instances these probably represented the fusion of many smaller adhesions. The counting method presents, therefore, only an exceedingly rough quantitative estimation of results. The significance of this will be pointed out later.

Results.—In the rabbit experiments, which were designed to study the formation of adhesions following both mechanical trauma and bacterial contamination, the results indicate a striking difference between the controls and the animals treated with heparin. In the mechanical trauma experiments, whereas all of 26 control animals showed adhesion formation, only one of ten heparin-treated animals showed any adhesions (Table I). In the contamination group the results were similar (Table II). All of ten controls showed adhesion formation while none of ten heparin-treated animals produced adhesions.

TABLE I

ADHESIONS FORMED AFTER MECHANICAL PERITONEAL TRAUMA

Results one week after traumatization of peritoneal surfaces in the rabbit and the introduction of the solutions indicated

Group	Total Number Rabbits	Number Rabbits Showing Adhesions	
Control			
No solution	10	10	
Normal saline	8	8	
Amniotic fluid	8	8	
Totals	26	26	(100%)
Experimental			
Heparin	10	1	(10%)

TABLE II

ADHESIONS FORMED AFTER CONTAMINATION OF THE PERITONEUM

Results two weeks after bacterial contamination of the peritoneum in the rabbit and the introduction of the solutions indicated

Group	Total Number Rabbits	Number Rabbits Showing Adhesions	
Control			
No solution	5	5	
Normal saline	5	5	
Totals	10	10	(100%)
Experimental			
Heparin	10	0	(0.0%)

Attention should be particularly directed to the fact that in the contamination experiments complete healing of an open wound of the appendix occurred when heparin was present in the peritoneal cavity from the start, although it might have been expected to have hindered sealing off of the experimental perforation.

In the dog experiments, designed to repeat the frequent surgical problem presented by adhesions needing division on account of intestinal obstruction, the results again strongly suggest the effectiveness of heparin in preventing adhesion formation (Table III).

TABLE III

REFORMATION OF ADHESIONS

Results two weeks following separation of previously produced peritoneal adhesions in the dog. The original adhesions resulted from bacterial contamination of the peritoneum. The adhesions following the introduction of heparin were uniformly single strands, whereas those in the controls were usually broad bands and sheets.

Group	Number of Dogs	Average Number of Adhesions Divided	Average Number of Adhesions Reformed	Average Percentage of Reformation
Control				
No solution	10	6.2	9.7	156%
Normal saline	5	5.8	13.6	234%
Amniotic fluid	5	13.8	17.4	126%
Totals	20	8.0	12.6	157%
Experimental				
Heparin	10	9.7	2.6	26%

In all the control dogs a greater number of adhesions reformed than were divided. When no solution was left in the peritoneal cavity, half as many again adhesions could be counted after division. It was striking that when normal saline solution was employed, more than twice as many adhesions recurred. Amniotic fluid presented the best numerical control results, in that only 25 per cent more adhesions reformed. In contrast with these control results, the heparin-treated animals presented only 25 per cent of the number of the original adhesions. The numerical difference is less striking than the observed difference at celiotomy. In the control animals, the reformed adhesions were largely extensive bands or sheets of adherence. In the heparin animals, the reformed adhesions were uniformly minute points of attachment, largely of omentum to the knots of silk ligatures. Although the numerical reformation of adhesions after amniotic fluid was the lowest in the control groups, actually the adhesions reforming after amniotic fluid were the most extensive of any group.

In a few dogs it was observed that, following the intraperitoneal injection of heparin in the present dosages, the coagulation time of the blood (capillary tube method) was lengthened from a normal average of two minutes to an average of ten minutes. The peak of the increased coagulation time occurred

two hours after injection, and the effect disappeared after approximately eight hours. Leukocyte counts of peritoneal fluid in several dogs during the first 24 hours after injection of heparin showed a rapidly developing polymorphonuclear leukocytosis, roughly corresponding in degree to that produced by saline and amniotic fluid.

Complications in these experiments referable to the intraperitoneal introduction of heparin were rare. In the rabbits no such complications were observed. In 24 dogs receiving heparin intraperitoneally in various dosages, three of them suffered massive intra-abdominal hemorrhage, from which they died. Postmortem examination revealed the peritoneal cavity filled with fluid blood. Two of these hemorrhages occurred during the preliminary studies with heparin, and one, early in the final experiments. One of the 24 dogs died of paralytic ileus. In this animal heparin was entirely ineffective. On account of this complication, the animal has not been counted in the percentages reported (Table III).

DISCUSSION.—There is no question that heparin has proven astonishingly effective in preventing adhesions under the conditions of these experiments. Although the number of experiments is limited, the use of heparin offers considerable promise for this hitherto unsolved surgical problem. The work has not progressed sufficiently to warrant application to the patient. Continued study of possible dangers, of effective dosages, and of the details of administration is being carried on. It is hoped that the results of this further investigation will permit clinical application.

There is no problem in which the clinical effectiveness of treatment is less easily estimated. An enormous number of cases must be treated with heparin, or any other substance, before a sufficient number of cases will come to receliotomy or postmortem, to offer a basis for judging the efficiency of the method employed. It would seem necessary, therefore, that primary acceptance of a method to prevent adhesions must rest upon laboratory evidence.

On the basis of the present experience, the chief danger of the employment of heparin seems to lie in the occurrence of intraperitoneal hemorrhage. Heparin prevents the formation of fibrin but does not dissolve or destroy it, as we have observed in test tube experiments. It is logical, therefore, to assume that hemorrhage should not be a real danger provided complete intraperitoneal hemostasis is effected before closure of the abdomen. It is assumed that in the three hemorrhages here reported, all of which occurred early in the study, this essential was not observed. Certainly, a wider animal experience will be necessary before the method can be condemned for this reason. On the other hand, such a further experience may prove intraperitoneal hemorrhage to be a hazard of important magnitude. Bleeding in the abdominal wound from mild general heparinization, following absorption of heparin from the peritoneum, has not occurred. It should be a no greater danger than is bleeding from wounds following heparinization in vascular surgery.

One of the interesting incidental observations in the rabbit is the healing of an experimental perforation of the appendix in the presence of heparin. Al-

though no definite conclusion can be reached on this point, this evidence suggests that suture of a wound in the bowel may not be a contraindication to the intraperitoneal use of heparin.

The primary object of this study relates to the clinical problem presented by intestinal obstruction from intraperitoneal adhesions. If the present work is confirmed, and hemorrhage does not prove to be too great a danger, one can predict that heparin may be useful after any clean celiotomy, and, as just noted, perhaps after a contaminated one. All surgeons have had the experience of operating for intestinal obstruction following such a simple intervention as that for chronic appendicitis. In other words, possibly dangerous adhesions are a potential sequela following any celiotomy. The abolition of this danger, small as it may be, will add one more factor to the safety of abdominal surgery.

There are other structures besides the peritoneum in which formation of fibrous adhesions may be deleterious. These include joints, tendon sheaths, pleura, pericardium, and the subarachnoid space. If heparin in the peritoneal cavity is found useful, it is conceivable that it may also be employed effectively in these other serous cavities. It is hoped to initiate work on some of these problems in the near future.

CONCLUSION

Heparin introduced into the peritoneal cavity of the dog and rabbit is effective in preventing the formation and reformation of adhesions.

BIBLIOGRAPHY

[1] Johnson, H. L.: Amniotic Fluid Concentrate in the Prevention of Adhesions. New Eng. Jour. Med., **199**, 661–664, 1928.

[2] Warren, Shields: Effects of Amniotic Fluid on Serous Surfaces. Arch. Path., **6**, 860–866, 1928.

[3] Trusler, H. M.: Peritonitis: An Experimental Study of Healing in the Peritoneum and the Therapeutic Effect of Amniotic Fluid Concentration. Proc. Staff Meetings, Mayo Clinic, **4**, 356, 1929.

[4] Lacey, J. T.: Prevention of Peritoneal Adhesions by Amniotic Fluid. ANNALS OF SURGERY, **92**, 281–293, 1930.

[5] Johnson, H. L., Coonse, C. K., Hazard, J. B., Forsee, D. S., and Aufranc, Otto: Amniotic Fluid Concentrate as an Activator of Peritoneal Immunity. Surg., Gynec., and Obstet., **62**, 171–181, 1936.

[6] Kubota, Takashi: Prevention of Peritoneal Adhesions. Japan Med. World, **11**, 226–229, 1922.

[7] Johnson, H. L.: Observations on the Prevention of Postoperative Peritonitis and Abdominal Adhesions. Surg., Gynec., and Obstet., **45**, 612–619, 1927.

[8] Walton, R. P.: Behavior of Papain in the Peritoneal Cavity. Jour. Pharmacol. & Exper. Ther., **43**, 487–497, 1931.

[9] Ochsner, Alton, and Garside, E.: Peritoneal Adhesions and Their Prevention by Ferments. Surg., Gynec., and Obstet., **54**, 338–361, 1932.

[10] Fausel, E. G.: Experimental Postoperative Intra-abdominal Adhesions, with Special Reference to the Use of Papain Solution. Med. Times and Long Island Med. Jour., **62**, 1–4, 1934.

[11] Rea, C. E., and Wangensteen, O. H.: Comparative Efficiency of Substances Employed in the Prevention of Intraperitoneal Adhesions. Proc. Soc. Exper. Biol. and Med., **31**, 1060–1063, 1934.

[12] Ochsner, Alton, and Storck, A.: Prevention of Peritoneal Adhesions by Papain; Clinical Study. ANNALS OF SURGERY, **104**, 736–747, 1936.

[13] Bogart, L. M.: Intra-abdominal Adhesions: An Experimental and Clinical Study (Use of Papain and Sodium Citrate Solutions in the Prevention). Arch. Surg., **34**, 129–148, 1937.

[14] Elliott, R. H. E., and Mellanby, F. L.: Papain and Peritoneal Adhesions. Surgery, **1**, 785–791, 1937.

[15] Donaldson, J. K.: Abdominal Adhesions and the Use of Papain: A Discussion and Experimental Study. Arch. Surg., **36**, 20-27, 1938.

[16] Miki, Hisoa, and Satani, Hideo: Heparin for the Prevention of Peritoneal Agglutination (Experimental Study). Mitt. a. d. Med. Akad. zu Kioto, **14**, 337–338, 1935.

[17] Widstrom, Gosta, and Wilander, Olaf: Treatment of Experimental Pleurisy in Rabbits with Heparin. Acta Med. Scand., **88**, 434–439, 1936.

[18] Jorpes, Erik: The Chemistry of Heparin. Biochem. Jour., **29**, 1817–1830, 1935.

[19] Vogel, K.: Clinical and Experimental Studies of Peritoneal Adhesions Occurring after Laparotomy. ANNALS OF SURGERY, **36**, 961–962, 1902.

[20] Malcolm, J. D.: On the Importance of the Practice of Washing Out the Peritoneal Cavity as a Means of Securing a Natural Disposition of the Intestines after an Abdominal Section. Lancet, **1**, 75–76, 1890.

[21] Gellhorn, George: Experimental Studies on Postoperative Peritoneal Adhesions. Surg., Gynec., and Obstet., **8**, 505–513, 1901.

[22] Richardson, E. H.: Synopsis on Studies on the Prevention of Peritoneal Adhesions, with a Contribution to the Treatment of Denuded Bowel Surfaces. Johns Hopkins Hosp. Bull., **22**, 283–289, 1911.

[23] Pope, Saxton: Prevention of Peritoneal Adhesions by the Use of Citrate Solution. ANNALS OF SURGERY, **63**, 205–207, 1916.

[24] Walker, W. H., and Ferguson, L. M.: Peritoneal Adhesions: Their Prevention with Citrate Solutions. ANNALS OF SURGERY, **63**, 198–204, 1916.

[25] Hertzler, A. E.: The Peritoneum. Vol. I. Chap. on Adhesions. C. V. Mosby Company, St. Louis, 1919.

[26] Williams, C. S., and Mann, F. C.: Postoperative Peritoneal Adhesions. Surg., Gynec., and Obstet., **34**, 674–676, 1922.

[27] Charles A. F., and Scott, D. A.: Studies on Heparin: I. The Preparation of Heparin. Jour. Biol. Chem., **102**, 425–429, 1933.

[28] Charles, A. F., and Scott, D. A.: The Preparation of Heparin from Beef Lung. Toronto Roy. Soc., Canada, **28**(V), 55, 1934.

[29] Mellanby, J.: Heparin and Blood Coagulation. Proc. Roy. Soc., London, (Series B) **116**, 1-9, 1934.

[30] Chargaff, E., and Olson, K. B.: Studies on the Chemistry of Blood Coagulation. VI. Studies on the Action of Heparin and Other Anticoagulants. The Influence of Protamine on Anticoagulation Effect *in Vivo*. Jour. Biol. Chem., **122**, 153–167, 1937.

[31] Quick, A. J.: Normal Antithrombin of the Blood and Its Relation to Heparin. Am. Jour. Physiol., **123**, 712–719, 1938.

[32] Best, C. H.: Heparin and Thrombosis. Proc. Staff Meetings Mayo Clinic, **14**, 81–84, 1939.

[33] Best, C. H., and Taylor, N. B.: Physiological Basis of Medical Practice. Chap. on Blood Coagulation. Williams and Wilkins & Co., Balt., 1939. 2nd ed.

[34] Murray, D. W. G., Jaques, L. B., Perrett, T. S., and Best, C. H.: Heparin and the Thrombosis of Veins Following Injury. Surgery, **2**, 163–190, 1937.

[35] Murray, D. W. G., and Best, C. H.: The Use of Heparin in Thrombosis. ANNALS OF SURGERY, **108**, 163–173, 1938.

[36] Murray, D. W. G., and Best, C. H.: Heparin and Thrombosis—The Present Situation. J.A.M.A., **110**, 118–119, 1938.

[37] Best, C. H.: Heparin and Thrombosis. Brit. Med. Jour., **2**, 977–981, 1938.

[38] Kelson, S. R., and White, Paul D.: Treatment of Subacute Bacterial Endocarditis. J.A.M.A., **113**, 1700–1701, 1939.

[39] Friedman, M., Hamburger, W. W., and Katz, L. N.: Use of Heparin in Subacute Bacterial Endocarditis. J.A.M.A., **113**, 1702–1704, 1939.

[40] Behan, R. J.: Intraperitoneal Adhesions: Their Origin and Prevention. Am. Jour. Med. Sci., **160**, 375–383, 1920.

[41] Blalock, A.: Peritonitis: Effects of the Administration of Salt Solution on the Amount of Fluid that Accumulates in the Peritoneal Cavity. Arch. Surg., **26**, 1098-1102, 1933.

[42] Jeley, G. P.: Peritonitis in Cats Produced by Intraperitoneal Injection of B. Coli Suspended in Mucin. Arch. Surg., **39**, 783–791, 1939.