

THE EFFECT OF HEPARIN UPON INTRA-ABDOMINAL ADHESIONS IN RABBITS

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IT IS GENERALLY RECOGNIZED that certain patients form intra-abdominal adhesions after operation or after inflammatory processes. These may produce intestinal obstruction, and at times require surgery. Often adhesions reform and, again, cause obstruction. Methods that might prevent formation or reformation of adhesions have been the subject of several investigations adequately reviewed by Boys¹ in 1942.

Certain of these methods have been of general interest. Rea and Wangenstein,² Gepfert,³ Totten,⁴ Merkle,⁵ and others, introduced amniotic fluid, saline, air, oil, glucose, blood, gum acacia, *etc.*, into the peritoneal cavity to mechanically separate damaged surfaces until reëpithelization might occur. Ochsner and Garside,⁶ Donaldson,⁷ Ward,⁸ and others, tried proteolytic enzymes, such as papain or trypsin to digest deposits of fibrin. Lehman and Boys^{9, 10} employed heparin to minimize the formation of fibrin. Clinical reports of the use of papain by Ochsner,¹¹ and of heparin by Lehman and Boys^{12, 13} and Massie,¹⁴ have offered encouragement, but the results are not easily evaluated.

Experiments have, therefore, been undertaken to further study the effect of heparin upon the formation and reformation of adhesions. Rabbits, usually male, have been used for all experiments.

EXPERIMENTAL METHODS

Operations for Production of Adhesions: Rabbits were anesthetized by intravenous sodium amytal. A midline incision was made, using sterile technic. The appendix was exposed and freed from the meso-appendix using fine silk No. 000000, for ligation of vessels. The antimesenteric surface was then seared by applying a thin, heated spatula. The peritoneum and the incision were closed by a continuous mattress suture of either fine silk or catgut. The skin was closed with interrupted silk. Operations were performed by several individuals employing this standard technic.

Operations for Division of Adhesions: Celiotomies were performed through the same midline incision two weeks, or more, after the searing and at varying intervals thereafter, to determine occurrence and extent of adhesions and to divide and study reformation of adhesions. Adhesions were divided, using blunt dissection for closely adherent surfaces or sharp dissection with division and ligation for longer bands.

The extent of adhesions was determined by estimating the length of bowel involved and at times also by comparison of serial photographs. The length of extensively-involved matted segments of bowel was empirically estimated as 20 cm. The abdomen and chest were examined by autopsy in all animals that died. Histologic examination was made on the appendices of all animals sacrificed or dying within ten days of the original searing, and on other viscera that exhibited gross pathologic change.

Technic of Control Experiments: Adhesions were examined at operation in two control series, but not divided. These adhesions were later observed at operation or autopsy. In a second control series the adhesions were divided without using any form of therapy and later reexamined.

Method of Study of Heparin and the Formation of Adhesions: The effect of heparin on formation of adhesions was studied by searing the appendix and immediately placing 15 mg. of heparin into the abdomen. A second intraperitoneal injection of 15 mg. of heparin was given 24 hours after operation. In each instance the heparin was diluted by 30 cc. of normal saline. Heparin in Pitkin's menstruum* (100 to 200 mg.) was placed in the abdomen at the time of searing in another group of rabbits.

Method of Study of Reformation of Adhesions.—Control Experiments: Reformation of adhesions was investigated in control experiments by administering 30 cc. of normal saline intraperitoneally at the time of division and then twice a day by needle puncture for 72 hours. Gelatin, 5 per cent in sterile H₂O, was similarly injected in another control series. The state of the adhesions was determined by subsequent operation or autopsy 7 days to 4 weeks, average 2 weeks, after division.

Methods of Study of Reformation of Adhesions.—Test Experiments:

(A) *Heparin in Saline:* Heparin was administered in a volume of 20 to 30 cc. of saline at the time of division of adhesions and subsequently at intervals during 72 hours by reinjection through the abdominal wall. Peripheral clotting time was determined on venous blood at intervals of two to four hours by the capillary-tube method. The initial 20 to 30 cc. of saline contained 5 to 60 mg. of heparin, usually 15 mg. Subsequent injections contained similar amounts of heparin. The usual total dose was around 200 mg. Intervals between injections were varied to maintain a prolongation of the clotting time always over two minutes and usually over 15. Maximum prolongation of clotting time was often one-half to two hours. Reformation of adhesions was studied during operation or autopsy in animals surviving seven to 60 days.

* The ingredients of the Pitkin menstruum are gelatin 15 to 30 per cent, dextrose 5 to 12 per cent, glacial acetic acid 0.5 per cent and sufficient distilled water to make 100 per cent. The formulae used in our paper were LP-8, LP-9, and LP-10. A reference for this information is: Loewe, *et al.*: Venous Thrombo-embolic Disease, J. A. M. A., Vol. 130, 388, February 16, 1946.

Heparin and saline (10 to 50 mg.) was also administered at the time of searing and again by intra-abdominal injection at 24 hours in a group of rabbits.

(B) *Heparin in Pitkin's Menstruum*: Twenty-five to 200 mg. was placed in the abdomen at the time of operation and subsequently injected during 72 hours at intervals determined by the peripheral clotting time. The usual total dose was around 300 mg. Prolongation of clotting time was maintained at levels similar to those described with heparin and saline. Heparin in Pitkin's menstruum (150 mg.) was used as a single dose at the time of searing and also at the time of division of adhesions in other rabbits.

EXPERIMENTAL RESULTS

FORMATION OF ADHESIONS

Production of Adhesions: One hundred and fifteen rabbits survived operation and searing of the antimesenteric surface of the appendix without infection. Of these, adhesions developed in 89. Twenty-six rabbits did not develop adhesions. Eighteen of the 26 were again treated by searing. Five developed minimal adhesions. Three developed adhesions only after the third searing.

Formation of Adhesions: Adhesions developed in 15 of 21 rabbits in which heparin had been placed in the abdomen at the time of searing of the appendix and again injected 24 hours later. The appendices of five of the six animals that did not develop adhesions were again seared and when reexamined there were no adhesions. Adhesions developed in three of five rabbits in which heparin in Pitkin's menstruum had been placed in the abdomen at the time of searing. In five additional instances single doses of heparin in Pitkin's menstruum were administered at the time of searing. Adhesions formed in three.

Duration of Adhesions: Seven rabbits were explored two weeks after searing, without dividing adhesions. Celiotomy 33 to 51 days later revealed no evidence of change in the amount or character of adhesions.

Control Experiments—Reformation of Adhesions:

Reformation of adhesions was studied in the 89 rabbits that developed adhesions after the first searing. Since these rabbits were subjected to a series of operations during which adhesions were examined or divided and treated, or not treated, by control or test substances, and since they often served for from two to seven consecutive experiments the results will be reported as groups of experiments and not as individual animals. Animals dying within a week of operation, or developing infection, are not included in the experimental results.

(A) *Division of Adhesions without Treatment*: Adhesions were divided in 18 experiments and subsequently reexamined. They reformed in all but one rabbit. The length of large and small intestine involved in adhesions before division varied from the attachment of a single band up to 20 cm.,

average 6.9. After division the length varied from one band to 20 cm., average 9.0 cm. The extent of the adhesions observed after redivision had increased over that before division in ten experiments and decreased in eight.

(B) *Normal Saline*: Adhesions were divided and treated initially by 30 cc. of saline and subsequently by intraperitoneal injections of saline during 72 hours in five experiments. In four, adhesions reformed. The extent of adhesion before division varied from 2 to 20 cm., average 8. After division and treatment by saline the length of involvement varied from 0 to 10 cm., average 5. The extent of adhesions after treatment increased in two experiments and decreased in three. The rabbit that did not have adhesions after saline treatment had 20 cm. before division.

(C) *Gelatin*: Gelatin was used after division of adhesions in six instances and in five adhesions redeveloped. Before division of adhesions the length of involvement was from 2 to 18 cm., average 7. After treatment the length varied from 0 to 10 cm., and averaged 6. The extent of involvement increased in three and decreased in three. The animal that did not redevelop adhesions had one 3 cm. band at the time of division.

Experiments with Heparin—Reformation of Adhesions:

(A) *Heparin in Saline*: In 20 experiments adhesions were divided and treated during 72 hours by heparin in saline. Adhesions reformed in 17. Before division and treatment adhesions involved 1 to 20 cm. of bowel, average 8. Afterward they involved 0 to 20 cm., average 6. The amount of involvement increased in ten, decreased in eight, and was unchanged in two. The three animals that had no adhesions had had 1, 4, and 10 cm., respectively, at the time of division.

In addition to the 20 successful experiments there were eight deaths. Three were caused by hemorrhage.

(B) *Heparin in Pitkin's Menstruum*: In 21 instances heparin in Pitkin's menstruum was employed during the first 72 hours after division of adhesions. They redeveloped in 19. The area of involvement before division and treatment varied from 2 to 20 cm., average 8.3 and afterward from 0 to 20, average 6.6 cm. The extent of adhesions increased in six, decreased in 11, and was unchanged in four. The two animals that did not redevelop adhesions had 4 and 10 cm., respectively, at the time of division of adhesions.

In four instances with single doses of heparin in Pitkin's menstruum given at the time of division of adhesions, all reformed them. The area of involvement varied from 6 to 15 cm., average 9, before treatment and afterward 2 to 20 cm., average 13. The extent increased in three and decreased in one.

In addition to the 25 successful experiments reported above there were 22 deaths during administration of heparin in Pitkin's menstruum. Of these 17 died of hemorrhage.

Pathologic Examination.—Autopsies were performed on 15 rabbits sacrificed at intervals within ten days of searing of the appendix. Autopsies were also performed on 30 animals dying during or just after a 72-hour period of

treatment with heparin. Treatment was begun immediately after division of adhesions.

Gross examination of the 15 sacrificed rabbits often revealed fibrinous adhesions about the appendix. Material for staining and preparation for microscopic study was obtained from cross-sections of the appendix. Microscopic preparations satisfactory for study of mesothelium were obtained in

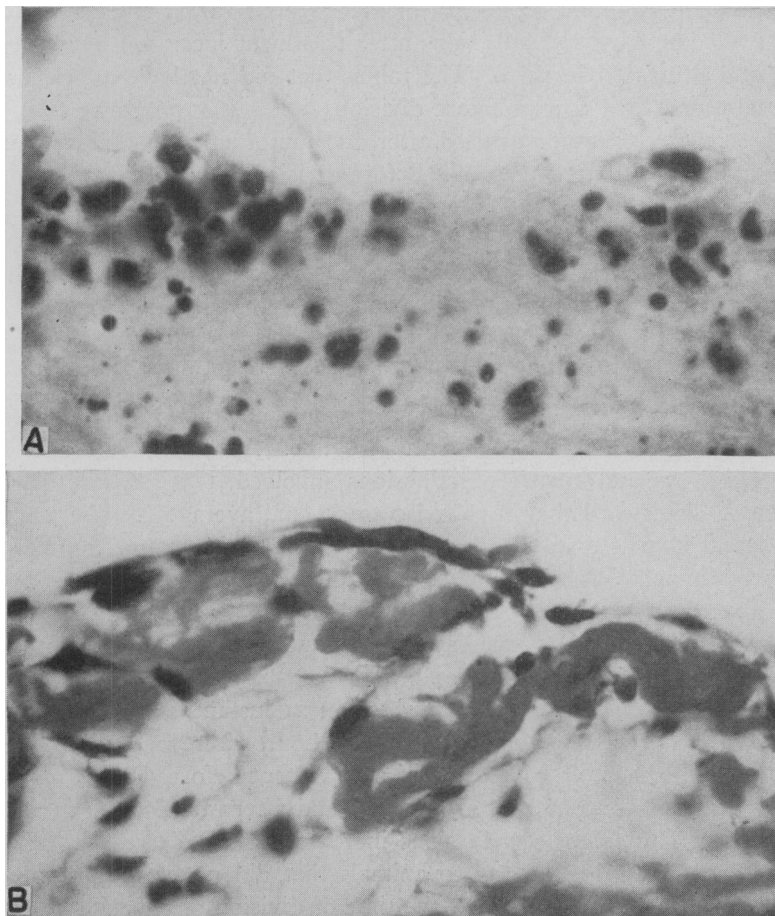


FIG. 1.—(A) Fibrinous surface of appendix 18 hours after searing.
(B) Fibrin covered by a single layer of flat cells resembling mesothelium 96 hours after searing.

only seven. Of these, two, six and 22 hours after searing, had no visible mesothelium. The burned surface was covered by fibrin (Fig. 1 A). Two, at 18 and 42 hours, had a few isolated cells resembling mesothelial cells on the surface of the fibrin over the burned area. Three, at 30, 72, and 96 hours had continuous sheets of single-layered cells, resembling mesothelial cells, over the granulating surface (Fig. 1 B).

INTRA-ABDOMINAL ADHESIONS

Gross examination of 30 rabbits that died during the period of heparin therapy revealed massive hemorrhage into the abdomen or into the wound in 20. Satisfactory microscopic examinations of the appendices at the area of division of adhesions were possible in five of nine rabbits studied. Fibrinous exudate or fibrin was present in each. Sections of lungs of 12 showed advanced pulmonary edema. Congestion was also present in five, and hemorrhage in three. Brain tissue was examined in three and there were small hemorrhages in the pia mater of the cortex of two. One of two hearts examined showed small subendocardial hemorrhages in the left ventricle.

TABLE I
SUMMARY OF STUDIES ON ADHESIONS

	No. of Experiments	No. that Developed Adhesions	% that Developed Adhesions	Change of Extent of Adhesions (Average)		Number of Animals Exhibiting Change		
				Before	After	Increase	Decrease	No Change
Formation of Adhesions:								
No treatment.....	115	89	77%
Heparin in saline..... (2 doses)	21	15	71%
Heparin in Pitkin's menstruum (1 dose)..	5	3*
Reformation of Adhesions:								
No treatment.....	18	17	94%	7 cm.	9 cm.	10	8	0
Heparin in saline..... (72 hours)	20	17	85%	8 cm.	6 cm.	10	8	2
Heparin in Pitkin's menstruum (72 hours)	21	19	90%	8 cm.	6 cm.	6	11	4
Heparin in Pitkin's menstruum (1 dose)..	4	4*	9 cm.	13 cm.	3	1	0
Saline alone.....	5	4*	8 cm.	5 cm.	2	3	0
Gelatin 5% aqueous solution.....	6	5*	7 cm.	6 cm.	3	3	0

* Series not large enough to warrant percentage.

DISCUSSION.—The summary in Table I demonstrates that the likelihood of developing adhesions was almost as great in the rabbits treated by heparin as in those that were not treated, or were treated by saline or gelatin. There was no significant difference in the average extent of involvement of bowel by adhesions or in the numbers of rabbits that had an increase or a decrease in the amount of adhesions.

It has been suggested by Lehman and Boys,^{9, 10} and others, that heparin might prevent or minimize the formation of fibrin and the development of adhesions. Our histologic studies in rabbits dying of hemorrhage during heparinization revealed fibrinous exudate or fibrin at the site of injury.

Although Brunn,¹⁵ Clarke,¹⁶ and Hertzler¹⁷ have studied regeneration of mesothelium, there is no definite evidence determining the rate of growth. Mesothelium has been described as originating from the edges of defects or as developing by a differentiation of connective tissue cells (Baily¹⁸). Since

young mesothelial cells are easily lost in preparation of tissue for microscopic study and since it is difficult to differentiate mesothelial cells from flattened wandering cells in cross-section preparations, our histologic studies have not solved these problems with certainty. It seemed, however, that reperitonealization may require a period of time longer than the maximum of 72 hours used for treatment in these and other experiments.

CONCLUSIONS

1. Heparin in large doses, continued up to 72 hours, has not prevented the formation or reformation of intraperitoneal adhesions or the deposition of fibrin on the surface of injured appendices in rabbits.
2. There was a spontaneous failure of development of adhesions in 23 per cent of the rabbits used in this experiment. There were also 6 per cent of the rabbits subjected to division of adhesions, and no other treatment, that did not redevelop adhesions. Heparin increased the likelihood of not developing adhesions by less than 8 per cent.
3. The extent of the adhesions that redeveloped after division and heparinization was equal to that observed in control experiments.

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BIBLIOGRAPHY

- ¹ Boys, F.: Prophylaxis of Peritoneal Adhesions: Review of Literature, *Surgery*, **11**, 118-168, 1942.
- ² Rea, C. E., and Wangenstein, O. H.: Comparative Efficacy of Substances Employed in Prevention of Intraperitoneal Adhesions. *Proc. Soc. Exper. Biol. and Med.*, 1060-1063, 1934.
- ³ Gepfert, J. R.: Intraperitoneal Use of Amniotic Fluid to Promote Smoother Post-operative Convalescence. *Amer. J. Surg.*, **32**, 40-44, 1936.
- ⁴ Totten, H. P.: The Intraperitoneal Use of Hypertonic Glucose Solution. *Surgery*, **8**, 456-463, 1940.
- ⁵ Merkle, H. J.: A New Technic for Instilling Amniotic Fluid Concentrate Intra-abdominally at the Close of Operations. *Amer. J. Surg.*, **65**, 210-220, 1944.
- ⁶ Ochsner, A., and Garside, E.: Peritoneal Adhesions. *Surg. Gynec. and Obst.*, **54**, 338-361, 1932.
- ⁷ Donaldson, J. K.: Abdominal Adhesions and the Use of Papain. *Arch. Surg.*, **36**, 20-27, 1938.
- ⁸ Ward, B. W.: Use of Papain in Prevention of Reformation of Peritoneal Adhesions. *J. Oklahoma M. A.*, **29**, 399-401, 1936.
- ⁹ Lehman, E. P., and Boys, F.: Heparin in the Prevention of Peritoneal Adhesions. *ANNALS OF SURGERY*, **112**, 969-974, 1940.
- ¹⁰ Lehman, E. P., and Boys, F.: Clinical Use of Heparin in the Peritoneum for the Prevention of Adhesions. *Arch. Surg.*, **43**, 933-945, 1941.
- ¹¹ Ochsner, A., and Storck, A. H.: The Prevention of Peritoneal Adhesions by Papain. *ANNALS OF SURGERY*, **104**, 736-747, 1936.
- ¹² Lehman, E. P., and Boys, F.: Experimental Prevention of Intraperitoneal Adhesions with Heparin: Third Report. *Surgery*, **12**, 236-241, 1942.

- ¹³ Boys, F., and Lehman, E. P.: Experimental Studies on Peritoneal Adhesions; Fourth Report—Sulfonamides With and Without Heparin. *ANNALS OF SURGERY*, **118**, 612-618, 1943.
- ¹⁴ Massie, F. M.: Heparin in Abdomen; Clinical Report. *ANNALS OF SURGERY*, **121**, 508-517, 1945.
- ¹⁵ Brunn, M. v.: Über die Entzündung seröser Häute, mit besonderer Berücksichtigung der Rolle der Serosa-Deckzellen. *Beitr. z. Pathologischen Anatomie*, **30**, 417-456, 1901.
- ¹⁶ Clarke, W. C.: Experimental Mesothelium. *Anat. Record*, **10**, 301-316, 1915.
- ¹⁷ Hertzler, A. E.: *The Peritoneum*. C. V. Mosby and Co., St. Louis, 1919.
- ¹⁸ *Baily's Textbook of Histology*, Williams and Wilkins Co., Baltimore, Maryland, p. 71, 1944.

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