Experimental Esophageal Lye Burns

II. Correcting Established Strictures with Beta-Aminopropionitrile and Bougienage

JOHN W. MADDEN, M.D., WILLIAM M. DAVIS, M.D., COOLEY BUTLER, II, M.D., ERLE E. PEACOCK, JR., M.D.

From the Departments of Surgery and Pathology, University of Ariona, College of Medicine, Tucson, Arizona 85724

THE SEQUELAE of chemical injury of the esophagus remain a major medical problem.[17] Ingestion of corrosive agents, particularly strong alkalies, may produce acute perforation and death or, more typically, chronic esophageal stenosis and significant morbidity.[21] When initial injury penetrates the entire esophageal wall or subsequent infection converts a deep partial thickness burn into a full thickness injury, free perforation with catastrophic complications may occur. Most burns, however, do not result in free perforation. Partial thickness injuries and, occasionally, even full thickness burns are walled off by surrounding tissues and heal. Although this normal response of esophagus to injury may prevent acute perforation, wound healing and scar formation create additional pathophysiology. During healing, the normally pliant esophagus may be converted into a rigid, narrow tube incapable of passing food. As necrotic tissue sloughs and the resultant ulcer heals, large deposits of dense scar tissue replace the normal submucosal and muscle layers. The presence of scar tissue alone may be of little consequence. However, if scar prevents the esophagus from dilating adequately or narrows the esophagus beyond critical limits, normal deglutition is impossible. Thus, the physical properties and form of esophageal scar tissue determine the physiological outcome of injury.

Standard treatment of lye ingestion is designed to alter form of esophageal scar tissue by mechanical bougienage and to alter inflammatory response and quantity of scar deposited by administering pharmacological doses of steroid hormones. Unfortunately, neither of these therapeutic measures are fully effective.[13] Limited data are available on the biology of visceral wound healing. However, if esophageal and cutaneous wound healing are analogous processes, failure of standard therapy can be explained biologically. Form of surface scars can be altered by mechanical means, but splints must be applied continuously for many months to achieve a permanent correction. In esophageal injuries, bougienage (a form of mechanical splinting) can only be performed intermittently. Each episode corrects rather than prevents form alterations, presumably creating new wounds and more scar. Although steroid hormones reduce acute inflammation, rate of protein synthesis, and rate of scar formation significantly, permanent effects on quantity or quality of scar tissue may not occur. Ultimately, wounds heal under the influence of systemic steroids with the accumulation of large amounts of scar. Systemic steroids change the rate of wound healing but may not alter the

Presented at the Annual Meeting of the American Surgical Association, Los Angeles, California, April 25–27, 1973.

Supported by Grant AM14047, National Institutes of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland 20014.

quantitative aspects of scar formation. In addition, both mechanical bougienage and pharmacological doses of steroids have significant mortality and morbidity. Because of these inherent failings of current therapy, we have investigated new approaches to the prevention and therapy of esophageal stenosis.

The physical characteristics of all mesenchymal tissues including scars are directly related to the physical properties of collagen fibers. Therefore, altering quantity or physical properties of scar collagen should alter the pathophysiology of esophageal injury. In order to be useful clinically, anticollagenous agents must specifically affect collagen without interfering with metabolism of other biologically important molecules and selectively affect collagen in pathological lesions without influencing structural integrity of normal mesenchymal tissues.[18] Selectivity can be achieved by using agents affecting only newly synthesized collagen in situations where collagen is turning over more rapidly in diseased than in normal tissues. Specificity can be achieved either by inhibiting unique steps in collagen maturation or by interfering specifically with collagen synthesis. Although several methods of inhibiting collagen synthesis are under study, none appear to be ready for clinical trial in the near future. Normal collagen maturation, however, can be inhibited specifically in animals and man using agents currently undergoing clinical evaluation.

The physical properties of newly synthesized scar tissue depend upon the amount of collagen synthesized, the physical weave of the collagen fibers, and the number of covalent bonds between collagen molecules. Although several types of cross bonds have been identified, covalent bonds derived from aldehyde precursors are the major stabilizing force in fiber formation.[25] Covalent bonding in newly synthesized collagen can be prevented specifically by a class of chemical compounds called lathyrogens. Beta-aminopropionitrile (BAPN), the most powerful known osteolathyrogen, specifically inhibits the action of lysyl oxidase and prevents the oxidative deamination of lysyl residues in collagen.[27] Because lysine derived aldehydes are necessary for the production of normal covalent bonds between molecules, collagen synthesized under the influence of BAPN fails to form covalent cross bonds. Lathyritic collagen forms fibers morphologically indistinguishable from normal collagen; lathyritic fibers, however, have significantly decreased tensile strength.[28] The systemic administration of BAPN in doses insufficient to produce generalized mesenchymal effects, reduces burst strength of healing skin wounds in rats, reduces the restrictive effect of adhesions around flexor tendon repairs in chicken's feet, and changes the type and amount of scar tissue in dimethylnitrosamine-induced hepatic cirrhosis in rats.[4,9,23] In addition, BAPN specifically inhibits covalent cross bonding in newly synthesized collagen in man.[14,24]

Recently, we have demonstrated that inhibiting lysyl oxidase activity prevents stenosis in dogs with acute esophageal lye burns.[5] BAPN alone, without addition of mechanical bougienage, is as effective in preventing esophageal stenosis as large doses of systemic steroid hormones and bi-weekly bougienage. The following studies were performed to test the hypothesis that inhibiting intermolecular covalent bonding in newly synthesized scar collagen with the addition of mechanical bougienage can reverse established esophageal stenosis.

Materials and Methods

The canine model of esophageal stenosis used in these studies has been described elsewhere.[5] Under Nembutal anesthesia, the distal esophagus was exposed through a left thoracotomy incision. Great care was taken not to disturb the longitudinal and segmental blood supply. A double lumen, plastic nasogastric tube inserted through the mouth was isolated within a 5 cm. esophageal segment with umbilical tapes. A 20% solution of sodium hydroxide was instilled into the isolated segment until a pressure of 30 cms. of water could be measured. After maintaining the pressure for 60 seconds, the sodium hydroxide was removed and the isolated segment irrigated with distilled water for 15 seconds at a pressure of 30 cm. of water. Washing was repeated three times. Before the chokers were removed, extent of the burn was marked with stainless steel wire sutures placed in the muscle and the submucosal layers at either end of the burned segment. Distance between sutures was measured carefully while the chest was open and by X-ray after the thoracotomy was closed.

Barium esophagograms were performed prior to burning and every 2 weeks throughout the experiment. Under general anesthesia, animals were positioned a standard distance from X-ray source and film cassette. Thin barium was introduced into the upper esophagus through a plastic catheter at a pressure of 30 cm. of barium-water mixture. Exposures were made when barium regurgitated into the mouth or when 500 ml. had been administered. The process was repeated twice. Esophageal diameter was determined by measuring the narrowest portion of the barium column between the wire sutures on both exposures and determining the mean.

At sacrifice, the esophagus was inflated with 10% neutral buffered formalin after ligating proximal and distal ends beyond the injured segment and was immersed for at least 48 hours in the same fluid. Longi-

tudinal and transverse transmural blocks were excised from the injured segment and examined histologically. Where the injury appeared eccentric, the area of maximum destruction was included in the longitudinal block.

Experimental Design

Forty adult mongrel dogs had standard esophageal lye burns as described. All were fed a soft solid diet for 4 weeks and had no other treatment. During this interval, four dogs died of acute esophageal perforation. At 4 weeks, barium esophagograms were analyzed and the 30 animals with the smallest esophgeal diameters were randomly divided into three groups. Group I received no therapy and served as controls for Groups II and III. Group II were begun on daily injections of Prednisolone (0.1 mg./Kg.). Group III received BAPN fumarate (100 mg. base/Kg.) as daily intraperitoneal injection. Previous data indicate that this dose of BAPN inhibits covalent bonding in newly synthesized collagen significantly without producing measurable effects on mature connective tissue.[5] In addition to daily drug injections, Groups II and III were dilated weekly under general anesthesia with a tapered 46 French mercury filled esophageal bougie (Maloney type). Groups II and III were treated for 6 weeks, beginning 4 weeks after the esophageal burn, and followed for an additional 20 weeks after the cessation of therapy (Fig. 1).

Our previous experience with this canine model demonstrated that only deep, muscle penetrating burns produced permanent esophageal stricture.[1] Therefore, at the conclusion of the experiment, each esophageal specimen was examined and the depth of burn established. The examiners had no knowledge of the previous treatment administered. Only animals with deep, penetrating muscle burns will be considered. Two of the controls, three of the steroid treatment group, and two of the BAPN group had superficial burns and were discarded.

Results

Mean esophageal diameters for all groups are presented in Table 1. During the 4 weeks prior to treatment, all animals lost weight and developed significant esophageal stenosis by esophagogram. At the beginning of treatment, esophageal diameters were comparable in all groups. During the 6-week treatment interval, mean esophageal diameters of the steroid plus bougienage group increased slightly but did not differ significantly from the control group. No further increase in mean esophageal diameter occurred during the 5-month follow-up period (Fig. 2). In contrast, the mean esophageal diameters of the BAPN plus

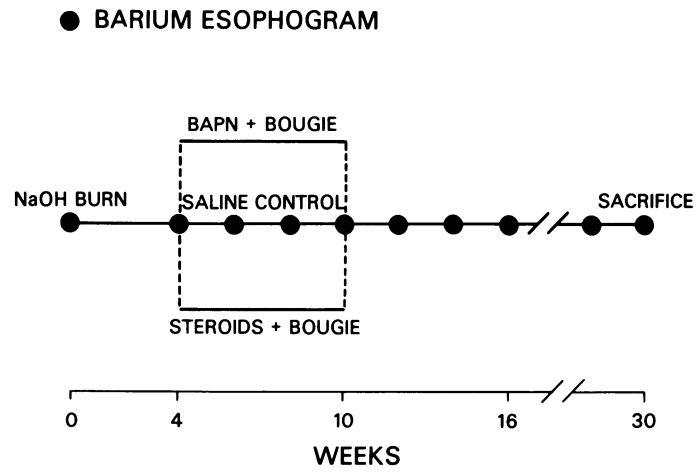


FIG. 1. Experimental design. At 30 weeks, dogs were sacrificed and only animals with deep muscle penetrating burns were considered.

bougienage group increased steadily during treatment. By the end of the treatment interval, mean esophageal diameter was significantly larger than both the steroid plus bougienage and control groups ($p < 0.02$). After treatment was discontinued, mean esophageal diameter continued to increase, reaching a maximum at 18 weeks, and remaining constant thereafter throughout the remainder of the 7-month observation period (Fig. 2).

Clinically, dogs in the three groups behaved differently. By the fourth week, despite ravenous appetites, all animals had difficulty swallowing a soft solid diet. Food swallowed quickly would be regurgitated a few moments later. After many attempts, however, some food would pass into the stomach. Control animals

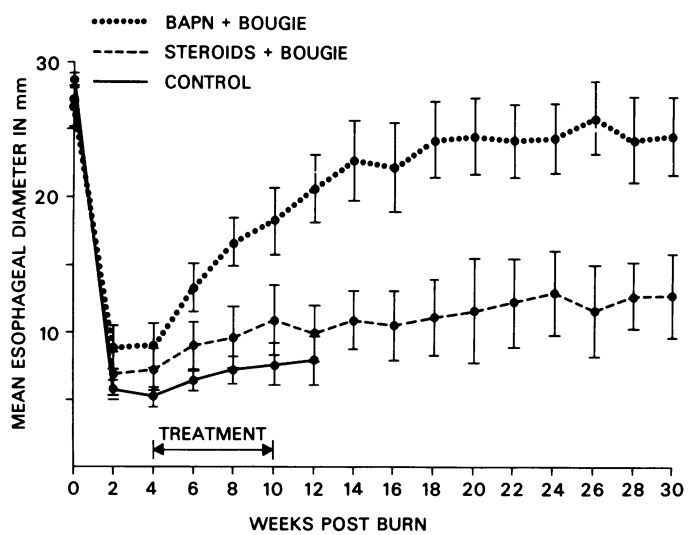


FIG. 2. Mean esophageal diameters and standard errors for the three groups. Although dogs were treated from the 4th through the 10th weeks, BAPN animals continued to improve for an additional 8 weeks.

TABLE 1. *Smallest Diameter of Esophagus*
(mean, mm ± SE)

	Group I Control (n=8)	Group II Steroids + Bougienage (n=7)	Group III BAPN + Bougienage (n=8)
Pre-burn	28.5 ± 0.6	27.2 ± 2.1	26.7 ± 1.5
2 Weeks	6.1 ± 0.8	7.0 ± 1.5	8.8 ± 1.5
4 Weeks	6.8 ± 0.8	7.3 ± 1.6	7.9 ± 1.6
6 Weeks†	6.5 ± 0.8	9.0 ± 1.8	13.3 ± 1.7
8 Weeks	7.3 ± 1.0	9.7 ± 2.3	16.7 ± 1.8
10 Weeks	7.7 ± 1.6	11.0 ± 2.5	18.3 ± 2.2*
12 Weeks	8.0 ± 1.8	10.0 ± 2.0	20.7 ± 2.3*
14 Weeks		11.0 ± 2.1	22.8 ± 2.7*
16 Weeks		10.6 ± 2.5	22.3 ± 2.1*
18 Weeks		11.2 ± 2.7	24.3 ± 2.6*
20 Weeks		11.7 ± 3.8	24.6 ± 2.6*
22 Weeks		12.3 ± 3.2	24.3 ± 2.5*
24 Weeks		13.0 ± 3.1	24.5 ± 2.4*
26 Weeks		11.7 ± 3.3	26.0 ± 2.5*
28 Weeks		12.8 ± 2.4	24.4 ± 3.0*
30 Weeks		12.8 ± 3.0	24.7 ± 2.7*

† Animals treated from Week 4 through Week 10
* Significantly greater than Groups I and II, $p < 0.02$

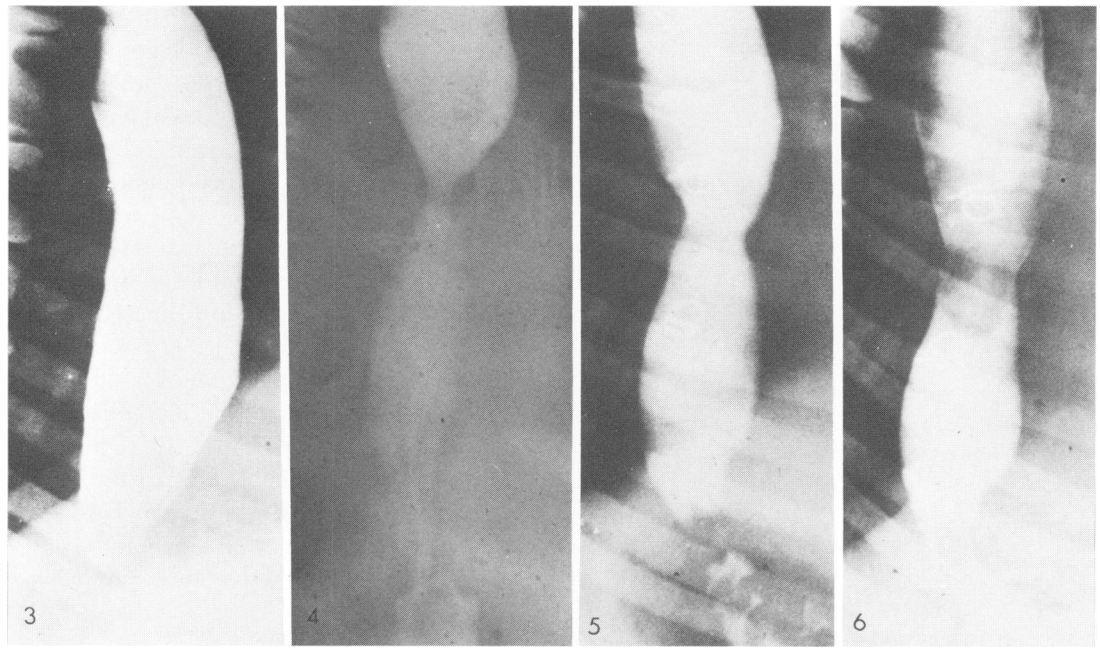
maintained this behavior until weight loss was significant or they lost interest in eating. By the 12th week, the majority of control animals had reached this state and were sacrificed. With the exception of one animal, the steroid plus bougienage group improved with the onset of therapy. Although all steroid plus bougienage animals continued to regurgitate, some food could be swallowed after several attempts. By the end of the 6-week treatment interval, steroid plus bougienage dogs continued to regurgitate, but maintained their weight. The lone exception could not maintain weight despite steroid plus bougienage treatment and was sacrificed in the sixth week of treatment; esophageal diameter during the final week was less than 4 mm. In contrast, BAPN plus bougienage treated animals improved steadily with the onset of therapy. (Figs. 3–6) By 6 weeks, all animals ate without regurgitating and maintained or increased their weight during the observation period.

Histologic examination of the esophagi at the conclusion of the experiment revealed that submucosa and muscle layers of all animals had been replaced by dense scar tissue. Examination of specimens without prior knowledge of treatment failed to reveal significant histological differences. Although thickness of regenerated epithelium and vascularity of scar varied slightly, differences observed within each group were greater than differences between groups. (Figs. 7–8) Even in retrospect, BAPN treated animals could not be distinguished from steroid treated dogs. No histologic abnormalities of collagen fibers were observed under the light microscope and the size and type of scar, estimated in Masson trichrome stained preparations, seemed the same in all groups. Two of the BAPN treated animals, however, developed generalized transmural thinning of the esophagus. Both animals had full thickness burns completely healed without stricture.

Discussion

Although methods of preventing esophageal stenosis following acute lye burns have been investigated extensively in animals and man, technics for correcting established strictures have received minimal study. In acute burns, steroids alone, BAPN alone, or steroids plus bougienage reduce stricture formation significantly.[5,7,15] Bougienage alone, however, does not prevent the development of esophageal stenosis.[8,15] Nevertheless, despite the inadequacies of bougienage, clinicians must rely on chronic mechanical dilatation once a fibrous stricture is established. If the esophagus receives a superficial injury and strictures are mild, bougienage provides lasting benefits. However, in severe penetrating injuries with extensive scarring and tight strictures, bougienage fails frequently.[6] Even with the addition of local steroid injections, chronic bougienage may not provide an adequate passage for food.[12]

In our canine model, dilating established strictures with the addition of systemic steroids failed to provide normal esophageal size or to restore normal eating patterns. Following each dilatation, steroid treated dogs seemed to swallow a soft solid diet more easily and to regurgitate less frequently. Improvement, however, was temporary. Within a few days, swallowing problems recurred. Mean esophageal diameters of the steroid plus bougienage group were slightly larger than the controls. Although statistically insignificant, this



FIGS. 3–6. Barium esophagograms of the dog who responded *least well* to BAPN therapy. (3) Prior to burning. (4) At 4 weeks, just prior to beginning treatment. (5) After 6 weeks of BAPN and bougienage treatment. Esophageal diameter is 3 times the pretreatment measurement. (6) After seven months of observation. Esophageal diameter is 4 times the pretreatment measurement. Despite the mild stenosis, the dog ate normally.

small difference may account for improved clinical behavior.

In contrast, BAPN plus mechanical bougienage reversed established esophageal stenosis, restoring normal esophageal patency and adequate physiological function. More important, once stenosis had been corrected, strictures did not recur in BAPN treated dogs. By the end of the treatment interval, animals ate without regurgitation and, by 18 weeks, feeding patterns in all dogs seemed entirely normal.

Because of our limited knowledge of how visceral wounds heal and how mechanical dilatation affects morphology and kinetic chemistry of scars, the mechanism by which lysyl oxidase inhibition plus bougienage reverses fibrous stricture is not clear. Data available from studies of cutaneous wound healing, however, provide at least two hypotheses. Despite their histologic appearance, dermal wounds remain metabolically active for prolonged periods.[20] New scar collagen is deposited and older material is removed at a rapid rate for many months. If the metabolic turnover of esophageal scar collagen remains elevated during the period of BAPN administration, newly deposited collagen would be lathyritic. Because lathyritic collagen lacks the great tensile strength of normally cross bonded fibers, mechanical dilatation could cause slippage of individual fiber bundles or rupture of non-covalent intermolecular bonds without creating a physical discontinuity in the scar. This hypothesis suggests that as esophageal scar collagen undergoes rapid metabolic turnover in the presence of a lysyl oxidase inhibitor, rigid esophageal scar becomes pliant and mechanical dilatation stretches the scar without creating new wounds. However, if rate of collagen turnover is not rapid enough to produce a lathyritic scar quickly, bougienage could rupture the stenotic segment creating a new esophageal wound. As the wound heals under the influence of BAPN, newly deposited collagen would be lathyritic. Subsequent mechanical dilatation could stretch or slip lathyritic fibers maintaining esophageal size without additional injury. Data from acute esophageal lye burn experiments suggest that in the presence of lathyritic scars even swallowing a soft solid diet dilates the esophagus and maintains patency.[5] In addition, although lathyrism has no affect on the contraction of cutaneous wounds, careful examination of esophagi from acute experiments indicates that lysyl oxidase inhibition may prevent wound contraction following esophageal injury.[2] Thus, a second hypothesis suggests that dilating a fixed fibrous stricture creates new wounds and, under the influence of BAPN, new scar formed remains pliable and easily stretched.

Both of these hypotheses are testable and point out the urgent need for careful study of biochemistry and mechanics of visceral wound healing. Preliminary experiments have demonstrated that visceral and cutaneous wounds differ significantly in rate of strength gain and in biochemical kinetics.[11] Currently, there are no quantitative data on visceral wound contraction.

Esophageal architecture differs from species to species. Several authors have stressed the differences between canine and human esophageal anatomy and physiology.[8] The canine model used in these experiments does not represent human esophageal stenosis. Our data demonstrate, however, that fibrous esopha-

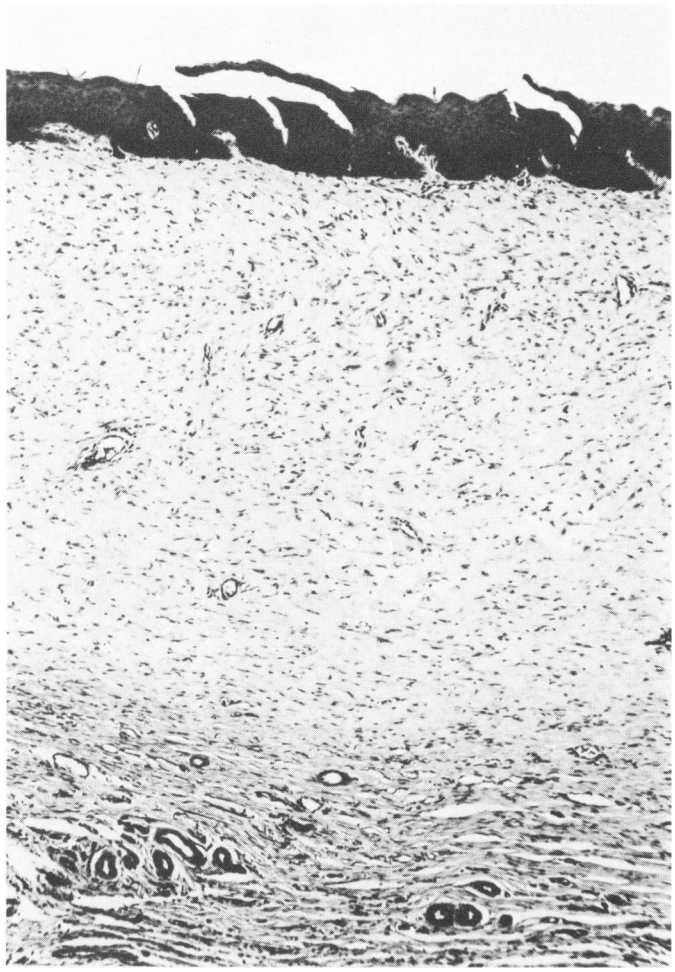


FIG. 7. Photomicrograph of esophagus from a steroid plus bougienage-treated dog 30 weeks post-burning. Dense scar tissue has replaced the entire esophageal wall including the muscularis. (Mallory's Trichrome, 105x magnification)

geal strictures created in the dog using these technics are stable over long time intervals and produce permanent physiological impairment.[1] Occasionally, we have noted a discrepancy between the functional diameter of the esophagus as measured by standard barium esophagograms and esophageal diameter measured in fixed postmortem specimens. Because of our interest in the effects of treatment on pathophysiology, we have stressed the functional rather than the fixed anatomical measurements.

Transmural thinning of the esophagus noted in two of our BAPN treated dogs is curious. This phenomenon, although unexplained, is not limited to BAPN treated animals. Control dogs, without treatment of any kind, have developed transmural thinning as early as 4 weeks following burning.[2] In addition, Rosenberg *et al*, noted esophageal thinning in rabbits treated with steroids following esophageal lye burns.[26] Generalized thinning may be due to rapid tissue remodeling secondary to the burn itself and deserves further study.

Data presented in this paper establish the principle that pathophysiology created by inappropriate fibrous tissue deposition can be altered significantly by interfering with normal scar collagen metabolsim. BAPN is only one example of several anticollagenous agents with clinical potential. Inhibiting collagen synthesis, either by chelating metals functioning as cofactors for proline hydroxylation or by introducing proline analogues incapable of hydroxylation, is an attractive area for study. Unfortunately, data on the *in vivo* effects of these agents are contradictory.[3,16,19,29] As yet, data demonstrating significant effects on wound healing by specifically inhibiting collagen synthesis *in vivo* are not available. Currently, agents specifically interfering with normal collagen maturation seem to be the most promising. In addition to BAPN and other lysyl oxidase inhibitors, d-penicillamine, an agent chelating lysine-derived aldehydes, produces lathyrism in animals and

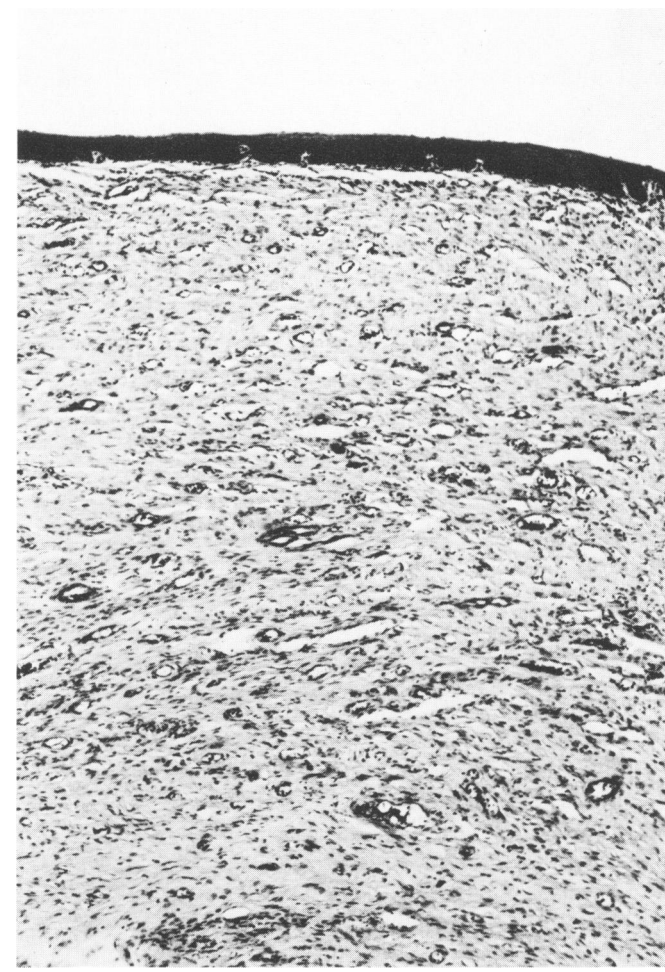


FIG. 8. Photomicrograph of esophagus from a BAPN plus bougienage-treated dog 30 weeks post-burning. The dense scar tissue replacing the entire wall is more vascular and slightly more cellular than specimen shown in Figure 7. Differences of even greater magnitude, however, occurred within groups. (Mallory's Trichrome, 105x magnification)

man.[10,22] Because of the potential clinical usefulness of these agents, further study in man seems warranted. Under FDA supervision, double-blind controlled studies of the effects of BAPN and d-penicillamine on pathophysiology of human diseases are in progress.

Summary

1. Instillation of 20% sodium hydroxide under carefully controlled conditions creates a standard injury of the mucosal, submucosal, and muscular layers of the canine esophagus. Healing, over a 4-week period, produces a fixed, fibrous stricture which results in severe dysphagia and significant inanition.

2. Treating 4-week old established strictures for 6 weeks with weekly bougienage and daily administration of Prednisolone improved the ability of dogs to swallow, but did not produce a significant increase in esophageal diameter nor restore normal eating patterns. After discontinuing therapy, neither clinical behavior nor esophageal diameters improved during a 20-week post-treatment observation period.

3. Treating 4-week old fibrous strictures for 6 weeks with weekly bougienage and daily administration of BAPN (a powerful lysyl oxidase inhibitor) produced a significant increase in esophageal diameter ($p < 0.02$) and restored normal deglutition. After discontinuing therapy, both the nutritional status of the animals and esophageal diameters continued to improve during a 20-week post-treatment observation period.

4. BAPN inhibits the formation of intermolecular covalent bonds in newly synthesized collagen. Substitution of collagen with a low cross bonding density for normal collagen in metabolically active scar tissue may be responsible for the significant differences in esophageal diameter and clinical behavior observed between steroid and BAPN groups.

References

1. Butler, C. II, Madden, J. W., Davis, W. M. and Peacock, E. E., Jr.: Morphologic Aspects of Experimental Esophageal Lye Stricture:1. Pathogenesis and Pathophysiologic Correlations. Proc. Soc. Exper. Bio. Med. (in press).
2. Butler, C. II, Madden, J. W., Davis, W. M. and Peacock, E. E., Jr.: Morphologic Aspects of Experimental Esophageal Lye Stricture:2. Effects of Steroid Hormones, Bougienage and Induced Lathyrisim on Acute Lye Burns. Proc. Soc. Exper. Biol. Med. (in press).
3. Chvapil, M., Madden, J. W., Carlson, E. C. and Peacock, E. E., Jr.: Effects of cis-Hydroxyproline on Collagen and Other Metabolites in Skin Wounds, Granuloma Tissue, and Liver of Mice and Rats. (in press).
4. Craver, J. M., Madden, J. W. and Peacock, E. E., Jr.: Biological Control of Physical Properties of Tendon Adhesions. Effect of Beta-aminopropionitrile in Chickens. Ann. Surg., **167**:697, 1968.
5. Davis, W. M., Madden, J. W. and Peacock, E. E., Jr.: A New Approach to the Control of Esophageal Stenosis. Ann. Surg., **176**:469, 1972.
6. Fatti, L., Marchand, P. and Crawshaw, G. R.: The Treatment of Caustic Strictures of the Esophagus. Surg. Obstet. Gynecol., **102**:195, 1956.
7. Haller, J. A., Jr., Andrews, H. G., White, J. J., Tamer, M. A. and Cleveland, W. W.: Pathophysiology and Management of Acute Corrosive Burns of the Esophagus: Results of Treatment in 285 Children. J. Ped. Surg., **6**:578, 1971.
8. Haller, J. A., Jr. and Bachman, B. A.: The Comparative Effect of Current Therapy on Experimental Caustic Burns of the Esophagus. Pediatrics, **34**:236, 1964.
9. Haney, A., Peacock, E. E., Jr., Madden, J. W.: Liver Regeneration and Hepatic Collagen Deposition in Rats With Dimethylnitrosamine–Induced Cirrhosis. Ann. Surg., **175**: 863, 1972.
10. Harris, E. D., Jr. and Sjoerdsma, A.: Effect of Penicillamine on Human Collagen and its Possible Application to Treatment of Scleroderma. Lancet, **ii**:996, 1966.
11. Hastings, J. C. and Van Winkle, W., Jr.: Organ Differences in Post-operative Healing. Surg. Forum, **23**:29, 1972.
12. Holder, T. M., Ashcraft, K. W. and Leape, L.: The Treatment of Patients with Esophageal Strictures by Local Steroid Injections. J. Ped. Surg., **4**:646, 1969.
13. Imre, J. and Kopp, M.: Arguments Against Long-term Conservative Treatment of Oesophageal Strictures Due to Corrosive Burns. Thorax, **27**:594, 1972.
14. Keiser, H. and Sjoerdsma, A.: Studies on Beta-aminopropionitrile in Patients with Scleroderma. Clin. Pharmacol. Ther., **8**:593, 1967.
15. Knox, W. G., Scott, J. R., Zintel, H. A., Guthrie, R. and McCabe, R. E.: Bougienage and Steroids Used Singly or in Combination in Experimental Corrosive Esophagitis. Ann. Surg., **166**:930, 1967.
16. Lane, J. M., Bora, F. W., Jr., Prockop, D. J., Heppenstall, R. B. and Black, J.: Inhibition of Scar Formation by the Proline Analog cis-Hydroxyproline. J. Surg. Res., **135**: 137, 1972.
17. Leape, L. L., Ashcraft, K. W., Scarpelli, D. G., Holder, T. M.: Hazard to Health-Liquid Lye. N. Engl. J. Med., **284**:578, 1971.
18. Madden, J. W.: Wound Healing: Biologic and Clinical Features, a chapter *in* Christophers Textbook of Surgery, Sabiston, D. ed., Philadelphia, Saunders, 1972.
19. Madden, J. W., Chvapil, M., Carlson, E. C. and Ryan, J. N.: Toxicity and Metabolic Effects of 3,4–Dehydroproline in Mice. Toxical. Appl. Pharmacol. (in press).
20. Madden, J. W. and Peacock, E. E., Jr.: Studies on the Biology of Collagen During Wound Healing, III. Dynamic Metabolism of Scar Collagen and Remodeling of Dermal Wounds. Ann. Surg., **174**:511, 1971.
21. Marchand, P.: Caustic Strictures of the Oesophagus. Thorax, **10**:171, 1955.
22. Nimni, M. E., Deshmukh, K., Gerth, N. and Bavetta, L. A.: Changes in Collagen Metabolism Associated with the Administration of Penicillamine and Various Amino and Thiol Compounds. Biochem. Pharmacol., **18**:707, 1969.
23. Peacock, E. E., Jr. and Madden, J. W.: Some Studies on the Effect of Beta-aminopropionitrile on Collagen in Healing Wounds. Surgery, **60**:7, 1966.
24. Peacock, E. E., Jr. and Madden, J. W.: Some Studies on the Effects of Beta-aminopropionitrile in Patients with Injured Flexor Tendons. Surgery, **66**:215, 1969.
25. Piez, K. A.: Cross Linking of Collagen and Elastin. Ann. Review Biochem., **36**:547, 1968.
26. Rosenberg, N., Kunderman, P. J., Vroman, L. and Moolten, S. E.: Prevention of Experimental Lye Strictures of the

Esophagus by Cortisone. Arch. Surg., 63:147, 1951.
27. Siegel, R. C., Pinnell, S. R., Martin, G. R.: Cross-Linking of Collagen and Elastin. Properties of Lysyl Oxidase. Biochem., 9:4486, 1970.
28. Tanzer, M. L. and Gross, J.: Collagen Metabolism in the Normal and Lathyritic Chick. J. Exper. Med., 119:275, 1964.
29. Whitson, T. C. and Peacock, E. E., Jr.: Effect of Alpha Alpha Dipyridyl on Collagen Synthesis in Healing Wounds. Surg. Gynec. Obstet., 128:1061, 1969.

Discussion

Dr. Harry H. LeVeen (Brooklyn): The results presented by the authors do confirm the value of lathrogenic drugs in the treatment of disabling strictures of the esophagus.

A year ago when a similar paper was presented to the society on this topic, I asked the authors why they had chosen BAPN rather than B-penicillamine. By this I meant to suggest that penicillamine would be a better choice than BAPN. At this time again I would like to put forth the suggestion with greater force.

We, like many others, have been studying the effects of penicillamine on collagen synthesis, especially in wound healing. Penicillamine has the advantage that it has already been used in humans, mainly for Wilson's disease, rheumatoid arthritis and cystine stones, but it has also been used in human diseases characterized by excessive fibrosis. Some patients have been treated with penicillamine for 5 or 10 years without adverse effects. Adverse effects which do occur are known and reversible. Penicillamine increases soluble collagen in tissues at the expense of insoluble collagen. BAPN is much less active in this regard. Dasler in 1961 showed that BAPN reduced the percentage of insoluble collagen and increased the percentage of soluble collagen in subcutaneously implanted vinyl sponges. (Metabolism 110:883, 1961) Nimni and Bavetta repeated this work using penicillamine instead of BAPN, the results were far greater than had been observed with BAPN. (Science 150:905, 1965) In Copenhagen, a surgeon has used penicillamine clinically to prevent keloid formation in susceptible patients. Because penicillamine therapy increases soluble collagen at the expense of insoluble collagen, Harris has even suggested its use in scleroderma, a disease where esophageal stenosis is often observed. Penicillamine has been suggested for use in cirrhosis and pulmonary fibrosis. Penicillamine would be more effective in the treatment of esophageal stricture and because so much more is known about its use, it could be used in human cases directly without further animal experimentation.

Dr. Stanley M. Levenson (Bronx): I would just like to comment on a point that Dr. Madden raised concerning a possible mechanism underlining the finding he has just reported and to recall to you that this really should come as no surprise to any of us.

Lind, more than 200 years ago, reported that when sailors become scorbutic, wounds they had received years before and had long since healed broke down without further trauma, the inference being of course that the wound was still an active, dynamic part of the body quite different from the ordinary uninjured connective tissue, even after many years.

Experimentally, that fact was confirmed in the laboratory about 25 or 30 years ago by a type of experiment analogous to Dr. Madden's. Laparatomy wounds were made in guinea pigs which were maintained on a normal intake of ascorbic acid (Proc. Soc. Exptl. Biol. Med. 82:95, 1953). After about 6 weeks the guinea pigs were placed on a vitamin C deficient diet. The laparatomy wounds, which had healed normally for the first 6 weeks weakened and some became hernias; microscopically one could see the collagen had disappeared.

I think what is happening in Dr. Madden's study is that when BAPN is begun 1 month after the insult, the scar tissue is turning over very dynamically, as Dr. Madden indicated when he said "this fresh scar tissue." As the collagen is reabsorbed, new collagen is laid down which is now under the influence of the BAPN. Now we have a situation which is analogous to that which Madden and Peacock reported last year, namely, that as collagen is laid down the BAPN will interfere with its cross-linking.

This is a very important observation and I would agree that it does have potential for many situations; materials such as penicillamine or other cross-linking inhibitors less toxic than BAPN may prove even more useful.

Dr. J. W. Madden (Closing): As far as Dr. LeVeen's specific questions are concerned, we have two double-blind controlled experiments in progress designed to see whether we can influence the patho-physiology of fibrotic disease in man using d-penicillamine.

The problem with using d-penicillamine in experimental animals is its expense. If we can demonstrate an effect with BAPN, experiments with d-penicillamine are then justified.

Currently, we are using d-penicillamine in an attempt to reverse chronic esophageal stenosis in this canine model.

The second point Dr. LeVeen made concerned the use of d-penicillamine in scleroderma. I believe he may have misinterpreted Harris's paper. Actually, there have been three other publications since Harris' initial work on d-penicillamine in scleroderma and most of them have indicated, in fact all of them indicate, that d-penicillamine has no usefulness in scleroderma.

There are specific criteria one can set down that human diseases must meet before anti-collagenous agents could be considered and scleroderma does not fit them.

As far as the increase in saline extractable collagen that d-penicillamine produces, d-penicillamine does seem to take apart Shiffs-base bonds, (the acid soluble collagen bonds) where BAPN does not.

Our experience with d-penicillamine in man indicates that in the doses the FDA allows us to give, it is a much less effective lathrogenic agent than BAPN

As far as Dr. Levenson's questions are concerned, we discuss in the manuscript in some detail the various biological possibilities of how BAPN might work.

Rapid turnover of scar collagen in the esophagus is one possibility, but Dr. Levenson, there is another equal possibility. If scar collagen does not turn over rapidly in the esophagus, then the initial bougienage could rupture the esophageal scar and create a new wound. Then you have rapid collagen synthesis directly under the influence of a lathrogenic agent. The new collagen synthesized would be very weak and have very little tensile strength. I believe that answers both questions.