Biological Control of Physical Properties of Tendon Adhesions:

Effect of Beta-Aminopropionitrile in Chickens

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In the past, the surgical approach to complete elimination of circumferential scar around tendon repairs has been almost brutally mechanical in concept and design.[1, 3, 6] Such an approach to control of scar tissue has revealed, however, that successful isolation of repaired tendons invariably results in failure of the tendon anastomosis to heal and in necrosis of the tendon on either side of the anastomosis.[11] It seems, therefore, that if the mechanical approach to restoration of gliding function and repaired tendons is to be successful, it must somehow take into consideration certain biological aspects of healing. One such aspect is the need for cell migration and nutrition in the anastomotic area while the rest of the tendon is shielded from the surrounding healing process. That such biological considerations can be met within the mechanical shield approach has been demonstrated by successfully allografting the entire flexor mechanism in over 20 human hands.[4, 7, 15] Although experience with composite tissue allografts has been valuable in understanding the fundamental biology of tendon healing and gliding function, and has provided useful digital flexion for some 20-odd patients with otherwise hopeless flexor tendon injuries, it is not a practical solution to the problem of the restrictive scar tissue which invariably surrounds the vast majority of injured and repaired long flexor tendons.

Another approach has been to ignore synthesis and location of postoperative scar while concentrating instead on the possibility of controlling physical properties of new fibrous tissue. After all, it is not synthesis of fibrous tissue which is detrimental to restoration of gliding function; it is the physical arrangement and internal cross-linking of collagen subunits that determines how effective adhesions will be in resisting longitudinal force. Thus the amount of scar tissue surrounding a tendon might not be of serious consequence if the internal and external arrangement of the various subunits were such that little or no resistance to external force was present. That the internal arrangement of collagen subunits in newly synthesized scar tissue can be manipulated to produce clinically significant alterations in physical properties of scar tissue seems possible in the light of recent investigations into the nature of chemical bonds which link collagen subunits together.[12, 13] This paper is a report of studies designed to determine if such alterations in inter- and intramolecular bonding can be produced without damage to other tissues, and if the effect of doing so would be clinically significant in restoring longitudinal motion to damaged flexor tendons.

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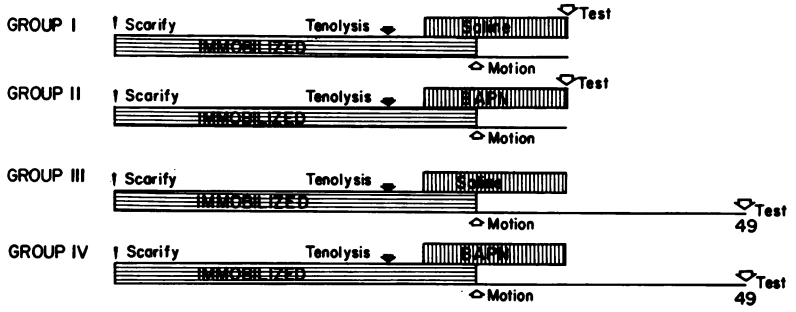


TABLE 1. *Summary of Experimental Design*

A class of substances (of which aminonitriles are the most potent) known as lathyrogens interfere with formation of aldehyde groups needed to form inter- and intramolecular cross links in collagen.[2] Administration of this substance to animals results in production of collagenous tissue which, among other characteristics, has very little physical strength. Lathyritic collagen in the aorta causes dissecting aneurysms; in long bones it produces osteolathyrism with scoliosis and other grotesque deformities; and in healing wounds it may be responsible for dehiscence following mechancial stress.

Although the evidence still is not conclusive, present data strongly suggest that the effect of lathyrogenic agents is to prevent —not destroy—inter- and intramolecular bonds.[17, 18] Thus Beta-aminopropionitrile acts predominantly on newly synthesized collagen. This is extremely important in the consideration of these agents for clinical use. It provides a sound basis for hypothesizing that newly synthesized collagen, such as that found in a healing wound, would be more sensitive to lathyrogens than mature, stable collagen in tissues such as aorta, bone, or skin. Thus it has seemed reasonable to search for an agent, dose, and route of administration which would selectively interfere with inter- and intramolecular bonding of newly synthesized scar tissue without affecting significantly mature collagen in other structures.

Review of what presently is known about various lathyrogenic agents indicates that Beta-aminopropionitrile (the most powerful known lathyrogen) should be investigated for its potential use in controlling scar tissue in human beings. In addition to being the most powerful known lathyrogen, Beta-aminopropionitrile also is the agent which has been used most frequently in laboratory animals and has the least number of general toxic side effects. We have found Beta-aminopropionitrile to be effective in altering the physical properties of scar tissue in healing incised and sutured wounds and around immobilized joints in rats.[5, 14] These data suggest that Beta-aminopropionitrile can be utilized safely for short peroids to control surgical scar tissue around tendons without interfering significantly with collagen metabolism in mature tissues.

Materials and Methods

Forty chickens of the Van Trees Cross strain approximately 12 weeks old and weighing 2 Kg. each were used in these experiments. As pointed out by Lindsay, the arrangement of flexor tendons, digital sheath, etc. in the long central digit of a chicken's foot is similar to that in a human hand—presence of a third long tendon corresponding to the interosseus muscle in human beings being the main difference.[9] The small size of tendons and sheath in younger chickens and calcification of soft

tissue in older ones makes both undesirable for tendon healing experiments. Chickens were fed commercial poultry mash, supplemented with terramycin (½ pound/400 pounds of feed). It is important that food and water be placed low enough on the cages so that chickens can eat and drink without having to stand erect; chickens with one or both feet in a plaster cast do not stand well to eat or drink. In addition, chickens with Beta-aminopropionitrile toxicity were often not strong enough to stand while eating and drinking; malnutrition and dehydration were responsible for the death of several chickens.

Surgical procedures were performed with aseptic technic and tourniquet hemostasis. Anesthesia consisted of intramuscular Nembutal (25 mg. administered 40 minutes prior to operation) and 1% Xylocaine injected just above the knee. Through a midlateral digital incision, a plantar skin flap was developed to expose the flexor sheath and fibrous retinaculum in the central digit. Sheath over a 3 cm. distance and sublimis tendon were excised. The vincula of the profundus tendon were not disturbed. A #4-0 braided steel suture was passed several times through the profundus tendon along a 3 cm. distance over the proximal and middle phalanges. See Figures 1 and 2. The wound was closed with a continuous nylon suture, and the foot was immobilized in flexion in a plaster cast. Three weeks after operation, the cast was removed and the wound was reopened through the previous incision. Tenolysis, consisting of complete excision of the vincula and all scar tissue surrounding the profundus tendon, was performed. Following tenolysis, the wound was closed and the foot was immobilized for 1 week.

Work of flexion was measured in the apparatus depicted in Figure 3. The amputated chicken's foot was mounted on a laboratory screw clamp in front of a plywood base. An incision was made in the proximal flexion crease of the ankle through which the flexor profundus tendon of the central digit was exposed. The tendon was divided and a #26 wire was attached to the distal end. The wire was passed over appropriate pulleys as shown in Figure 3, and a 3-liter receptacle was suspended from the end. Soft tissue was cut away from the opening in the ankle so that a smooth course was provided for tendon and wire.

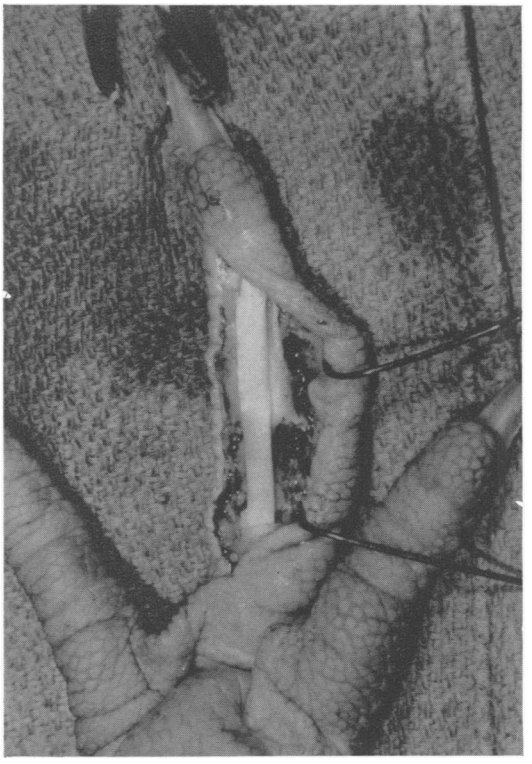
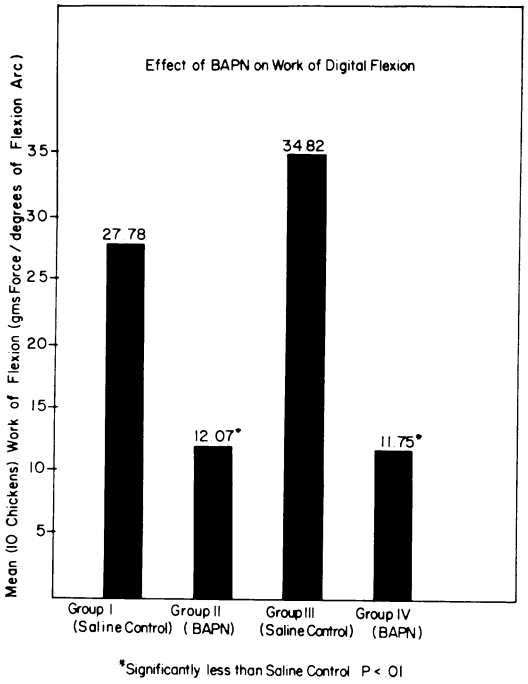


FIG. 1. Exposed flexor profundus tendon in central digit of chicken foot.

Because there were variable degrees of flexion contracture in the interphalangeal joints, a counterweight of 50 Gm. was suspended from the tip of each digit by overhead pulleys as shown in Figure 3. The counterweight assured that measurement of the flexion arc would start with a constant extensor force in all experiments. A yoke was attached to a transverse wire inserted through the distal phalanx to assure

TABLE 2.



that the counterweight would exert a constant force as the digit changed position and direction of movement. A card was mounted behind the foot so that a permanent record of the entire flexion arc could be traced while work of flexion was being measured. Flexion arc is reported as the sum of the number of degrees of flexion obtained in all three joints. Water was allowed to flow at a constant rate of 1,350 cc./min. into the receptacle, but flow was stopped as soon as movement ceased at any point in the flexion arc or when the tip of the digit touched the extremity. Chicken feet were tested in random order, and work of flexion was measured by the same investigator. A code was used to identify the chickens so that the invesitgator would not know whether a chicken had received Beta-aminopropionitrile while the foot was being tested.

Chickens were divided into four groups. See Table 1. Group I chickens were operated upon as described above, had a tenolysis after 3 weeks immobilization, and were tested for work of flexion two weeks after tenolysis. The foot was immobilized for the first week after tenolysis; cage activity was permitted during the last week of the experiment. This group received 3 cc. of saline intramuscularly for 12 days, beginning 3 days after tenolysis, and served as controls for Group II.

Group II chickens were operated upon and treated exactly the same as Group I chickens except that a 300 mg. daily dose of Beta-aminopropionitrile was injected intramuscularly during the last 12 days of the experiment. Beta-aminopropionitrile was started 3 days after tenolysis and was continued for 12 days until the chickens were sacrificed. This experiment was designed to measure the immediate effect of a 12-day course of Beta-aminopropionitrile on work of digital flexion.

Group III chickens were operated upon and treated exactly the same as chickens in Group I except for a 4-week delay between tenolysis and measurement of work of flexion. Immobilization was carried out during the first week after tenolysis only; intramuscular saline injections were started on the third day after tenolysis and were continued for 12 days. During the last 2 weeks of the experiment, chickens in Group III did not receive any medication and were allowed cage activity. The chickens in Group III served as a control for Group IV.

Chickens in Group IV were operated upon and treated exactly the same as chickens in Group III except that a 300 mg. daily dose of Beta-aminopropionitrile was administered for 12 days, beginning on the third day after tenolysis. During the last 2 weeks of the experiment, chickens did not receive Beta-aminopropionitrile and were allowed normal cage activity. This experiment was designed to examine the delayed effect (2 weeks after administration) of Beta-aminopropionitrile on work of digital flexion.

Results

The dose of Beta-aminopropionitrile was derived by preliminary toxicity studies. Fowl are extremely sensitive to lathyrogenic agents, yet they vary considerably in their general response. See Figure 4. An average dose of 300 mg. of Beta-aminopropionitrile administered intramuscularly produced lethargy and muscular weakness; fatal complications could be avoided by titrating the dose to the individual chicken's clinical response. Normal vigor and muscular activity were regained within 48 hours after the dose was reduced or the drug discontinued. A clinical impression was that chickens which developed the most lethargy and weakness showed the most striking lathyrogenic effect in scar tissue. No evidence of a lathyrogenic effect in mature collagenous tissue was observed. An interesting observation was that toxicity to Beta-aminopropionitrile also was manifest in the color of the comb in about half of the birds; transformation from a bright red to a pale pink color often heralded severe lethargy and weakness. Anemia was not responsible for change in color of the comb. Figure 4 demonstrates the generalized effect of Beta-aminopropionitrile in unusually susceptible chickens.

The results from measuring and calculating work of digital flexion in all four groups are depicted in Table 2. Analysis of data from Group I reveals that, 2 weeks following tenolysis, a mean force of 3,133 Gm. was required to produce only 118.1° of mean flexion arc. The mean work of flexion in this group was 27.78.

Analysis of data from Group II reveals that a mean force of 1,737 Gm. was required to flex the digit through a mean arc of 155.5°. The mean work of flexion was 12.07. The difference between BAPN-treated and control chickens was highly significant. $p < 0.01$.

Analysis of data from Group III revealed that, 4 weeks following tenolysis, a mean force of 2,950 Gm. was required to produce only 86.8° of mean flexion arc. The mean work of flexion in this group was 34.82. The relatively large amount of work required to produce a small flexion arc strongly suggests that the secondary healing process results in an increased rate of change in tendon scar 4 weeks after a tenolysis procedure.

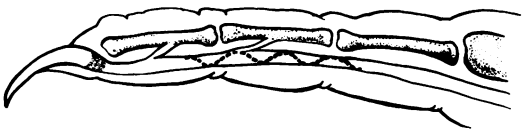


FIG. 2. Location of braided wire suture in flexor profundus tendon of chicken digit.

Analysis of data from Group IV revealed that, 2 weeks after Beta-aminopropionitrile was discontinued, a mean force of 1,714 Gm. was required to produce a mean flexion arc of 142°. The mean work of flexion in these chickens was 11.75. The difference between BAPN-treated and control chickens in this experiment was highly significant. $p < 0.01$.

Dissection of the chicken feet revealed that the testing process had not resulted in dehiscence of fibrous adhesions to any great degree. Less than 300 Gm. difference between the results of the first and subsequent measurements of force required to flex the digits indicated that stretching and change of direction of adhesions were more responsible for longitudinal tendon motion than was frank dehiscence of scar tissue.

There was a striking difference between the physical characteristics of the scar tissue in saline controls and BAPN-treated chickens. Scar tissue surrounding tendons in BAPN-treated animals was gelatinous in consistency and hemorrhagic in color. The collagenous tissue literally could be wiped out of the flexor tendon canal in the feet of some chickens; the hemorrhagic color was produced by hemorrhage secondary to ruptured blood vessels caused by unusual motion of the tendon without fibrous tissue restraint.

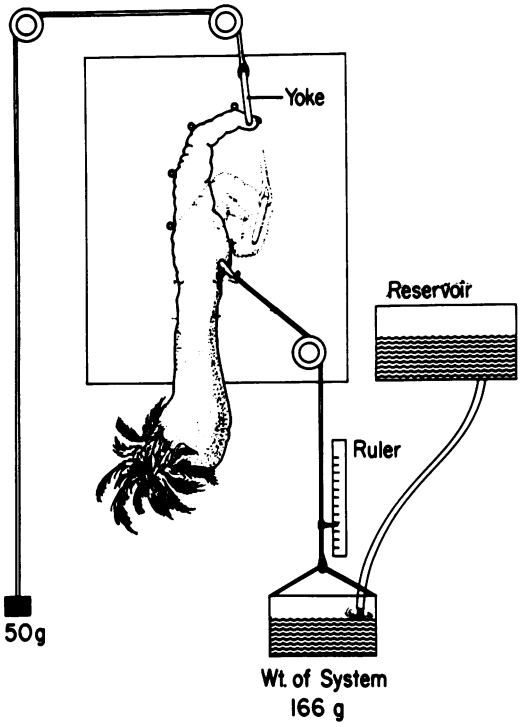


FIG. 3. Diagram of apparatus designed to measure work required to flex digit.

Discussion

In addition to the special suitability of the long central digit for studying tendon healing, chickens were chosen for these experiments because of their known susceptibility to aminonitriles. Most fowl have unusually high blood pressure and apparently have an inherently weak aorta. Aneurysms are common in fowl, and rupture of the aorta is a frequent complication of aminonitrile poisoning.[17] Because Beta-aminopropionitrile presently is believed to act only in preventing (not destroying) inter- and intramolecular cross-links, the supposition has been that the turnover of collagen in a young fowl aorta is relatively rapid. Thus, as normally cross-linked collagen is replaced with lathyritic collagen, the structures become gradually weaker until dissection or blowout occurs. Although the same type of process undoubtedly produces abdominal hernias, separation of tendons from bones, scoliosis, etc. in other laboratory animals, the time required for lathyritic deformities to occur is much longer in rodents and other laboratory animals than in the aorta of fowls. Thus chickens appeared ideal to test the relative sensitivity of wound tissue to Beta-aminopropionitrile.

The basis for any hope that the lathyrogenic phenomenon might be useful in controlling scar tissue in human beings is the fact that the healing wound contains more susceptible collagen than other fibrous tissue. Because the turnover rate of collagen in the healing wound is so much more rapid (as evidenced by proline uptake studies and collagenolytic enzyme activity) than non-wounded tissue, the hope that wound collagen could be made selectively lathyritic without significantly affecting the physical characteristics of unwounded collagen apparently was realistic.[10, 16] Although toxicity to Beta-aminopropionitrile as represented by lethargy, muscle weakness, and change in comb color was fairly prominent in some birds, a dose of Beta-aminopropionitrile was found which produced a marked lathyrogenic effect in newly synthesized scar tissue around tendons without producing a serious deformity in mature tissue. Moreover, systemic effects such as lethargy and muscle weakness could be reversed by reducing the dose of the drug.

The soft tissue wounds of lathyritic chickens did not dehisce as long as sutures were intact. The cellular, vascular, and globular protein contributions to healing apparently were not affected by Beta-aminopropionitrile. These aspects of the healing process (completely independent of the collagen system) provide approximately 120 Gm. of strength to a 1 cm. section of rat or guinea pig skin wound. When sutures were removed from the chicken's feet, the wounds in the digit could be disrupted easily by applying horizontal tension.

An experimental model planned around tenolysis rather than a free tendon graft or repair of a divided tendon avoids the possibility that a lathyrogenic effect in the scar between tendon ends would cause dehiscence of the anastomosis. Selection of such a model does not mean, however, that application of the lathyrogenic principle to tendon repair is not realistic. Actually, the suture material in a tendon anastomosis imparts a significant amount of tensile strength for a longer period of time than is generally realized. At 21 days (the time when motion usually is started in human hands) most of the tensile strength of the tendon anastomosis is provided by suture material. Thus the main reason for immobilizing a repaired tendon for 21 days is not so that strong fibrous union between coapted ends will occur before motion is started but to permit adequate blood supply to develop so that the materials for repair and sustenance can be delivered to the injured area. It seems likely, therefore, that a high friction anastomosis such as end-weave or other type of splice complemented by permanent sutures would not require a mature fibrous scar to provide structural integrity when motion is attempted. In human hands, such an anastomosis has been performed routinely and, at a time when experiments on early motion were being conducted, relatively large physical stress could be placed across the anastomoses without dehiscence during the first 21 days. An end-to-end anastomosis probably would not be secure in a lathyritic individual, however, as gap formation between tendon ends following end-to-end anastomosis is common. Because we were interested primarily in studying circumferential scar around damaged tendon, and because a chicken's foot is not suited for an end-weave type of anastomosis, an incontinuity injury followed by secondary tenolysis was selected to test the possibility of utilizing the lathyrogenic phenomenon to improve gliding function.

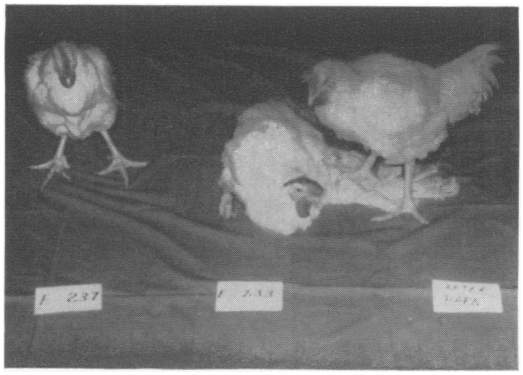


FIG. 4. Variation in general response of chickens to administration of Beta-aminopropionitrile. All three chickens received the same dose of Beta-aminopropionitrile. Chicken No. E237 apparently was unaffected; chicken No. E233 shows marked lethargy and weakness; the third chicken showed similar lethargy to chicken No. E233 but has recovered completely 8 hours following cessation of the drug.

Previous attempts to influence collagen metabolism with lathyrogenic agents in chronic conditions in human beings such as scleroderma and Wilson's disease have provided valuable information.[8, 19] These conditions are characterized by abnormal amounts of fairly mature collagen, however, and the lathyrogenic principle cannot be expected to exert as dramatic an influence as it might in conditions where rapid synthesis and assembly of new connective tissue is occurring. The healing wound is such a condition, in that for a short period of time biologic activity is near embryonic from the standpoint of kinetics of protein synthesis. Thus the surgeon, with his opportunity to influence early healing in acute wounds, may have the best possible model to test the lathyrogenic phenomenon in human beings. Control of abdominal adhesions, pericardial fibrosis, hepatic cirrhosis, tubular organ stenosis, etc. are but a few of the many surgical conditions and complications where short-term control of the physical properties of newly synthesized collagen could exert a significant influence. Data obtained so far on the chicken tendon model supports the hypothesis that specialized surgical wounds are a fertile

area to look for new applications of an old phenomenon—lathyrism.

Summary and Conclusions

1. The immediate and delayed effects of Beta-aminopropionitrile on the physical properties of tendon adhesions have been determined in chicken feet.

2. Beta-aminopropionitrile produced lethargy and weakness in some chickens but did not produce fatal complications.

3. Immediately following cessation of Beta-aminopropionitrile, significantly less work (Gm. of force/degrees of flexion arc) was required to produce longitudinal tendon motion. $p < 0.01$.

4. Two weeks following administration of Beta-aminopropionitrile, work required to develop digital flexion was still significantly less than in untreated chickens. $p < 0.01$.

5. Examination of the flexor mechanism by gross and histological means reveal that Beta-aminopropionitrile-treated tendons were surrounded by copious amounts of scar tissue but the physical characteristics of the collagen were altered significantly.

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