THE USE OF HYALURONIDASE BY IONTOPHORESIS IN THE TREATMENT OF GENERALIZED SCLERODERMA*

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This is a preliminary report on the use of hyaluronidase in the treatment of generalized scleroderma. The hyaluronidase was applied through iontophoresis. This method of administration was selected as large areas of the body surface could be treated without pain or discomfort.

Hyaluronidase (1, 2) is an enzyme prepared from bovine testes and other sources which has the property of hydrolyzing hyaluronic acid. Hyaluronic acid has a role in the formation of collagen. Its solutions are highly viscous. It exists as a gel in many of the body tissues where it serves as a cement or ground substance between the cells and is a barrier to the diffusion of invasive substances. On hydrolysis of hyaluronic acid by hyaluronidase, there is lessening of the viscidity of the gel and a consequent reduction in the resistance of fluid absorption.

Because of the possible disturbance of the hyaluronidase-hyaluronic acid system in scleroderma, either through a defect or deficiency in hyaluronidase production or an excess of anti-hyaluronidase substance or of hyaluronic acid, the following studies were undertaken.

METHOD AND MATERIALS

Two cases of chronic severe generalized scleroderma were treated. As there were no reports in the literature as to the practicability of the application of hyaluronidase by iontophoresis, considerable preliminary investigation was necessary. Repeated studies were made on 6 normal persons and on 2 patients with scleroderma to determine the most satisfactory technic for administration and the effectiveness of transfer of the hyaluronidase from the active electrode into the skin. After these were determined, treatment was instituted. Frequent observations were made during the period of treatment.

Hahn (3) in electrophoretic studies found that hyaluronidase at a pH of 5.2 was positively charged and migrated from the anode to the cathode. Tint (4) in further investigations found a pH of 5.4 more satisfactory for a greater degree of transfer. The following studies were therefore made with the hyaluronidase dissolved in a .1 M acetate buffer solution which had a pH of 5.4. At the onset, .1 mgm. of hyaluronidase (900 Turbidity reducing units per mgm.) was applied. This was later increased to 1 mgm. of the same strength. Two mgm. (1300 TRU/mgm.) was later used in treatment. The strength of the current and duration of application were gradually increased from 3 milliamperes in 5 minutes to 15 milliamperes for 15 minutes. It was found that 15 milliamperes for 15 minutes while satisfactory for the cruder preparations of hyaluronidase created too much local heat and destroyed some of

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the more purified preparations. Seven milliamperes at 10 minutes were determined as the optimum strength and time for treatments. All applications were made on the flexor surfaces of the arms or forearms.

The opposite member was used as the control. No immediate systemic effects were ever noted. The only immediate local effect was redness under the active electrode. This would persist for as long as 2 hours. Occasionally hives similar to those produced on histamine ionization were observed under this electrode. These persisted for about 30 minutes. They differed from histamine in that itching was not a feature. No subjective sensations were ever elicited except occasionally a sensation of warmth at the active electrode during the application of current. This was never great. It was usually present when the current was in excess of 10 milliamperes or over 10 minutes duration. The degree of hyaluronidase penetration was determined by the disappearance time of a .1 cc. normal saline intradermal-subdermal wheal. These were injected in the treated and untreated opposite extremity which was used as a control. Twenty-five gauge needles were used for the injections in all studies. With practice, exactly identical wheals were raised in all individuals. Histamine wheals were also used but had to be discontinued due to the pain created. Evans blue dye was occasionally used.

Initial studies were made with the acetate buffer solution alone at the positive pole with currents ranging up to 10 milliamperes for 10 minutes. It was found that the disappearance rate of the saline wheal on application of current with the buffer solution alone as compared to a control area was similar. With the higher currents, a slight increase in surface temperature was noted. When present, this was confined to a very small area, usually about 2 cm. x 2 cm. The vasodilatation never persisted beyond 30 minutes. Wheals placed in the center of greatest vasodilatation did not show any difference in disappearance time. Disappearance times varied between individuals from 20 minutes to over one hour. They were consistent in each individual.

Increasing concentrations of hyaluronidase were applied in all cases at the onset. Nine hundred turbidity reducing units per mgm. were used in the following pilot studies.

.01 mgm. hyaluronidase, currents varying from 3 milliamperes in 5 minutes to 10 milliamperes at 10 minutes. There was no difference in disappearance time of the saline wheal in the treated or control areas.

.1 mgm. hyaluronidase. There was a slight increase in isolated instances of the disappearance rate in the treated areas.

1 mgm. hyaluronidase. The wheal disappeared in the treated area in every case at a faster rate than the control area.

2 mgm. hyaluronidase. The wheal disappeared in every case in 25% of the time taken in the control area.

In treatment, the initial applications were made with 1 mgm. of 900 turbidity reducing units per mgm. dissolved in the buffer solution. This was later increased to 2 mgm. of the 900 TRU/mgm. and later to 2 mgm. of 1300 TRU/mgm.

The following cases were treated:

Case 1. Female, age 24, generalized scleroderma of 3 years duration, increasing in severity. A total of 40 applications to the forearms were given over a period of 3 months. No untoward reactions were noted. The disappearance rate of intradermal wheals in the treated area and untreated control area was checked every 2 weeks. There was progressive decrease of the disappearance time of the wheal in the treated areas. After a 6 weeks period of treatment, the control area also showed a decrease in disappearance time but not as great as the treated area. The skin throughout the body became softer. The waxy appearance decreased. The sensitivity to cold decreased. This was the first time since the onset of the scleroderma that the patient went through a winter without pain, stiffness, limitation of motion, Raynaud's syndrome or malaise. The sclerodactylia bilaterally and tightness in the treated forearm were especially benefited. Although the greatest objective improvement was in the treated area, subjective improvement was generalized. One tenth cc. intradermal .4%

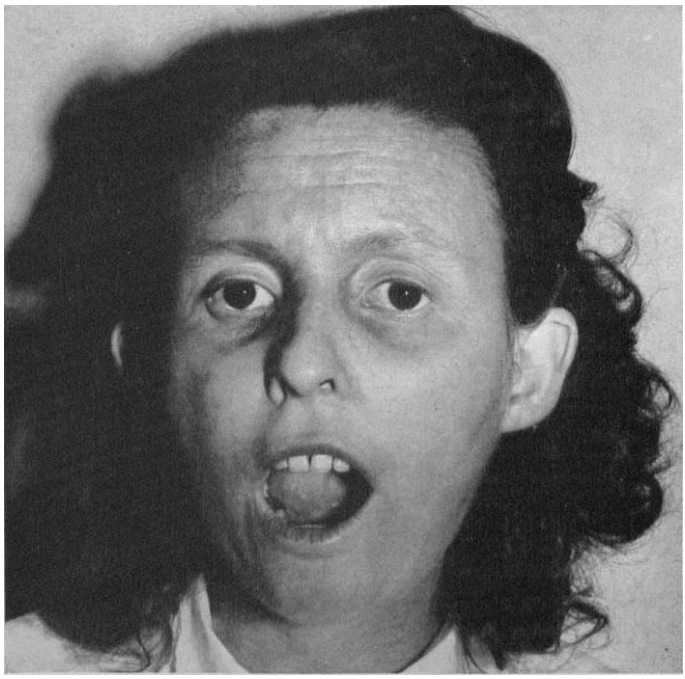

FIG. 1. Before treatment

Evans blue was injected into the treated and control area after 12 applications and after 20 applications. In each instance, the area of dispersion of the dye was significantly greater in the treated area. The area of dispersion was 30% greater in the treated area at the first check. The second injection of Evans blue showed a 50% greater dispersion in the treated area as compared to the control area.

No further treatments were given for 9 months. As the mouth became constricted interfering with nutrition and dental care, hyaluronidase was applied to the cheeks and about the mouth. Alternate sides were treated at weekly intervals. At the onset, the maximum the teeth could be separated on opening the mouth was 2.0 cms. and the lips 3.0 cms. After a total of 18 applications given over a period of 4 months, the teeth could be separated by 2.3 cms. and the lips 4.5 cms. (Figs. 1–2). The sunken cheeks became full and normal facial expression returned. The fluid content of the treated tissues appeared increased. The local

improvement has persisted for 8 weeks following cessation of therapy. However, the scleroderma process appeared to be progressing in the pulmonary tissues as revealed by x-rays and by subjective symptoms.

Case 2. Female, age 57, severe generalized scleroderma of 15 years duration with multiple ulcerations and flexion contractures of all the fingers. This patient also showed during her course of treatment, a remarkable increase in the resistance to cold and increased general well being. During the winter of administration of hyaluronidase, she was free of all the uncomfortable symptoms of stiffness and Raynaud's syndrome that were previously present. Softness of the skin and normal color returned. Considerable subjective relief has been maintained for 9 months following cessation of therapy. She received 33 treatments over a period of 3 months.

In each case, complete blood studies, urinalysis and x-ray studies of the hands and fingers during the period of observation produced no significant changes.

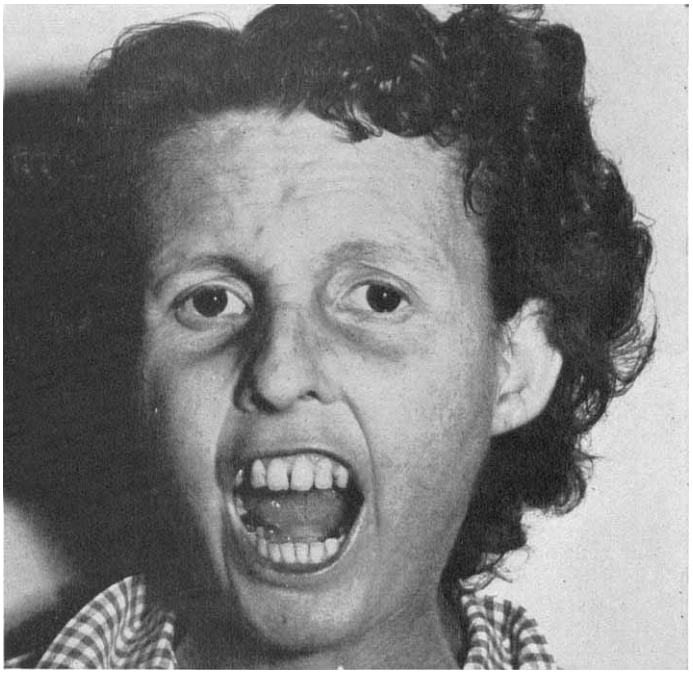

FIG. 2. After 18 local applications of hyaluronidase

It was noted that towards the end of the original 3 months treatment, the effectiveness of the hyaluronidase appeared to decrease in each case. Rotation of application sites corrected this.

Within one week's cessation of treatment to the forearm, there was a partial return of sensitivity to cold, malaise and of the saline wheal disappearance time to that observed at the onset of treatment. The softness of the skin with the apparent increase in fluid content persisted for several months. In neither case did the Raynaud's syndrome return in the original severity.

DISCUSSION

The interpretation of the apparently good results is difficult to evaluate at this time especially in view of the great number of substances which had been

used in treatment previously and which also have been reported of benefit. Treatment of scleroderma by iontophoresis is not new. Potassium iodide (5) and mecholyl (6) have been applied by this method. Dihydrotachysterol (AT-10) (7), promin (8) and ammonium chloride with benadryl (9) have also been reported to be of value. Rarely have these substances or methods proven to be successful in hands other than their originators.

From the evidence obtained from these two cases of scleroderma, it appears that a deficiency of hyaluronidase is one of the etiological factors. This fits in with the current concept of a collagen disturbance in this disease. Some of the pathological tissue changes in scleroderma appear to be reversible. The effects of the hyaluronidase introduced by this method are not lasting. The results of treatment appear promising. Many problems arise with the use of an enzyme. Stability is difficult to maintain especially due to the factors of humidity, heat and impurities. Other factors which probably also entered into this investigation were: (a) A possible loss of hyaluronidase activator due to excessive purification. This might have been a factor in the hyaluronidase administered later in treatment when the more purified preparations were used. (b) Production of antihyaluronidase by the tissue cells in frequent contact with the hyaluronidase. This was avoided in later investigations by rotating the site of the active electrode. (c) Destruction of the hyaluronidase at the active electrode through the heat produced by the ionizing current. This was corrected by reducing the current and time of application.

It is realized that the increased disappearance time of an intradermal and subdermal saline wheal is not too accurate a method for the determination of hyaluronidase. It is not a quantitative method. Other methods, however, were not available. The increased disappearance time of the saline wheals closely paralleled the clinical improvement.

SUMMARY

1. Hyaluronidase was effectively transferred into the tissues through the unbroken skin, of normals and of generalized scleroderma by iontophoresis. This was proven by the increased disappearance rate of saline intradermal-subdermal wheals and the increased area of dispersion of intradermal Evans blue dye.

2. Two cases of generalized scleroderma were treated with hyaluronidase administered through iontophoresis for a period of 3 months with clinical improvement manifested by increased softness and flexibility of the tissues, decreased sensitivity to cold, improvement in skin color and return of general well being. On cessation of therapy, malaise and sensitivity to cold returned in one week; improvement in tissue softness, flexibility and skin color persisted up to 3 months.

3. The beneficial effects of hyaluronidase in generalized scleroderma, a collagen disease, substantiate the concept that there is a disturbance in the hyaluronidase-hyaluronic acid system.

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