# THE DETERMINATION OF HYALURONIDASE IN URINE

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(Received June 14, 1955. Submitted by Active Member of the Academy of Medical Sciences of the USSR, A. L. Myasnikov)

In recent years many proofs have been found regarding the participation of the hyaluronidase-hyaluronic acid enzyme system in the permeability of tissues.

Taking into account the fact that the problem of tissue permeability has a definite significance in understanding the etiology and pathogenesis of the disease process, we set ourselves the problem of determining the frequency and amount of change in the activity of this enzyme during rheumatism by means of investigating the hyaluronidase in urine.

## EXPERIMENTAL METHOD

Hyaluronidase was determined in urine by delaying the coagulation of mucin and lowering the viscosity of the protein-hyaluronic acid solution.

The first reaction is based on the ability of the protein-hyaluronic acid combination to coagulate after weak acetic acid is added. In the presence of hyaluronidase, this ability disappears and no coagulum is formed.

The hyaluronic acid was obtained from umbilical cords.

In our investigations the standard sample was usually 0.3 ml.

The experiment was set up in the following manner. Into 5 agglutination tubes was poured from 0.1 to 0.5 ml of urine, then 0.3 ml of hyaluronic acid was added to each test tube. This solution was made up to 1 ml by double distilled water. The tubes were shaken well and put into a thermostat for 20 minutes at a temperature of 37°. After incubation, they were cooled for 2-3 minutes, then 0.2 ml of 20% acetic acid solution was added to them and they were again mixed; after 2 minutes the results were determined.

Each experiment was accompanied by three controls: a test tube with a standard sample of hyaluronic acid, a test tube with 0.5 ml of urine and 0.2 mg of heparin or 0.2 ml of chloroform (inhibitors of hyaluronidase) and a test tube with 0.5 ml of urine which had been boiled earlier.

When hyaluronidase is absent in the test tube, the mucin coagulates; absence of coagulation or its destruction when lightly shaken indicates the presence of hyaluronidase.

The reaction of lowering the viscosity of the solution is based on the ability of the hyaluronidase to lower the viscosity of the hyaluronic acid as a result of its depolymerization.

In order to establish the lowering of the viscosity, we used a leucocyte mixer [4]. The outflow time for the buffered solution of hyaluronic acid with urine deactivated by heparin and with boiled urine (control) was determined. Then the time required for the same solution combined with urine to flow out was studied. The

noticeable decrease in the outflow time of the test sample compared with the control indicated that hyaluronidase was present. The determination was carried out every 5 minutes for 20 minutes at 37°. Usually the arithmetic average of 3-4 determinations was taken as the outflow time.

When the necessary conditions are met and an exact account kept of the time, the methodology of this modification is quite suitable for the determination of hyaluronidase activity.

## EXPERIMENTAL RESULTS

Preliminary investigations showed that boiled urine, as well as urine deactivated by heparin (control), does not lose the ability to depolymerize hyaluronic acid in the majority of cases. This indicates that the depolymerization of hyaluronic acid by urine occurs not simply as a result of the enzyme, hyaluronidase.

Taking into account the actual significance of these observations, we investigated the urine of healthy people and the urine of patients with rheumatism, as well as hypertonic disease, and those subjected to radiation; the same relationship was established.

In all, 70 persons were investigated. The data obtained are presented in the **Table.**

Depolymerizing Capability of Urine, Determined by Delaying Mucin Coagulation

| Depolymerization | Number of Tests | Control | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | chloroform | | heparin | | boiling | | standard sample of hyaluronic acid | |
| | | depolymerization | | | | | | | |
| | | present | absent | present | absent | present | absent | present | absent |
| Absent | 19 | – | 19 | – | 19 | 7 | 12 | – | 19 |
| Present | 51 | – | – | 41 | 10 | 41 | 10 | – | 51 |
| Total | 70 | 19 | | 70 | | 70 | | 70 | |

Account was kept of the results in the following manner. In the test tubes with urine and no hyaluronidase, a coagulum was formed after the addition of acetic acid; we considered this a negative reaction. Absence of coagulation in the presence of hyaluronidase was a positive reaction.

As is obvious from the Table, in 51 samples out of 70 the ability of urine to depolymerize hyaluronic acid was found, but since this ability was preserved in 41 cases out of 51 when the urine was boiled, we cannot explain the depolymerization of hyaluronic acid by urine by the presence of hyaluronidase in it, since the enzyme hyaluronidase is thermolabile and is destroyed by boiling. But in those 10 cases in which the urine lost the ability to depolymerize hyaluronic acid (in all the test tubes a typical coagulum of mucin was obtained), the depolymerization of hyaluronic acid by natural urine occured at the expense of the hyaluronidase. In these cases heparin also inactivated the urine's ability of depolymerize, and since it is a specific inhibitor of hyaluronidase, the fact that depolymerization occurred due to hyaluronidase was once more confirmed in these tests of urine.

The fact, that out of 19 patients whose urine did not contain depolymerizing materials before boiling, in 7 cases the urine after boiling acquired the ability to depolymerize hyaluronic aid, is brought out.

The impression is formed that boiling implements the liberation of some materials which have a mucolytic action.

Confirming our assumption that the urine's ability to depolymerize hyaluronidase is increased as a result of boiling, are the data that were obtained when investigating the hyaluronidase activity of the urine by means of the decreased viscosity of the protein – hyaluronic acid compound (average data from 15 determinations).

The time required for the buffered solution of hyaluronic acid with boiled urine to flow out was 5.4 seconds, urine with heparin was 7 and unboiled urine prior to incubation was 7.1 (the standard sample of hyaluronic acid took 7 seconds).

In subsequent measurements in the course of the experiment some decrease in the viscosity of the hyaluronic acid was observed.

Thus, our data indicate that urine can have the property of hyaluronidase in a few cases. Basically, hyaluronic acid is depolymerized by urine due to impurities in it.

In the literature there are indications [1, 2, 3, 4 and others] that a number of substances - ascorbic acid, glutathione, cysteine, hydrogen peroxide, azoproteins, pyrogallol, and others – are able to depolymerize hyaluronic acid, i. e., to decrease its viscosity nonspecifically.

Urine is the end product of metabolism and, naturally, can contain a number of substances able to change the viscosity of hyaluronic acid. Therefore, from our point of view, it is impossible to judge the hyaluronidase activity of a system on the basis of a study of urine.

We have come to this conclusion on the basis of analysis of much material.

## LITERATURE CITED

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