Study of Ether Soluble Factor from Urine Capable of Reducing the Viscosity of Gelatin

Lawrence H. Docta
STUDY OF ETHER SOLUBLE FACTOR FROM URINE CAPABLE OF REDUCING THE VISCOSITY OF GELATIN

II APPARATUS
A. Constant temperature bath
B. Koch all glass viscometer
C. Calibration of viscometer
D. Koch adenogen extractor

By
Lawrence H. Docta

A Thesis Submitted to the Faculty of the College of Liberal Arts Marquette University in Partial Fulfillment of the Requirements for the Degree of Bachelor of Science

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Preface and Acknowledgements

I INTRODUCTION

A. Presentation of Problem
The work of Hermann L. Karl and Mr. McNay and Leonard

B. Resume of Previous Work on Viscosity Reducing Factors
Driss on the viscosity reducing factor of urine, interested this

writer.

C. Viscosity

D. Gelatin and its viscosity

Under the direction of Dr. J. R. Koch and Mr. E. Karl,
Leonard Driss found that one factor in urine capable of reducing
viscosity of gelatin was ether soluble. A further study of this
ether soluble factor supported its existence.

II APPARATUS

A. Constant temperature bath

B. Koch all glass viscometer

C. Calibration of viscometer

D. Koch estrogen extractor

III PROCEDURE AS USED

A. A Typical Experiment

1. Extraction of factor

2. Preparation of solutions

3. Charging viscometer

4. Calculating percentage drops

IV DATA, CALCULATIONS AND GRAPH

V SUMMARY AND CONCLUSIONS

VI SUGGESTIONS FOR FURTHER STUDY

VII ANNOTATED BIBLIOGRAPHY
The work of Hermann L. Karl and Wm. McAsey and Leonard Driss on the viscosity reducing factor of urine, interested this writer.

Under the direction of Dr. J. R. Koch and Mr. H. Karl, Leonard Driss found that one factor in urine capable of reducing viscosity of gelatin was ether soluble. A further study of this ether soluble factor suggested itself.

The writer expresses acknowledgement to Dr. J. R. Koch and Mr. Hermann L. Karl for their guidance and suggestions throughout.
A. PRESENTATION OF PROBLEM

Leonard Driss (7) reported that urine contained an ether soluble factor capable of reducing the viscosity of a gelatin solution.

This thesis intends to further investigate this point and to determine the nature of the viscosity reducing factor. Also, to further investigate the phenomena reported by Driss (7) that male urines obtained soon after emission had a greater amount of this viscosity reducing factor than normal urine.

I INTRODUCTION

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In all these researches it was proven that these factors could always be destroyed by heating.

A search of the literature brings out the fact that only at Marquette has gelatin been used to measure these active factors of urine; in fact, only at Marquette have these properties of urine been measured.

(11) Karl, Hermann L., Masters Thesis, Marquette University, (1940) "A Possible Prenatal Sex Determination and a Viscometeric Determination for the Proteolytic Activity of Various Urines Gelatin as a Substrate."


B. RESUME OF PREVIOUS WORK ON VISCOSITY REDUCING FACTORS OF URINE

In 1938, Hermann L. Karl (11), while seeking a method of determining fetal sex during pregnancy, found that urines of the mothers varied in their ability to reduce the viscosity of gelatin solution.

Mr. Wm. McAsey (13), under Mr. Karl's guidance, worked with urines of tuberculosis patients; found that the factor capable of reducing the gelatin viscosity could be precipitated from urine with acetone.

Leonard Driss (7) found that two factors are present in urines that reduce viscosity of gelatin, one that can be precipitated with acetone, and the other can be extracted with ether.

In all these researches it was proven that these factors could always be destroyed by heating.

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C. VISCOSITY

The viscosity of liquids and solutions is a quality comparatively easy to measure. If some substance affects the viscosity of another substance, one can measure this effect quantitatively by determining the viscosity change.

The viscosity of a liquid can be defined as the force per unit area necessary to maintain a unit velocity gradient between two parallel planes at unit distance apart. Viscosity can be calculated from the following equation: (14)

\[ N = \frac{T}{dv \over dx} \]

where: \( T \) = (tau) force per unit area acting parallel to the planes equals the shearing force in dynes per square centimeters.

\[ \frac{dv}{dx} \] = velocity gradient perpendicular to the plates in centimeters per second.

\( N \) = viscosity in gram per centimeters per second.

The absolute unit of viscosity is the poise, defined as the viscosity of a material which requires a shearing force of one dyne per square centimeter to maintain a velocity gradient of one centimeter per second between two planes one centimeter apart (8). This unit was named after the French physician J.L.M. Poiseuille, who, in 1846, published a study of flow of liquids through narrow capillaries.

Many methods exist for measuring viscosity, but for this thesis one method is superior to the others by virtue of its simplicity and accuracy. This is the measurement of the rate of flow of a liquid through a capillary under constant pressure.

To obtain reliable results of viscosity measurements, certain conditions must be controlled. First, is purity of the liquid. It is very important that liquid be free from dust, filter fibers, or any other impurity which may jam the capillary and hinder the flow.

Viscosity changes very rapidly with temperature. It is, therefore, essential to carry out all measurements in a media which can be controlled to about 0.1°C.

Capillary effects may cause a reduction in the effective pressure and similarly introduce an error in measurements. It is necessary to use liquids having similar surface tension for comparison of viscosity measurements.

Then, too, interpretation of viscosity data is rendered difficult by three factors. The most important is hydration. The other two factors are the effect of the charge of the particles upon their intrinsic viscosity, and the effect of the velocity gradient in the viscometer upon the magnitude of the apparent coefficient.

In this thesis absolute viscosity is not determined but kinematic viscosity. It has been shown (9) that for short tube capillary viscometers discharging into air, the Kinematic (i.e., the ratio of absolute viscosity to density) of a liquid can be expressed in terms of the time of discharge, using the equation:

$$\frac{\text{Kinematic viscosity}}{\text{Density}} = At - \frac{B}{t}$$

where: $u$ absolute viscosity  
$v$ density  
$t$ discharge time of a definite volume  
$A$ and $B$ constants of viscometer

These constants are determined by measuring the discharge time, $t$, with suitable liquids of known viscosity, density and surface tension.

(9) Herschel, W.H., Technologic Papers of the Bureau of Standards No. 100, Nov. 9, 1917

(12) Koch, J.R., Orthmann, A.C., and Degenfelder, M.A., J. Am. Leather Chemists Assoc., 34, No. 9, p. 489-514, 1949
D. GELATIN AND ITS VISCOSITY

Gelatin, according to Dawidowsky (6), is produced from hides, skins and bones. It is distinguished by its purity, has a slight yellow tint, and is very hard and elastic. In cold water it softens, swells up, becomes opaque, but does not dissolve. In hot water it dissolves completely.

As stated in Alexander (1), evidence compiled regarding the chemical nature of gelatin is given by products of its hydrolysis. Skroup and Von Bühler found that on hydrolysis with hydrochloric acid, gelatin yielded the following substances: glycine, lysine, phenylalanine, arginine, proline and oxyproline, and other amino acids. Whether the amino acids exist in the gelatin molecule as such or were formed from the disintegration of large molecules, cannot with certainty be decided at present. Dawidowsky also stated that the chemical nature of gelatin is entirely changed by concentrated sulfuric and nitric acid. Dilute acids have no appreciable effect on gelatin. Meyer (15) reports that some gelatins contain a mucopolysaccharide hyaluronic acid.

According to Bingham (3), the viscosity of a gelatin solution is influenced by time, temperature, concentration, mechanical aggregation, hydrolysis, enzymatic action, addition of salts, acids and alkalies, and a wide variety of non-electrolytes.

(1) Alexander, J., Glue and Gelatin, Chemical Catalogue Co., New York, (1923) p. 30
In regard to pH, Bogue (4) says that when ordinary gelatin is placed in an electric field, the gelatin is found to be ionized and migrates to the anode. If a small amount of acid is added, the migration becomes less and less, and eventually ceases altogether. This point is known as the isoelectric point and is for gelatin defined by a hydrogen ion concentration of $2 \times 10^{-5}$ or pH 4.7. It is Loeb's theory (1), that at the isoelectric point 4.7, gelatin combines neither with an ion or cation of electrolytes. At pH 4.7, it combines with cations, forming metal gelatinates; and with anions, forming gelatin chlorides. Loeb goes on to say, as stated by Jerome Alexander (1), that the viscosity of a gelatin solution reaches its minimum at the isoelectric point, and that it rises on either side thereof, but with different rates, according to the particular anion or cation with which it is brought into combination.

It is desired to emphasize at this time, however, that in all probability, ionized particles of gelatin impart to a solution a greater viscosity than do the natural unchanged molecules. This effect is closely connected with hydration, and is shown by the fact that at the isoelectric point the swelling and the viscosities are also at their minimum level.

Bogue (5) investigated the relation between the viscosity and the concentration of gelatin sols and found that variation in the hydrogen ion concentration caused wide variation in

viscosity and in the volume occupied by a unit weight of gelatin. Isoelectric gelatin has the lowest viscosity and lowest degree of solvation. Gelatin chloride (H ion concentration is $3.1 \times 10^{-6}$) has the highest, and calcium gelatinate (H ion concentration is $2.5 \times 10^{-6}$) is intermediate. Solvation and viscosity appear to be parallel functions, according to Bogue.

Whether the purely chemical or the physical explanation be assumed, these facts indicate the patent influence on viscosity or changes in the size of particles constituting the dispersed gelatin.

Bogue (4) in his book, after a critical view of several theories, concludes that a sol consists of slightly hydrated swollen particles united in a short chain. When the temperature falls, the threads increase in length and number, and the power of water absorption increases, resulting in an increase of viscosity. A solid jelly results when the relative volume occupied by the swollen molecular threads becomes so great that freedom of motions is lost, and the adjacent heavily swelled aggregates coher.

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A. CONSTANT TEMPERATURE BATH

A glass aquarium of about fifteen gallons capacity served as a constant temperature water bath, and electrical heating coils connected to 110 volt A.C. were used to heat the water. The coils consisted of one large coil and four smaller ones. If no warm water was available, the large coil was used to heat the water up to 40°C, and the smaller coils were used to keep the temperature constant at 40°C.

A fuse was also used in the circuit as a switch to supply 110 volts across the large coil for the initial heating to 40°C. By unscrewing the fuse, heaters 1, 2, and 3 would be put in series across the line, and the water in the bath would slowly drop below 40°C. A thermostat was then used to throw heater number 4 parallel with heater number 3, which gave more heat so that the bath temperature would very slowly rise above 40°C. By these two opposite actions of the coils, the temperature was kept constant to within 0.2°C. This arrangement is shown in Diagram I.
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The Koch viscometer was found by the writer to be very workable in following changes of viscosity in relation to time. The major feature is that it is a closed system, therefore showing less chance for errors from loss of liquid or recontamination with air. With this instrument, viscosity readings could be taken.

The construction of the viscometer is simple to visualize. It consists of a length of auxiliary tubing about 6.5 ft., inside of which is a tube of wide glass tubing. A glass stopcock having a funnel opening is attached at one end, and a capillary tube is attached at the other end to allow the flow of liquid from one container to the other, simply by tilting over the viscometer. The entire viscometer is mounted in an upright position, as shown in the diagram.

Diagram I
B. KOCH ALL GLASS VISCOMETER

The Koch viscometer was found by the writer to be very workable in following changes of viscosity in relation to time. The most important features are: having a closed system, thereby leaving less chance for errors from loss of liquid or from contamination from dust, and the ease with which continual viscosity readings could be taken.

The construction of the viscometer is simple to visualize. It consists of a length of capillary tubing about 3.5 cm. long sealed into a section of wide glass tubing AA' as shown in diagram II. This in turn is sealed at each end to an Erlenmeyer flask of 200 cc. volume. Lastly, a side arm having a funnel opening is attached; the capillary is cut off at end to allow free flow of liquid from one chamber to the other by simply turning over the viscometer.

Lead weights are attached to the extreme ends of the flasks to keep viscometers in an upright position when placed in the water bath.
Koch Viscometer

In a capillary viscometer discharging into air, the kinematic viscosity (i.e., the ratio of absolute viscosity to density) of a liquid can be expressed in terms of the time of discharge (t) using the following equation:

\[ \text{Kinematic Viscosity} = \frac{v}{\eta} = \frac{A}{t} \]

where:
- \( v \) is the kinematic viscosity
- \( \eta \) is the absolute viscosity
- \( A \) and \( B \) are constants of the viscometers

These constants \( A \) and \( B \) were according to the size and shape of the viscometers and were determined by measuring the discharge time, with suitable liquids of known viscosity and density.

Method used for calculation was developed by Koch, et al., (12), and used by Tikuisis (7) and Pringsheim (13) in their work.

Solutions used were: 30% acetic acid at 25°C; 40% sucrose solution at 50°C; and one at 40°C. The water and the 40% sucrose at 30°C were used to determine the constants of the viscometer and the sucrose solution at 40°C was used as a check.

The values obtained for \( t \) were substituted into above equation and solved simultaneously for constants \( A \) and \( B \). The calculation of \( K \), \( V \), and 40% sucrose solution were checked with values in literature.


(13) Tikuisis, F., Eastern Trade, Massachusetts University (1940). "A measure of the proteolytic effect of pepsin or various enzymes using new digestion-rates viscometer."

(7) Osler, B. B. Bachelor Thesis, Marquette University (1948). "The
C. CALIBRATION OF VISCOMETERS

For a short capillary viscometer discharging into air, the kinematic viscosity (i.e., the ratio of absolute viscosity to density) of a liquid can be expressed in terms of the time of discharge \( t \), using the following equation:

\[
\text{Kinematic Viscosity } K.V. = \frac{u}{v} \quad \text{At} - \frac{B}{t}
\]

where:
- \( K.V. \) is the kinematic viscosity
- \( u \) is the absolute density
- \( v \) is the density
- \( t \) is the discharge time of a definite volume
- \( A \) and \( B \) are constants of the viscometers

These constants, \( (A \) and \( B) \), vary according to the size and shape of the viscometer, and are determined by measuring the discharge time, \( t \), with suitable liquids of known viscosity and density.

Method used for calibration was developed by Koch, et al. (12), and used by Tikula (16) and Driss (7) in their work. Solutions used were 50 mls. of water at 25°C.; 40% sucrose solution at 30°C.; and one at 40°C. The water and the 40% sucrose at 30°C. were used to determine the constants of the viscometer and the sucrose solution at 40°C. was used as a check.

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(9) Hershel, W.H., Technologic Papers of the Bureau of Standards No. 100, Nov. 9, (1917)
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(16) Tikula, F., Masters Thesis, Marquette University (1940) "A measure of the proteolytic effect of pepsin on various gelatins using new digestion-flask viscometer."
D. KOCHESTROGEN EXTRACTOR

This is a large glass apparatus used in continuous extraction of materials soluble in a vaporizable, immiscible solvent of low density. It is composed of a large, round bottom flask, an Erlenmeyer flask, and a condenser. Underneath the condenser, a tube delivers the solvent to the material being extracted in the round bottom flask. A diagram of this apparatus is more readily followed than a written description (see Diagram III).
A. A TYPICAL EXPERIMENT

1. Extraction of Ether Soluble Factor: Seven hundred cc. of a morning urine sample were placed in the round bottom flask of the Koch Extractor. Ether was added to fill flask, additional ether was distilled over from Erlenmeyer flask which contained 250 mls. of ether, thereby filling column up to return arm. Rate of distillation was controlled by regulating vacuum and heat. The urine sample was then extracted at a constant temperature, below 40°C., for twenty-four hours. Ether extract from the Erlenmeyer flask at the end of extraction was then distilled under reduced pressure and below 40°C. to a concentrated volume of 20 mls. This last volume was allowed to evaporate at room temperature to dryness. The residue had a yellow-orange color and an odorous odor due to the urinary porphyrins, and consisted of long, thin needle crystals.

III PROCEDURE AS USED

2. Preparation of Solutions: A gelatin solution was made by weighing out eight grams of Seidel's gelatin and mixing it with 92 milliters of pH 5.0 distilled water. The gelatin solution was then tempered to 40°C., and after one hour of standing in the water bath, it became a clear homogeneous liquid.

The ether soluble factor solution was prepared by dissolving the residue from extraction with 30 mls. of 5.0 pH distilled water. This solution was then tempered to 40°C. in the water bath for one hour.

3. Filling the Viscosimeters: In preparing the ether soluble factor solution for a run, 30 mls. of it were mixed with 30 mls. of the gelatin solution. As rapidly as possible, 50 mls. of this solution was pipetted into a viscometer and placed
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3. Charging the Viscometers.- In preparing the ether soluble factor solution for a run, 30 mls. of it were mixed with 30 mls. of the gelatin solution. As rapidly as possible, 50 mls. of this solution was pipetted into a viscometer and placed in
the water bath at 40°C. Immediately after this preparation, a control was prepared by mixing 30 mls. of water pH 5.0 with 30 mls. of gelatin solution. Fifty mls. of this solution was then pipetted into a viscometer and placed in the water bath at 40°C. A run was made on the control as soon as it was placed in the bath. The kinematic viscosity of the first run was taken as the initial kinematic viscosity for the factor gelatin mixture. Fifteen minutes after the first run on the control a run on the factor gelatin mixture and the control was made. Runs on both were continued at this time interval for one hour. After the completion of a run, the change in pH of factor gelatin mixture was determined with the Beckman pH meter. In the runs of Series B, some of the samples were tested for carbohydrate hydrolysis products with Benedict's solution, also for amount of protein hydrolysis by Biuret Test and Ninhydrin Test.

4. Calculation of Percentage Drop in Kinematic Viscosity.- The first run of the blank was taken as the initial kinematic viscosity. The difference in the viscosity units between the first run on the blank and the last run on the solution being studied for factor's activity, was divided by the initial kinematic viscosity. The quotient was multiplied by 100. The product is the per cent drop in kinematic viscosity for the solution containing the gelatin and the factor. For runs in which there was an appreciable drop in the viscosity of the control, the calculation of the per cent of drop in kinematic viscosity was modified to correct for this effect. In such
cases the difference in the viscosity units between the last run on the control and the last run on the factor solution was divided by the final kinematic viscosity. This quotient was multiplied by 100.
## TABLE 1

**Date of run** - 3/2/49  

**Viscometers:**  
- B - Control  
- C - contained ether soluble factor  

**On Graph:**  
- Curve C - curve of ether soluble factor  
- Curve B - control

| Time (min) | Sec. | K.V. | Time (min) | Sec. | K.V. | K.V. %  
|-----------|-----|------|-----------|-----|------|---------  
| 0 0       | 54  | 54.2 | 0 0       | 60  | --   | ++        
| 15        | 54  | 54.0 | 15        | 45  | 42.0 | 32.3      
| 30        | 54  | 54.0 | 30        | 45  | 43.5 | 29.1      
| 45        | 54  | 54.0 | 45        | 45  | 43.2 | 28.9      
| 60        | 54  | 54.0 | 60        | 45  | 41.8 | 28.6      

pH end of run Viscometer C - 4.3  

% drop in K.V. in one hour - 175
SERIES A

TABLE I

Date of run - 3/2/49

Viscometers:

B - Control

C - contained ether soluble factor

On Graph:

Curve C - curve of ether soluble factor

Curve B - control

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<tr>
<th>Time</th>
<th>Sec.</th>
<th>K.V. X10</th>
<th>Time</th>
<th>Sec.</th>
<th>K.V. X10</th>
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<td>54.4</td>
<td>34.0</td>
<td>60</td>
<td>41.8</td>
<td>28.3</td>
</tr>
</tbody>
</table>

pH end of run Viscometer C - 4.3

% drop in K.V. in one hour  17%
TABLE II

Date of run - 3/4/49

Viscometers:

B - control

C - contains ether soluble factor

On graph:

Curve B - control

Curve C - Ether soluble factor

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<tr>
<th>Time (min)</th>
<th>Sec. K.V. X 10</th>
<th>Time (min)</th>
<th>Sec. K.V. X 10</th>
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<td>60</td>
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<td>60</td>
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</tr>
</tbody>
</table>

pH at end of run Viscometer C - 4.15

% drop in K.V. in one hour 28.65%

N.B. Sample taken 8 hours after emission.
SERIES A

TABLE III

Date of run - 3/9/49

Viscometers:

B - control
A - ether soluble factor
C - ether soluble factor

On graph:

Curve B - control
Curve A - ether soluble factor
Curve C - ether soluble factor

<table>
<thead>
<tr>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
<th>Time</th>
<th>Sec.</th>
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<td>30.0</td>
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<td>40.6</td>
<td>26.4</td>
</tr>
</tbody>
</table>

pH at end of run viscometer A - 4.35
pH at end of run viscometer B - 4.2
%
% drop in K.V. in one hour - viscometer A - 12.5%
% drop in K.V. in one hour - viscometer B - 21.39%

N.B. Sample in viscometer C taken eight hours after emission.
Date of run - 3/11/49

Viscometers:

B - control
A - ether soluble factor
C - ether soluble factor

On graph:

Curve B - control
Curve A - ether soluble factor
Curve C - ether soluble factor

<table>
<thead>
<tr>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
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<td>41.0</td>
<td>27.4</td>
</tr>
</tbody>
</table>

pH end of run viscometer A = 4.9
pH end of run viscometer C = 4.3
% drop in K.V. over one hour - viscometer A = 1.19%
% drop in K.V. over one hour - viscometer C = 18.69%
Date of test: 3/11/49

Viscometer: A
- control
Viscometer: B
- other soluble factor
Viscometer: C
- other soluble factor

On graph:
- Curve A - other soluble factor
- Curve B - control
- Curve C - other soluble factor

End of run viscometer A - 4:2
End of run viscometer B - 4:3

% drop in K.V. over one hour - viscometer A - 15.9%
% drop in K.V. over one hour - viscometer C - 11.1%

Time in Minutes
SERIES A

TABLE V

Date of run - 3/12/49

Viscometers:

B - control
A - ether soluble factor
C - ether soluble factor

On graph:

Curve B - control
Curve A - ether soluble factor
Curve C - ether soluble factor

<table>
<thead>
<tr>
<th>Time</th>
<th>K.V.</th>
<th>Time</th>
<th>K.V.</th>
<th>Time</th>
<th>K.V.</th>
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<tbody>
<tr>
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<td>60</td>
<td>42.0</td>
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</table>

pH end of run viscometer A - 4.2
pH end of run viscometer C - 4.3

% drop in K.V. over one hour - viscometer A - 15.9%
% drop in K.V. over one hour - viscometer C - 11.1%
SERIES A
TABLE VI

Date of run - 3/15/49

Viscometers:

B - control
A - ether soluble factor
C - ether soluble factor

On graph:

Curve B - control
Curve C - ether soluble factor
Curve A - ether soluble factor

<table>
<thead>
<tr>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
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<td>34.9</td>
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<td>28.8</td>
</tr>
</tbody>
</table>

pH end of run viscometer A - 4.0
pH end of run viscometer C - 4.3

% drop in K.V. over one hour - viscometer A - 24.4%
% drop in K.V. over one hour - viscometer C - 20.07%
SERIES B
TABLE VII

Date: 3/19/49

Data

<table>
<thead>
<tr>
<th>Sample 1</th>
<th>Viscometer A</th>
<th></th>
<th>Viscometer B</th>
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<th>Viscometer C</th>
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<tbody>
<tr>
<td>Interval</td>
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<td>K.V.</td>
<td>Interval</td>
<td>Time</td>
<td>K.V.</td>
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<td>54.2</td>
<td>34.2</td>
</tr>
</tbody>
</table>

pH 4.3

pH 4.2

Results:

Negative Benedict's test on samples

Sample 1  15.3% K.V. Drop
Sample 2  15.9% K.V. Drop
Results of Neg. Bencze's test on paper samples: Sample 3 = 21.76% K.V. Drop; Sample 4 = 12.6% K.V. Drop. Sample 3 taken 8 hours after emission.

(Time in Minutes)
Date: 3/23/49

Data

<table>
<thead>
<tr>
<th>Viscometer A</th>
<th>Viscometer B</th>
<th>Viscometer C</th>
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</thead>
<tbody>
<tr>
<td>Sample 3</td>
<td>Control</td>
<td>Sample 4</td>
</tr>
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<td>Time</td>
<td>Interval Time</td>
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</tr>
<tr>
<td>45 &quot;</td>
<td>46.8</td>
<td>45 &quot;</td>
</tr>
<tr>
<td>60 &quot;</td>
<td>46.6</td>
<td>60 &quot;</td>
</tr>
</tbody>
</table>

pH 4.0

Results:

Negative Benedict's test on samples

Sample 3  21.78% K.V. Drop
Sample 4  10.6% K.V. Drop

Sample 3 taken 8 hours after emission.

[Graph showing time in minutes]
## Series B

### Table IX

**Date:** 4/19/49

**Data:**

<table>
<thead>
<tr>
<th>Viscometer A</th>
<th>Viscometer B</th>
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</thead>
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<tr>
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<td>K.V.</td>
<td>Interval</td>
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<tr>
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<td>0</td>
</tr>
<tr>
<td>15</td>
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<tr>
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<td>45</td>
</tr>
<tr>
<td>60</td>
<td>53.2</td>
<td>60</td>
</tr>
</tbody>
</table>

**pH:** 4.3

**Results:**

Negative Bivret, Ninhydrin and Benedict's test on samples

- Sample 5 9.43% K.V. Drop
- Sample 6 23.14% K.V. Drop

Sample 6 taken 8 hours after emission.
**Time in Minutes**

Graph showing the relationship between time (in minutes) and K.V. (Kilovolts). The graph has three lines labeled A, B, and C, with points marked at intervals of 15, 30, 45, and 60 minutes. The K.V. values range from 30 to 50.

**Results**

- **Sample A**: 13.10% N.V. Drop, treated with hydrazine and Benedict's test on sample. Sample was taken 8 hours after emission. It was divided into two portions, "A" and "B". Portion "A" was heated in a water bath at 100°C for an hour.
### SERIES B

#### TABLE X

**Date:** 4/22/49

**Data:**

<table>
<thead>
<tr>
<th>Viscometer A &quot;A&quot;</th>
<th>Viscometer B Control</th>
<th>Viscometer C &quot;B&quot;</th>
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</thead>
<tbody>
<tr>
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<td>K.V.</td>
</tr>
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<tr>
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</tr>
<tr>
<td>60</td>
<td>51.0</td>
<td>29.7</td>
</tr>
</tbody>
</table>

**pH**

- pH 4.3
- pH 4.9

**Results:**

Negative Bivret, Ninhydrin and Benedict's test on samples

- Sample "A" 13.16% K.V. Drop
- Sample "B" 4.7% K.V. Drop

Sample was taken 8 hours after emission. It was divided into two portions, "A" and "B". Portion "B" was heated in a water bath at 100°C for an hour.
SERIES B

TABLE XI

Date: 4/26/49

Data:

<table>
<thead>
<tr>
<th>Viscometer A</th>
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<th>Viscometer C</th>
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</thead>
<tbody>
<tr>
<td>Sample 8B</td>
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<td>Sample 8A</td>
</tr>
<tr>
<td>Interval</td>
<td>Interval</td>
<td>Interval</td>
</tr>
<tr>
<td>Time</td>
<td>Time</td>
<td>Time</td>
</tr>
<tr>
<td>K.V.</td>
<td>K.V.</td>
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</table>

<table>
<thead>
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</thead>
<tbody>
<tr>
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<td>4.1</td>
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</tbody>
</table>

Results:

Negative Bivret, Ninhydrin and Benedict test.

Sample 8A 19.98% K.V. Drop
Sample 8B 5.76% K.V. Drop

This sample received same treatment as sample 7.
There exists in urines a viscosity reducing factor that is soluble in water.

Urines obtained after emission contain a much larger amount of this factor than normal urines.

From negative results of Ninhydrin and Biuret tests, it can be assumed that the factor does not cause a viscosity reduction of gelatin to alpha amino acids.

Results of Benedict's test are unreliable since copper, a heavy metal, causes precipitation of the protein thus obscuring the test. This reaction perhaps removes copper ions before they are reduced to the copper sulfate which is essential for the detection of free aldehyde groups or products of depolymerization of polysaccharides. Hence, the negative results of this test are not of much value whatsoever.

Viscosity reducing factor of urines can be inactivated by heat.

It is not definite if this factor increases the acidity of the gelatin solution by its activity. Perhaps this substance is strongly acidic and could possibly be the cause of the lowering of the pH. Since the solution of factor in 5.0 pH was not again checked for pH after solution of factor
There exists in urines a viscosity reducing factor that is soluble in ether.

Urines obtained after emission contain a much larger amount of this factor than normal urines.

From negative results of Ninhydrin and Biuret tests, it can be assumed that the factor does not cause a viscosity reduction of gelatin to alpha amino acids.

Results of Benedict's test are unreliable since copper, a heavy metal, causes precipitation of the protein thus obscuring the test. This reaction perhaps removes cupric ions before they are reduced to the cuprous state which is essential for the detection of free aldehyde groups of products of depolymerization of polysaccharides. Hence, the negative results of this test are not to be accepted, and any deductions may have no value whatsoever.

Viscosity reducing factor of urines can be inactivated by heat.

It is not definite if this factor increases the acidity of the gelatin solution by its activity. Perhaps this substance is strongly acidic and could possibly be the cause of the lowering of the pH. Since the solution of factor in 5.0 pH was not again checked for pH after solution of factor
VI SUGGESTIONS FOR FURTHER WORK

Check pH changes in pH 5.0 distilled water when ether-extracted material is added, and compare this pH with pH of gelatin solution after one hour of reaction.

Check the sugar tests on the gelatin solution acted on by the factor; find a way of eliminating the protein material that interferes with heavy metals solutions used in test.

Determine effect of pH on ability of factor to reduce viscosity of gelatin.

Test the effect of this factor on reduction of viscosity of Hyaluronic acid at a pH 5.0.

Determine if glucuronic acid is liberated when factor acts on hyaluronic acid.
Check pH changes in pH 5.0 distilled water when ether extracted material is added, and compare this pH with pH of gelatin solution after one hour of reaction.

Check the sugar tests on the gelatin solution acted on by the factor; find a way of eliminating the protein material that interferes with heavy metals solutions used in test.

Determine effect of pH on ability of factor to reduce viscosity of gelatin.

Test the effect of this factor on reduction of viscosity of Hyaluronic acid at a pH 5.0.

Determine if glucuronic acid is liberated when factor acts on hyaluronic acid.

   This book deals with the structure, reactions and practical applications of gelatin.


   Barr discusses methods used in viscometry and the theory and difficulties involved.


   Explains the theory involved in various types of viscometry and conditions which affect viscosity.


   From a study of gelatin solutions, Bogue determines the relationships between viscosity and concentration.


   Bogue explains the effect of hydrogen ion concentration, heat and other conditions on gelatin.


   Chemistry and technology of gelatin.


   Referred to section dealing with the definition of viscosity and conditions affecting it.


   Herschel in his paper derives the relationship of absolute viscosity to kinematic viscosity.


    Capillary effects cause of errors in the measurements of viscosity.

This book deals with the structure, reactions and practical applications of gelatin.


Barr discusses methods used in viscometry and the theory and difficulties involved.


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Bogue explains the effect of hydrogen ion concentration, and the effect of stirring, heat and other conditions on gelatin.


Chemistry and technology of gelatin.


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9. Hershel, W. H., Technologic Papers of the Bureau of Standards, No. 100, Nov. 9, (1917)

Hershel in his paper derives the relationship of absolute viscosity to kinematic viscosity.


Capillary effects cause of errors in the measurements of viscosity.


Mark interprets viscosity form a molecular point of view.


As study of mucopolysaccharide content of various skins.
