The Influence of Certain Factors in Urine on the Viscosity of Gelatin

Leonard Driss

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THE INFLUENCE OF CERTAIN FACTORS IN URINE ON THE VISCOSITY OF GELATIN

By

Leonard Driss

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

MILWAUKEE, WISCONSIN July, 1948
After reading the Masters Thesis of Mr. Herman L. Karl, the writer became interested in this problem and then took up the study of the proteolytic activity of normal urine. Mr. Karl worked on the urine of pregnant women. Using gelatin as a substrate, he hoped to find a difference in proteolytic activity between the urine of a woman pregnant with a male fetus and the urine of a woman pregnant with a female fetus. His results were not conclusive but suggested that the problem of proteolytic action of urine should be studied under normal conditions.

The writer is indebted to Dr. John R. Koch for his supervision of this work and to Mr. Herman L. Karl for his guidance and his suggestions throughout.
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A. Presentation of the Problem

The aims of this thesis are four-fold:

I. To determine the apparent proteolytic activity of normal urine from day to day.

II. To determine the apparent proteolytic activity of a sample of blood donor after injection.

III. To determine whether the other extracts of normal urine possess apparent proteolytic activity.

IV. To determine the effect of hyaluronidase on the viscosity of solution.

This study of enzymatic action is not a new one. Karl (9) has shown that almost every blood from adult subjects is proteolytically active. Others have worked on enzymatic action and many of the assumptions made this work greatly, i.e., pH of 4, use of pancreatin.

The work on hyaluronidase is relatively new. As described in the South Dakota State College of Medicine and Pharmacy (8), a pathology in recent years used hyaluronidase in connection with cancer chemotherapy. This enzyme exists in the testes, spleen and skin. Dr. Jardine collected a large amount of this material exposed to various tissues. His experiments using a solution of fresh splenic cells mixed with

---

(9) Karl, Herman L., Hyaluronidase, Washington University, 1943.
(10) Maxey, Frank, Hyaluronidase, Washington University, 1943.
A. Presentation of the Problem

The aims of this thesis are four-fold:

I. To determine the apparent proteolytic activity of normal urine from day to day.

II. To determine the apparent proteolytic activity of a sample of 8-12 hours after emission.

III. To determine whether the ether extracts of normal urine possess apparent proteolytic activity.

IV. To determine the effect of hyaluronidase on the viscosity of gelatin.

This study of enzymatic action is not a new one. Karl (9) has shown that normal urine taken from adult subjects is proteolytically active. Maxey (10) also worked on enzymatic action and many of his suggestions aided this work greatly, i.e., pH of 4, and 8% gelatin.

The work on hyaluronidase is relatively new. As described in the South Dakota Journal of Medicine and Pharmacy (8), a pathologist, Albert Harris, used hyaluronidase in connection with cancer detection. This enzyme exists in the testes, spleen and skin. Dr. Harris believed a large amount of this material existed in cancerous tissue. His experiments using a solution of fresh umbilical cord mixed with

(9) Karl, Herman L., Masters Thesis, Marquette University, 1940.
(10) Maxey, Frank, Bachelor Thesis, Marquette University, 1942.
rabbit serum as a substrate to show enzymatic activity were relatively successful. This thesis attempts to find how successful gelatin would be as a substrate for hyaluronidase determination.

The attempt to extract enzyme by the extraction method with ether was also encouraged by Dr. Harris (18). Up to this time, it was believed that extraction of an enzyme by ether was not possible, but Dr. Harris's work encouraged work along this line. The enzyme was extracted with ether by the use of an hormone extraction apparatus.
B. Viscosity

In Bingham (3), viscosity is defined by Maxwell as follows:

"The viscosity of a substance is measured by the tangential force on a unit area of either of two horizontal planes at a unit distance apart required to move one plane at a unit velocity in reference to the other plane, the space being filled with the viscous substance."

Bingham (3) goes on to say that there are at least several distinct methods which are susceptible to mathematical treatment, so that absolute viscosities may be obtained. The possible methods for measuring viscosity may be classified under three headings:

I. The measurements of the resistance offered to a moving body (usually a solid in contact with the viscous liquid).

II. The measurement of the rate of flow of the viscous liquids.

III. Methods in which neither the flow nor the resistance to flow are measured. The method herein used is number two on the list; the measurement of the rate of flow of a viscous liquid through a vertical tube of a small diameter.

Poiseuille (2) showed how the viscosity of a liquid varied with the structure of the capillary in the viscometer. This was best shown by his equation

\[ n = \frac{Pr^4}{8vL} \]

(2) Barr, Guy, A Monogram of Viscometry, Oxford University Press, London (1931).
When \( P \) = pressure of surrounding medium
\[ v = \text{volume of liquid} \]
\[ t = \text{time of flow} \]
\[ r = \text{radius of capillary} \]
\[ L = \text{length of capillary} \]
\[ n = \text{viscosity} \]

Besides the capillary, change in temperature has a great effect on the viscosity of a liquid. Barr has shown that viscosity of gases at \( 0^\circ C \) may increase by as little as \( 0.2\% \) per \( 1^\circ C \) rise in temperature, but that of liquid decreases at a more rapid rate. For example, castor oil at \( 20^\circ C \) (\( n = 986 \text{ Cp.} \)) will have a rate of decrease of \( 8.4\% \) per \( 1^\circ \), while for pitch at \( 20^\circ C \) (\( n = 3 \times 10^8 \text{ poises} \)) the figure becomes more than \( 30\% \). As a general rule, the effect of temperature is more marked the higher the viscosity.

The poise is the unit of viscosity. According to Getman and Daniels (7) it is "the viscosity of a hypothetical liquid such that a force of one dyne per square centimeter causes two parallel liquid surfaces one square centimeter in area and centimeter apart to slide past one another with a velocity of one centimeter per second."

C. Gelatin and Its Viscosity

Gelatin, according to Dawidowsky (5), 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 Buhler found that on hydrolysis with hydrochloric acid, gelatin yielded the following substances: glycine, lysine, alanine, phenylalanine, leucine, aspartic acid, glutamic acid, histidine, arginine, proline and oxyproline. Whether these amino acids exist in the gelatin molecule as such as were formed from the disintegration of large molecules cannot with certainty be decided at present. Dawidowsky (5) 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.

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.

---

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 (11). It is Loeb's theory (1) that at the isoelectric point of 4.7, gelatin combines neither with anion or cation of electrolyte. At pH 4.7, it forms only with cations, forming metal gelatinate; and at pH 4.7 forms with anions, forming gelatin chloride. Loeb goes on to say, as stated by 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 (4) investigated the relation between the viscosity

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(1) Alexander, Jerome, op. cit., 36.
(1) Alexander, Jerome, ibid., 196.
and 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 = $3.1 \times 10^{-3}$) has the highest, and calcium gelatinate (H ion concentration = $2.5 \times 10^{-6}$) 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, concluded that a sol consists of slightly hydrated swollen particles united in a short chain. When 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 motion is lost, and the adjacent heavily swollen aggregates cohere.

(4) Bogue, Robert, op. cit., 106.
D. Enzymes

Tauber (15) defines an enzyme as a catalyst produced by the living cell, but whose action is independent of the living cell, and which is destroyed if its solution is heated long enough.

Fearson (6) states that beside having the general endowment of all catalysts, namely, power of survival, continuity of effect and independence of equilibrium, enzymes are especially sensitive to temperature. This is shown by two important characteristics, heat inactivation and heat acceleration. This heat inactivation is ascribed to irreversible changes in the colloid enzyme, comparable with the heat coagulation of higher proteins. Heat acceleration is shown by the existence of an optimal temperature range for every reaction, although limits of the range are modified by various factors, including the gradual inactivation of the enzyme by prolonged heating.

Enzymes are also sensitive to pH. All enzymes display a region of optimal H^+ ion concentration which vary about from 1.5 pH for pepsin up to a pH of 10 for trypsin. The majority act best within a range of pH of 4.5 - 7.5.

There are maximal and minimal limits of pH beyond which an enzyme does not act. Exposure to these extremes is often marked by destruction of the catalyst in a manner comparable

with the thermal inactivation.

Proteolytic enzymes (12) catalyze the hydrolytic breakdown of proteins or of their primary cleavage products. They are secreted by bacteria, molds, and all animal and vegetable tissue, and are known by the names of proteases or peptidases. They are classified according to the material they attack, although all the proteolytic enzymes cause hydrolysis of the peptide linkage.

\[ -C-NH \]

Some enzymes only attack the proteins themselves, while others only attack peptides - products of partial hydrolysis of proteins. The former are called proteinases or endopeptidases; the latter are called peptidases or exopeptidases.

Sumner (14) stated that the pH optimum for a proteolytic enzyme will vary somewhat depending upon what protein is serving as a substrate. The optimum is also influenced by the purity and even by the buffer employed.

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Constant temperature water bath:

A glass aquarium of about fifteen gallons capacity served as a 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 that temperature.

A fuse was also used in the setup 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 to heater number 1, 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 Number I.
Constant temperature water bath:

A glass aquarium of about fifteen gallons capacity served as a 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 that temperature.

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Koch All-Glass Viscometer:

The instrument used in this work is an especially designed digestion-flask viscometer which possesses the important features of accuracy and ease of workability. The viscosity determinations can be carried out conveniently in a constant temperature bath. The viscometer is constructed of two 200 cc Erlenmeyer flasks fused together at the neck. A capillary tube about 35 millimeters long extends from the discharge chamber into the receiving chamber. A side arm connects one flask with the other in such a manner that the liquid can be transferred from one chamber into the other. In this arm is a funnel-shaped opening, through which the liquid can be introduced very conveniently. The viscometers are kept in an upright position in the water bath by means of lead weights attached to both extremities of the instrument.

Koch Estrogen Extractor:

This is a large glass apparatus used in extracting enzymatic material from a liquid. It is composed of a large round-bottom flask, Erlenmeyer flask and a condenser. There is a tube underneath the condenser which is used to deliver the extracting material to the material being extracted. A diagram of the apparatus is more readily followed than a verbal description (see Figure II).
Koch Estrogen Extractor

Figure No. II
A method used to calibrate viscometers is described by Timulka [17]. He used the equation

\[ \eta = KV = AT - \frac{B}{T} \]

\( KV \) = Kinematic viscosity (16). It is the ratio of the absolute viscosity (\( \eta \)) to the density of the substance used (\( \rho \)).
\( T \) = efflux time in seconds
\( A \), \( B \) = constants for each viscometer varying according to the capillary and shape of the viscometer.

From this equation, constants of the viscometers can be determined.

The solutions used were 50 ml. water at 25°C, 40% sucrose at 30°C, 40% sucrose at 40°C. The water and the 40% sucrose at 30°C were used as a check.

III CALIBRATION OF VISCOMETER

A viscometer containing water at 25°C was put in a constant temperature bath and runs were made. Then 40% sucrose was put in the viscometer and runs were made at 30°C. An average of five runs - fifteen minutes apart - was used for the effluxing time for each solution. Then, knowing the kinematic viscosity of water at 25°C and 40% sucrose at 30°C, and the efflux time of each of their respective temperatures, the constants could be calculated. The kinematic viscosities of different materials can be obtained from the Chemical Rubber Company Handbook.

(17) Timulka, Frank, Masters Thesis, Marquette University, 1940.
A method used to calibrate viscometers is described by Tikulka (17). He used the equation

\[ \frac{U}{C} = KV = AT - \frac{B}{T} \]

*KV* = Kinematic viscosity (16). It is the ratio of the absolute viscosity (*U*) to the density of the substance used (*C*).

*T* = efflux time in seconds

*A*  
*B* = constants for each viscometer varying according to the capillary and shape of the viscometer.

From this equation, constants of the viscometers can be determined.

The solutions used were 50 ml. water at 25°C.; 40% sucrose at 30°C.; 40% sucrose at 40°C. The water and the 40% sucrose at 30°C. were used to determine the constants of the viscometer and the sucrose at 40°C. was used as a check.

A viscometer containing water at 25°C. was put in a constant temperature bath and runs were made. Then 40% sucrose was put in the viscometer and runs were made at 30°C. An average of five runs - fifteen minutes apart - was used for the effluxing time for each solution. Then, knowing the kinematic viscosity of water at 25°C. and 40% sucrose at 30°C. and the efflux time of each of their respective temperatures, the constants could be calculated. The kinematic viscosities of different materials can be obtained from the Chemical Rubber Company Handbook.

(17) Tikulka, Frank, Masters Thesis, Marquette University, 1940.
The mathematics used in solving for the constants were just simply solving for two unknowns in two equations.

Example:

KV of water at 25°C. = .8479
KV of 40% sucrose at 30°C. = 3.739
Average efflux time of water = 31.8 sec.
Average efflux time of 40% sucrose = 49.5

\[ KV \text{ water} = AT - \frac{B}{T} \]
\[ KV \text{ sucrose} = AT - \frac{B}{T} \]
\[ .8497 = A \frac{31.8}{31.8} - \frac{B}{31.8} \]
\[ 3.73 = A \frac{49.5}{49.5} - \frac{B}{49.5} \]

Solving:

\[ A = .1095 \]
\[ B = 83.783 \]

After the constants for a viscometer are calculated, they are checked by making a run of 40% sucrose at 40°C. With the average efflux time and the constants, the kinematic viscosity is determined. The calculation KV is checked with the KV of the 40% sucrose in literature. They should check closely if constants are correct.
Viscosimeter Method

The method used for determining proteolytic activity was the method used by Maxey (10). An early morning sample of urine was taken and 150 ml. of it was mixed with 150 ml. of acetone, the precipitating reagent. If the precipitate did not form, a little caustic was added until it did. The precipitate contained the proteolytic matter of the urine.

The solution containing the precipitate was put into rubber-lined centrifuge tubes and centrifuged for five minutes. The supernatant liquid was decanted off, and the centrifuge tubes were refilled with more acetone urine solution. The material was centrifuged until all of the precipitate was separated from the urine-acetone mixture. The rubber linings of the centrifuge were turned inside out, depositing the precipitate into a beaker. The use of the rubber lining helped facilitate the complete removal of the proteolytic matter. The rubber lining was washed with a little water, and the washings were allowed to run into the beaker containing the precipitate. Dilute hydrochloric acid was then added until the precipitate just disappeared; caustic and dilute hydrochloric acid were added carefully until the pH was adjusted to 4.2. The solution of the proteolytic matter was then put into a water bath of 40° C. until ready for use.

A gelatin solution was made by weighing out eight gr. of Seidel gelatin and mixing it with ninety-two ml. of water.

(10) Maxey, Frank, Bachelor Thesis, Marquette University, 1942.
Viscometer Method:

The method used for determining proteolytic activity was the method used by Maxey (10). An early morning sample of urine was taken and 160 ml. of it was mixed with 160 ml. of acetone, the precipitating reagent. If the precipitate did not form, a little caustic was added until it did. This precipitate contained the proteolytic matter of the urine. The solution containing the precipitate was put into rubber-lined centrifuge tubes and centrifuged for five minutes. The supernatant liquid was decanted off, and the centrifuge tubes were refilled with more acetone urine solution. The material was centrifuged until all of the precipitate was separated from the urine-acetone mixture. The rubber linings of the centrifuge were turned inside out, depositing the precipitate into a beaker. The use of the rubber lining helped facilitate the complete removal of the proteolytic matter. The rubber lining was washed with a little water, and the washings were allowed to run into the beaker containing the precipitate. Dilute hydrochloric acid was then added until the precipitate just disappeared; caustic and dilute hydrochloric acid were added carefully until the pH was adjusted to 4.2. The solution of the proteolytic matter was then put into a water bath of 40°C. until ready for use.

A gelatin solution was made by weighing out eight gr. of Seidel gelatin and mixing it with ninety-two ml. of water.

(10) Maxey, Frank, Bachelor Thesis, Marquette University, 1942.
The gelatin was then attempered to 40°C., and after one hour of standing, it became a clear homogenous liquid. It was determined by Maxey that 8% gelatin solution was best for the determination.

In preparing the proteolytic solution for a run, 30 ml. of it was mixed with 30 ml. of 8% gelatin. Immediately after this preparation, a blank was prepared by mixing 30 ml. of 8% gelatin with 30 ml. of 4.2 pH water which was previously attempered to 40°C. With great speed, 50 ml. was pipetted out of each solution and put into respective viscometers. A run was made on the blank just as soon as it was placed in the constant temperature bath. The kinematic viscosity of this first run was taken as the initial kinematic viscosity for the proteolytic solutions. Fifteen minutes after the run on the blank a run on the proteolytic material and the blank was made. Runs on both were continued at that time interval until four runs on the proteolytic material were made. After that, the per cent drop in kinematic viscosity was determined.
Proteolytic activity by extraction method:

Two or three early morning samples were needed for this method. 700 ml. of urine were placed in the round bottom flask of the extractor; and ether, the extracting substance, was added until it reached the side arm. About 300 ml. of ether were placed into the large Erlenmeyer flask which had a thermometer propped up in it. Heat was applied to the Erlenmeyer flask by means of a sand-electric hot plate. The pressure was reduced in the flask by the use of an aspirator. The heat and pressure were adjusted so that the temperature inside the Erlenmeyer flask was approximately 35°C. At this temperature the ether boiled and the escaping vapors were condensed by the condenser. The condensed ether forced its way to the bottom of the flask containing the urine by means of a long tube with a fritted glass diffuser and diffused up through the urine into the ether layer above it. This increase in ether volume forced some ether from the round bottom to the Erlenmeyer flask via the side arm. This extraction was continued for 24 hours, but it was not at any time carried out without interruption.

After the extraction, the ether volume was concentrated to about 150 ml. by the regular method for distilling ether and the rest of the ether was removed by placing the beaker of it in a hot water bath. At first, the water was kept hot, but when the material in the beaker was cut down to about 50 mls. the temperature of the water was kept a little below 40°C. The residue, after all ether was removed, was diluted up to
30 ml. of water, pH adjusted to 4.2, and then a run was made on it with a viscometer as described previously.

In order to determine whether material extracted had enzymatic characteristics, the ether after extraction for the 24 hours was divided into two parts. One part, after evaporation of the ether, the residue was put into an oven at 110° for one hour. The other portion was treated normally. Both residues were then diluted up to 30 ml., pH adjusted, and runs were made on them.

One other operation was performed. The urine - after it was extracted with ether - was precipitated by acetone. A run was made on a portion of this urine as described above in the viscometer method. This was done to see if any proteolytic activity was left in the urine after extraction.
Table 1

Date of run - 12/3/47
Date of sample - 12/3/47

Viscometers:

C - contained proteolytic material of urine mixed with 6% gelatin.
B - was blank.

On graph:

Curve A - curve of proteolytic material.
Curve B - blank.

DATA CALCULATIONS GRAPHS

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<th>K.V.</th>
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<tr>
<td>7:15</td>
<td>45.6</td>
<td>34.00</td>
</tr>
</tbody>
</table>

1 drop in K.V. = 4.96%
Table I

Date of run - 12/3/47
Date of sample - 12/3/47

Viscometers:

C - contained proteolytic material of urine mixed with 8% gelatin.
B - was blank.

On graph:

Curve A - curve of proteolytic material.
Curve B - blank.

<table>
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<th>Time</th>
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<th>K.V.</th>
<th>B Time</th>
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<td></td>
<td></td>
<td>7:15</td>
<td>55.6</td>
<td>35.40</td>
</tr>
</tbody>
</table>

% drop in K.V. = 4.98%

Run A
Run-A

Table II

Date of run - 12/5/47
Date of sample - 12/9/47

Viscosimeters:
- contained proteolytic material of urine precipitated from acetone andmixed with 8% gelatin.
- was blank.

On graph:
- Curve A - pure urine material.
- Curve B - blank.

Time in Minutes

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<td>60</td>
<td>35.0</td>
<td>34.5</td>
<td>34.0</td>
<td>33.5</td>
</tr>
</tbody>
</table>
Table II

Date of run - 12/9/47
Date of sample - 12/9/47

Viscometers:

C - contained proteolytic material of urine precipitated from acetone and mixed with 8% gelatin.

B - was blank.

On graph:

Curve A - curve of proteolytic material.

Curve B - blank.

<table>
<thead>
<tr>
<th>Time</th>
<th>C</th>
<th>K.V.</th>
<th>Time</th>
<th>B</th>
<th>K.V.</th>
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<td>7:55</td>
<td>41.0</td>
<td>27.40</td>
<td>7:40</td>
<td>55.5</td>
<td>35.30</td>
</tr>
<tr>
<td>8:10</td>
<td>40.5</td>
<td>26.30</td>
<td>7:55</td>
<td>55.2</td>
<td>35.0</td>
</tr>
<tr>
<td>8:25</td>
<td>39.8</td>
<td>25.10</td>
<td>8:10</td>
<td>55.0</td>
<td>34.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8:25</td>
<td>53.0</td>
<td>34.8</td>
</tr>
</tbody>
</table>

% drop in K.V. = 28.8%

Run B
Run-B

Table III

Date of run = 12/13/47
Date of sample = 12/13/47

Viscosimeters:
- Contains proteolytic material of urine precipitated by extraction mixed with 8% gelatin.
- Was blank.

On graph:
- Curve A = blank.
- Curve B = contains proteolytic material.

% drug in K.V. = 15.5%

Run C

Time in Minutes
Table III

Date of run - 12/13/47
Date of sample - 12/13/47

Viscometers:

C - contained proteolytic material of urine precipitated by acetone mixed with 8% gelatin.

B - was blank.

On graph:

Curve A - curve of proteolytic material.

Curve B - blank.

<table>
<thead>
<tr>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:45</td>
<td>42.5</td>
<td>29.30</td>
<td>12:30</td>
<td>53.5</td>
<td>33.20</td>
</tr>
<tr>
<td>1:00</td>
<td>42.1</td>
<td>28.80</td>
<td>12:45</td>
<td>53.4</td>
<td>33.10</td>
</tr>
<tr>
<td>1:15</td>
<td>41.5</td>
<td>27.90</td>
<td>1:00</td>
<td>53.1</td>
<td>32.70</td>
</tr>
<tr>
<td>1:30</td>
<td>41.2</td>
<td>27.50</td>
<td>1:15</td>
<td>53.0</td>
<td>32.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1:30</td>
<td>53.0</td>
<td>32.60</td>
</tr>
</tbody>
</table>

% drop in K.V. = 16.5%

Run C
Run-C

Table IV

Date of run = 12/20/47
Date of sample = 12/20/47

Viscometers:

- Curve A - curve of proteolytic material
- Curve B - blank

The vessel contained proteolytic material of urine mixed with 8% gelatin was blank.

On graph:

- % drop in K.V. = 8.08%

Run D

Time in Minutes
Table IV

Date of run - 12/20/47
Date of sample - 12/20/47

Viscometers:
  C - contained proteolytic material of urine mixed with 8% gelatin.
  B - was blank.

On graph:
  Curve A - curve of proteolytic material.
  Curve B - blank.

<table>
<thead>
<tr>
<th>Time (Sec)</th>
<th>K.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:30</td>
<td>44.8</td>
</tr>
<tr>
<td>1:45</td>
<td>44.3</td>
</tr>
<tr>
<td>2:00</td>
<td>44.0</td>
</tr>
<tr>
<td>2:15</td>
<td>43.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time (Sec)</th>
<th>K.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:15</td>
<td>54.9</td>
</tr>
<tr>
<td>1:30</td>
<td>54.5</td>
</tr>
<tr>
<td>1:45</td>
<td>54.4</td>
</tr>
<tr>
<td>2:00</td>
<td>54.4</td>
</tr>
<tr>
<td>2:15</td>
<td>54.2</td>
</tr>
</tbody>
</table>

% drop in K.V. = 8.08%

Run D
Run-D

Table V

Date of run - 12/24/47
Date of sample - 12/24/47

Vesicatory:
- contained proteolytic material of urine mixed with 15% gelatin.
- was blank.

On graph:
- curve A - curve of proteolytic material.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>K.V.</th>
<th>Time (min)</th>
<th>K.V.</th>
<th>Time (min)</th>
<th>K.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>15</td>
<td></td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td></td>
<td>28</td>
<td></td>
<td>31.35</td>
<td></td>
</tr>
<tr>
<td>24.2</td>
<td></td>
<td>28.15</td>
<td></td>
<td>31.35</td>
<td></td>
</tr>
<tr>
<td>24.2</td>
<td></td>
<td>28.2</td>
<td></td>
<td>31.35</td>
<td></td>
</tr>
<tr>
<td>24.2</td>
<td></td>
<td>28.25</td>
<td></td>
<td>31.35</td>
<td></td>
</tr>
<tr>
<td>24.2</td>
<td></td>
<td>28.25</td>
<td></td>
<td>31.35</td>
<td></td>
</tr>
<tr>
<td>24.2</td>
<td></td>
<td>28.25</td>
<td></td>
<td>31.35</td>
<td></td>
</tr>
<tr>
<td>24.2</td>
<td></td>
<td>28.25</td>
<td></td>
<td>31.35</td>
<td></td>
</tr>
</tbody>
</table>

$\Delta$ drop in K.V. = 13.746

Time in Minutes
Table V

Date of run - 12/24/47
Date of sample - 12/24/47

Viscometers:

C - contained proteolytic material of urine mixed with 8% gelatin.
B - was blank.

On graph:

Curve A - curve of proteolytic material.
Curve B - blank.

<table>
<thead>
<tr>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:10</td>
<td>43.1</td>
<td>30.30</td>
<td>12:55</td>
<td>54.4</td>
<td>34.20</td>
</tr>
<tr>
<td>1:25</td>
<td>42.8</td>
<td>30.00</td>
<td>1:10</td>
<td>54.2</td>
<td>33.90</td>
</tr>
<tr>
<td>1:40</td>
<td>42.4</td>
<td>29.20</td>
<td>1:25</td>
<td>54.2</td>
<td>33.90</td>
</tr>
<tr>
<td>1:55</td>
<td>42.2</td>
<td>29.00</td>
<td>1:40</td>
<td>54.0</td>
<td>33.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1:55</td>
<td>54.0</td>
<td>33.70</td>
</tr>
</tbody>
</table>

% drop in K.V. = 13.74%

Run E
Run-E

Table VI

Date of Run: 12/30/40
Date of sample: 12/30/40

Viscosity:

The solution contained precipitated material of urine mixed with 6% gelatin.

On bright light:

Curve A = curve of precipitated material
Curve B = blank

KV

Time in Minutes

A

B

11
40
36
32
28
24
15 30 45 60
Table VI

Date of run - 12/26/48
Date of sample - 12/26/48

Viscometers:

C - contained proteolytic material of urine mixed with 8% gelatin.
B - was blank.

On graph:

Curve A - curve of proteolytic material
Curve B - blank.

<table>
<thead>
<tr>
<th>Time</th>
<th>C</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sec.</td>
<td>K.V.</td>
<td>Sec.</td>
</tr>
<tr>
<td>1:55</td>
<td>42.8</td>
<td>29.80</td>
</tr>
<tr>
<td>2:10</td>
<td>42.0</td>
<td>28.70</td>
</tr>
<tr>
<td>2:25</td>
<td>41.6</td>
<td>27.80</td>
</tr>
<tr>
<td>2:40</td>
<td>41.0</td>
<td>27.10</td>
</tr>
</tbody>
</table>

% drop in K.V. = 19.54%
Run-F

Table VII

Date of run: 12/30/47
Date of sample: 12/30/47

Viscosimeters:
- contained proteolytic material of urine, taken
  after exercise, mixed with 5% gelatin.
  - see blank.

On graph:
- Curve A = curve of proteolytic material.

Time in Minutes

<table>
<thead>
<tr>
<th>Time</th>
<th>K.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>33.90</td>
</tr>
<tr>
<td>30</td>
<td>34.20</td>
</tr>
<tr>
<td>45</td>
<td>34.20</td>
</tr>
<tr>
<td>60</td>
<td>35.00</td>
</tr>
</tbody>
</table>
Table VII

Date of run - 12/30/47
Date of sample - 12/30/47

Viscometers:

C - contained proteolytic material of urine, taken after emission, mixed with 8% gelatin.

D - was blank.

On graph:

Curve A - curve of proteolytic material.

Curve B - blank.

<table>
<thead>
<tr>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:30</td>
<td>41.4</td>
<td>27.70</td>
<td>2:15</td>
<td>55.2</td>
<td>35.00</td>
</tr>
<tr>
<td>2:45</td>
<td>41.0</td>
<td>27.40</td>
<td>2:30</td>
<td>54.5</td>
<td>34.20</td>
</tr>
<tr>
<td>3:00</td>
<td>40.8</td>
<td>26.80</td>
<td>2:45</td>
<td>54.5</td>
<td>34.20</td>
</tr>
<tr>
<td>3:15</td>
<td>40.6</td>
<td>26.40</td>
<td>3:00</td>
<td>54.5</td>
<td>34.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3:15</td>
<td>54.2</td>
<td>33.90</td>
</tr>
</tbody>
</table>

% drop in K.V. = 21.42%

Run G
Run-G

Table VIII

Date of run = 12/11/67
Date of sample = 12/19/67

Viscous nature of sample contained precipitable material of urine mixed with 8% gelatine was plated.

On graph:

Curve A = viscous material.
Curve B = same as curve A material.

<table>
<thead>
<tr>
<th>Time in Minutes</th>
<th>K.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>6:00</td>
<td>43.0</td>
</tr>
<tr>
<td>6:15</td>
<td>42.4</td>
</tr>
<tr>
<td>6:30</td>
<td>42.1</td>
</tr>
<tr>
<td>6:45</td>
<td>42.2</td>
</tr>
<tr>
<td>7:00</td>
<td>43.0</td>
</tr>
<tr>
<td>7:15</td>
<td>53.9</td>
</tr>
<tr>
<td>7:30</td>
<td>54.6</td>
</tr>
<tr>
<td>7:45</td>
<td>34.20</td>
</tr>
</tbody>
</table>
Table VIII

Date of run - 12/31/47
Date of sample - 12/31/47

Viscometers:

C - contained proteolytic material of urine mixed with 8% gelatin.
B - was blank.

On graph:

Curve A - curve of proteolytic material.
Curve B - blank.

<table>
<thead>
<tr>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>6:00</td>
<td>43.0</td>
<td>30.20</td>
<td>5:45</td>
<td>55.1</td>
<td>34.90</td>
</tr>
<tr>
<td>6:15</td>
<td>42.4</td>
<td>29.20</td>
<td>6:00</td>
<td>55.0</td>
<td>34.80</td>
</tr>
<tr>
<td>6:30</td>
<td>42.3</td>
<td>29.10</td>
<td>6:15</td>
<td>55.0</td>
<td>34.80</td>
</tr>
<tr>
<td>6:45</td>
<td>42.2</td>
<td>29.00</td>
<td>6:30</td>
<td>54.6</td>
<td>34.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6:45</td>
<td>54.5</td>
<td>34.20</td>
</tr>
</tbody>
</table>

% drop in K.V. = 14.90%

Run H
Run-H

Date of run: 2/4/48
Date of sample: 2/4/48
Viscometers:
contains concentrated material of urine mixed with 8% gelatin.

On graph:
Curve A - urine sample. 60 mL.
Curve B - normal material.

Time in Minutes

[Graph with plotted points and axes labeled]
### Table IX

Date of run - 1/2/48  
Date of sample - 1/2/48

Viscometers:  
- **C** - contained proteolytic material of urine mixed with 8% gelatin.  
- **B** - was blank.

On graph:  
- Curve A - curve of proteolytic material.  
- Curve B - blank.

<table>
<thead>
<tr>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4:15</td>
<td>42.0</td>
<td>28.70</td>
<td>4:00</td>
<td>53.7</td>
<td>33.20</td>
</tr>
<tr>
<td>4:30</td>
<td>41.7</td>
<td>28.10</td>
<td>4:15</td>
<td>53.4</td>
<td>33.00</td>
</tr>
<tr>
<td>4:45</td>
<td>41.2</td>
<td>27.40</td>
<td>4:30</td>
<td>53.2</td>
<td>32.90</td>
</tr>
<tr>
<td>5:00</td>
<td>41.0</td>
<td>27.10</td>
<td>4:45</td>
<td>53.0</td>
<td>32.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5:00</td>
<td>53.0</td>
<td>32.50</td>
</tr>
</tbody>
</table>

% drop in K.V. = 16.23%

Run I
Run-I

The diagram shows the decay of KV over time in minutes. The x-axis represents time in minutes ranging from 15 to 60, and the y-axis represents the KV values ranging from 41 to 24.

Two curves are plotted: one labeled 'A' and another labeled 'B'. The 'A' curve starts at a higher KV value and decreases gradually over time, while the 'B' curve starts at a lower KV value and also decreases gradually.

The diagram suggests a study of KV decay over time, possibly in a controlled experiment or reaction.
Table X

Date of run - 1/10/48
Date of sample - Run J - 1/9/48
Run K - 1/10/48

Viscometers:
C - contained proteolytic material of urine after emission mixed with 8% gelatin. 1/9/48
A - contained proteolytic material of urine with 8% gelatin.
B - was blank.

On graph:
Curve A - curve of proteolytic material.
Curve B - blank.

<table>
<thead>
<tr>
<th>Time</th>
<th>C</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sec.</td>
<td>K.V.</td>
<td>Sec.</td>
<td>K.V.</td>
</tr>
<tr>
<td>4:30</td>
<td>41.7</td>
<td>56</td>
<td>34.80</td>
</tr>
<tr>
<td>4:45</td>
<td>41.2</td>
<td>55</td>
<td>33.80</td>
</tr>
<tr>
<td>5:00</td>
<td>41.0</td>
<td>54.8</td>
<td>33.60</td>
</tr>
<tr>
<td>5:15</td>
<td>40.9</td>
<td>54.6</td>
<td>33.30</td>
</tr>
</tbody>
</table>

% drop in K.V. = 19.00%
Run J

% drop in K.V. = 4.48%
Run K
Run-J

Time in Minutes

K.V.
Run-K

Table XI

Date of run - 1/14/48
Date of sample - Run L - 1/13/48
Run K - 1/14/48

The liquid contained proteolytic material of urine mixed with 8% gelatin.

The liquid contained proteolytic material of urine taken after gelatin was mixed with 8% gelatin.

On graph:
- curve of proteolytic material:
- blank.

<table>
<thead>
<tr>
<th>Time in Min.</th>
<th>K.V.</th>
<th>Time in Min.</th>
<th>K.V.</th>
<th>Time in Min.</th>
<th>K.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4:00</td>
<td>44.8</td>
<td>4:00</td>
<td>52.5</td>
<td>3:40</td>
<td>55.1</td>
</tr>
<tr>
<td>4:15</td>
<td>44.5</td>
<td>4:15</td>
<td>52.2</td>
<td>4:00</td>
<td>55.1</td>
</tr>
<tr>
<td>4:30</td>
<td>44.0</td>
<td>4:30</td>
<td>52.0</td>
<td>4:15</td>
<td>55.1</td>
</tr>
<tr>
<td>4:45</td>
<td>45.7</td>
<td>4:45</td>
<td>51.5</td>
<td>4:30</td>
<td>55.1</td>
</tr>
</tbody>
</table>

% drop in K.V. = 12%
Run L = 1 grammet G

Time in Minutes
Table XI

Date of run - 1/14/48
Date of sample - Run L - 1/13/48
Run M - 1/14/48

Viscometers:

C - contained proteolytic material of urine mixed with 8% gelatin.
A - contained proteolytic material of urine taken after emission mixed with 8% gelatin.
B - was blank.

On graph:

A - curve of proteolytic material.
B - blank.

<table>
<thead>
<tr>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4:00</td>
<td>44.8</td>
<td>32.80</td>
</tr>
<tr>
<td>4:15</td>
<td>44.5</td>
<td>32.30</td>
</tr>
<tr>
<td>4:30</td>
<td>44.0</td>
<td>31.70</td>
</tr>
<tr>
<td>4:45</td>
<td>43.7</td>
<td>31.20</td>
</tr>
</tbody>
</table>

% drop in K.V. = 8.56%
Run L - Viscometer C

% drop in K.V. = 12%
Run M - Viscometer A
Run-M
Run-o
Run-N

Table XIII

Time: 4:44 in - 2/2/48
Date of sample: 2/2/48

Viscometers:
- contained proteolytic material of urine taken after
  emission and mixed with 8% gelatin.
- was blank.

On graph:
- Curve A = blank.
- Curve B = blank.

Time | K.V.  | Time | K.V.  |
-----|-------|------|-------|
4:44 | 43.2  | 4:25 | 39.6  |
4:50 | 42.6  | 4:40 | 38.4  |
5:10 | 42.3  | 4:55 | 38.4  |
5:25 | 42.2  | 5:10 | 38.4  |

% drop in K.V. = 24.10%
Table XIII

Time of run - 2/2/48
Date of sample - 2/2/48

Viscometers:

C - contained proteolytic material of urine taken after emission and mixed with 8% gelatin.

B - was blank.

On graph:

Curve A - curve of proteolytic material.

Curve B - blank.

<table>
<thead>
<tr>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4:40</td>
<td>43.2</td>
<td>30.50</td>
<td>4:25</td>
<td>58.6</td>
<td>38.50</td>
</tr>
<tr>
<td>4:55</td>
<td>42.6</td>
<td>29.90</td>
<td>4:40</td>
<td>58.4</td>
<td>38.30</td>
</tr>
<tr>
<td>5:10</td>
<td>42.3</td>
<td>29.30</td>
<td>4:55</td>
<td>58.4</td>
<td>38.30</td>
</tr>
<tr>
<td>5:25</td>
<td>42.2</td>
<td>29.00</td>
<td>5:10</td>
<td>58.4</td>
<td>38.30</td>
</tr>
</tbody>
</table>

% drop in K.V. = 24.10%

Run P
Run-P

Table XIV

Date of run - 2/9/48
Date of sample - 2/5/48

Viscosimeter contained proteolytic material of urine taken after emission and mixed with 8% gelatin.

% drop in K.V. = 16.96%

Time in Minutes

K.V.
44
40
36
32
28
24

15 30 45 60
Date of run - 2/9/48  
Date of sample - 2/9/48  

Viscometers:

- **C** - contained proteolytic material of urine taken after emission and mixed with 8% gelatin.
- **B** - was blank.

On graph:

- **A** - curve of proteolytic material.
- **B** - blank.

<table>
<thead>
<tr>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5:00</td>
<td>43.0</td>
<td>30.20</td>
<td>4:45</td>
<td>54.5</td>
<td>34.20</td>
</tr>
<tr>
<td>5:15</td>
<td>42.5</td>
<td>29.10</td>
<td>5:00</td>
<td>54.4</td>
<td>34.10</td>
</tr>
<tr>
<td>5:30</td>
<td>42.2</td>
<td>28.90</td>
<td>5:15</td>
<td>54.4</td>
<td>34.10</td>
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<tr>
<td>5:45</td>
<td>41.8</td>
<td>28.30</td>
<td>5:30</td>
<td>54.4</td>
<td>34.10</td>
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<td></td>
<td></td>
<td>5:45</td>
<td>54.4</td>
<td>34.10</td>
</tr>
</tbody>
</table>

% drop in K.V. = 16.96%  

Run Q
Run 9

Date of run - 2/21/48
Date of sample - 2/17/48

Materials:
A - contained proteolytic material of urine after extraction with ether and 8% gelatin.
B - was blank.

Graph shows curve of proteolytic material over time. Curve B = blank.

Time in Minutes: 15, 30, 45, 60

% Drop in K.V. = 16.75%
Table XV

Date of run - 2/21/48
Date of sample - 2/17/48

Viscometers:

C - contained proteolytic material of urine after extraction with ether and 8% gelatin.

B - was blank.

On graph:

Curve A - curve of proteolytic material.

Curve B - blank.

<table>
<thead>
<tr>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>3:55</td>
<td>43.5</td>
<td>30.80</td>
<td>3:40</td>
<td>56.4</td>
<td>36.30</td>
</tr>
<tr>
<td>4:10</td>
<td>43.1</td>
<td>30.30</td>
<td>3:55</td>
<td>56.3</td>
<td>36.20</td>
</tr>
<tr>
<td>4:25</td>
<td>42.9</td>
<td>29.90</td>
<td>4:10</td>
<td>56.2</td>
<td>36.10</td>
</tr>
<tr>
<td>4:40</td>
<td>42.7</td>
<td>29.70</td>
<td>4:25</td>
<td>56.0</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>4:40</td>
<td>56.0</td>
<td>35.80</td>
</tr>
</tbody>
</table>

% drop in K.V. = 16.79%

Run R
Run-R

Diagnosis: 
Date of Sample: 

Viscoseters:

- contained and enzyme solution of urine and 6% gelatin. The mixture was then extracted with ether and ethanol. The precipitate was precipitated by

On graph:

Curve A:

Curve B:

Time in Minutes

Points:

15 30 45 60
Table XVI

Date of run - 2/28/48
Date of sample - 2/17/48

Viscometers:

C - contained proteolytic material of urine and 8% gelatin. The urine had already been extracted with ether and material in this viscometer was precipitated by acetone to see if there was any proteolytic material left in the urine.

C - was blank.

On graph:

Curve A - curve of proteolytic material.
Curve B - blank.

<table>
<thead>
<tr>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:40</td>
<td>42.5</td>
<td>29.10</td>
</tr>
<tr>
<td>2:55</td>
<td>42.3</td>
<td>29.00</td>
</tr>
<tr>
<td>3:10</td>
<td>42.2</td>
<td>28.90</td>
</tr>
<tr>
<td>3:25</td>
<td>42.2</td>
<td>28.90</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:25</td>
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<td>34.30</td>
</tr>
<tr>
<td>2:40</td>
<td>54.6</td>
<td>34.30</td>
</tr>
<tr>
<td>2:55</td>
<td>54.4</td>
<td>34.10</td>
</tr>
<tr>
<td>3:10</td>
<td>54.4</td>
<td>34.10</td>
</tr>
<tr>
<td>3:25</td>
<td>54.4</td>
<td>34.10</td>
</tr>
</tbody>
</table>

\% drop in K.V. = 14.12\%

Run S
Run-S

Table XVII

Date of run - 3/19/48
Date of sample - 3/18/48
3/17/48

Vials A and B contained proteolytic material of urine obtained by extracting with ether and 8% gelatin.

A - blank.
B - blank.

On graph:

- A - proteolytic curve.
- B - blank.

% drop in K.V. = 12.30%
Table XVII

Date of run - 3/19/48
Date of sample - 3/18/48
3/17/48

Viscometers:

C - contained proteolytic material of urine obtained by extracting with ether and 8% gelatin.

B - was blank.

On graph:

Curve A - proteolytic curve.
Curve B - blank.

<table>
<thead>
<tr>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4:15</td>
<td>44.7</td>
<td>32.60</td>
<td>4:00</td>
<td>56.</td>
<td>35.80</td>
</tr>
<tr>
<td>4:30</td>
<td>43.9</td>
<td>31.50</td>
<td>4:15</td>
<td>55.9</td>
<td>35.70</td>
</tr>
<tr>
<td>4:45</td>
<td>43.6</td>
<td>31.00</td>
<td>4:30</td>
<td>55.8</td>
<td>35.60</td>
</tr>
<tr>
<td>5:00</td>
<td>43.3</td>
<td>30.60</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>5:00</td>
<td>55.2</td>
<td>35.00</td>
</tr>
</tbody>
</table>

% drop in K.V. = 12.30%

Run T
Run-T

Time in Minutes

K.V.

A

B

Date

Dated

Viscosity

mm

On 1:20

2:05

1:55

1:35

1:20

24

28

32

36

10

14

mm

viscosity of urine which had
already been analyzed with ether. This material
was then dried for determination by analysis.
Table XVIII

Date of run - 3/26/48
Date of sample - 3/17/48
3/18/48

Viscometers:

C - contained proteolytic material of urine which had already been extracted with ether. This material in the viscometer was precipitated by acetone.

B - was blank.

On graph:

Curve A - proteolytic curve.
Curve B - blank.

% drop in K.V. = 6.31%
Run U

<table>
<thead>
<tr>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:20</td>
<td>45.1</td>
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<td>1:05</td>
<td>54.9</td>
<td>34.70</td>
</tr>
<tr>
<td>1:35</td>
<td>44.9</td>
<td>33.00</td>
<td>1:20</td>
<td>54.8</td>
<td>34.60</td>
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<tr>
<td>1:50</td>
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<td>32.20</td>
<td>1:35</td>
<td>54.8</td>
<td>34.60</td>
</tr>
<tr>
<td>2:05</td>
<td>44.3</td>
<td>32.10</td>
<td>1:50</td>
<td>54.8</td>
<td>34.60</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>2:05</td>
<td>54.6</td>
<td>34.30</td>
</tr>
</tbody>
</table>
Run-U

Date of run - 3/31/49
Date of sample - 3/30/49.

K.V.

Time in Minutes

15 30 45 60

A B

Graphs:
Curve A - proteolytic curves of urine heated.
Curve B - blank.

Notes:
- contained portion of proteolytic material of urine obtained through extraction on ether.
- contained portion of proteolytic material of urine obtained during collection on ether. This portion was put in 10% aque for one hour.
Table XIX

Date of run - 3/31/48
Date of sample - 3/28/48
3/29/48

Viscometers:

C - contained portion of proteolytic material of urine obtained through extraction with ether.
A - contained portion of proteolytic material of urine obtained through extraction with ether. This portion was put in an oven for one hour.
B - was blank.

On graph:

Curve C - proteolytic curve of urine treated normally.
Curve A - proteolytic curve of urine when heated.
Curve B - blank.

<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>
</tr>
</thead>
<tbody>
<tr>
<td>5:55</td>
<td>37.7</td>
<td>21.80</td>
<td>5:40</td>
<td>56.3</td>
<td>36.20</td>
<td>5:55</td>
<td>50.7</td>
<td>29.80</td>
</tr>
<tr>
<td>6:10</td>
<td>37.3</td>
<td>21.30</td>
<td>5:55</td>
<td>56.1</td>
<td>36.10</td>
<td>6:10</td>
<td>50.2</td>
<td>28.60</td>
</tr>
<tr>
<td>6:25</td>
<td>37.1</td>
<td>21.00</td>
<td>6:10</td>
<td>56.0</td>
<td>35.80</td>
<td>6:25</td>
<td>49.9</td>
<td>28.30</td>
</tr>
<tr>
<td>6:40</td>
<td>37.1</td>
<td>21.00</td>
<td>6:25</td>
<td>56.0</td>
<td>35.80</td>
<td>6:40</td>
<td>49.9</td>
<td>28.30</td>
</tr>
<tr>
<td></td>
<td>6:40</td>
<td>56.0</td>
<td>35.80</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

% drop in K.V. (normal) = 40.86%
% drop in K.V. (oven) = 16.20%

Run V
Table XX

Date of run - 4/2/48
Date of sample - 3/28/48

Viscometers:

C - contained proteolytic material of urine which was already extracted with ether. This proteolytic material was precipitated by acetone.

B - was blank.

On graph:

Curve A - proteolytic curve
Curve B - blank

<table>
<thead>
<tr>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>3:55</td>
<td>42.5</td>
<td>29.40</td>
<td>3:40</td>
<td>55</td>
<td>34.80</td>
</tr>
<tr>
<td>4:10</td>
<td>41.5</td>
<td>27.80</td>
<td>3:55</td>
<td>54.8</td>
<td>34.60</td>
</tr>
<tr>
<td>4:25</td>
<td>41.3</td>
<td>27.60</td>
<td>4:10</td>
<td>54.5</td>
<td>34.50</td>
</tr>
<tr>
<td>4:40</td>
<td>41.2</td>
<td>27.40</td>
<td>4:25</td>
<td>54.5</td>
<td>34.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4:40</td>
<td>54.5</td>
<td>34.50</td>
</tr>
</tbody>
</table>

% drop in K.V. = 20%

Run W
Run-W

Time in Minutes

KV

Time
3:00
3:15
3:45
22
28
32
36
40
44
Date of run - 4/10/48
Date of sample - 4/7/48

Viscometers:

C - contained portion of proteolytic material obtained through extraction with ether.
A - contained portion of proteolytic material which was heated in an oven for one hour.
B - was blank.

On graph:

Curve A - curve of proteolytic material.
Curve C - curve of proteolytic material heated in oven.
Curve B - blank.

<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>
</tr>
</thead>
<tbody>
<tr>
<td>3:00</td>
<td>37.6</td>
<td>21.70</td>
<td>3:00</td>
<td>47</td>
<td>24.9</td>
<td>2:45</td>
<td>51.4</td>
<td>31.20</td>
</tr>
<tr>
<td>3:15</td>
<td>37.5</td>
<td>21.60</td>
<td>3:15</td>
<td>47</td>
<td>24.9</td>
<td>3:00</td>
<td>51.2</td>
<td>31.00</td>
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<tr>
<td>3:30</td>
<td>37.4</td>
<td>21.30</td>
<td>3:30</td>
<td>46.9</td>
<td>24.8</td>
<td>3:15</td>
<td>51.1</td>
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</tr>
<tr>
<td>3:45</td>
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<td>21.30</td>
<td>3:45</td>
<td>46.9</td>
<td>24.8</td>
<td>3:30</td>
<td>51.1</td>
<td>30.5</td>
</tr>
</tbody>
</table>

% drop in K.V. = 29.40%
% drop in K.V. (oven) = 18.30%

Run X
Run-X

Table X

Time of run: 4/12/48
Date of sample: 4/7/48

- contained proteolytic material from urine already extracted by ether.
- was blank.

On graph:
- Curve A - proteolytic curve.
- Curve B - blank.

<table>
<thead>
<tr>
<th>Time (Sec.)</th>
<th>K.V.</th>
<th>Time (Sec.)</th>
<th>K.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>34.2</td>
<td>45</td>
<td>33.4</td>
</tr>
<tr>
<td>30</td>
<td>34.0</td>
<td>60</td>
<td>33.4</td>
</tr>
<tr>
<td>15</td>
<td>33.4</td>
<td>45</td>
<td>33.4</td>
</tr>
<tr>
<td>30</td>
<td>33.4</td>
<td>60</td>
<td>33.4</td>
</tr>
</tbody>
</table>

S drop in K.V. = 39.8

Time in Minutes
Table XXII

Time of run - 4/12/48
Date of sample - 4/7/48
4/8/48

Viscometers:

C - contained proteolytic material from urine already extracted by ether.

B - was blank.

On graph:

Curve A - proteolytic curve.

Curve B - blank.

<table>
<thead>
<tr>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
</tr>
</thead>
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<tr>
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<td>9:05</td>
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<td>34.1</td>
</tr>
<tr>
<td>9:35</td>
<td>37.6</td>
<td>21.80</td>
<td>9:20</td>
<td>54.3</td>
<td>34.0</td>
</tr>
<tr>
<td>9:50</td>
<td>37.2</td>
<td>21.10</td>
<td>9:35</td>
<td>54.1</td>
<td>33.7</td>
</tr>
<tr>
<td>10:05</td>
<td>37.0</td>
<td>20.90</td>
<td>9:50</td>
<td>53.8</td>
<td>33.4</td>
</tr>
</tbody>
</table>

\[ \text{% drop in K.V.} = 39.80\% \]

Run Y
Run-Y

The action of the enzyme hyaluronidase on gelatin:

1. An mg. of hyaluronidase were mixed with 50 ml. of water. An attempt was then made to find the amount of this water-gelatin mixture which would give an appreciable drop in density of density of gelatin solution. Equal volumes of hyaluronidase solutions and 5% gelatin solution gave a satisfactory result. 1 mg. of this water solution of the enzyme was enough and this proved to be satisfactory for the action of hyaluronidase.
The action of the enzyme hyaluronidase on gelatin:

Ten mg. of hyaluronidase were mixed with 50 mls. of water. An attempt was then made to find the amount of this water-enzyme mixture which would give an appreciable drop in the kinematic viscosity of a gelatin solution. Equal volumes of hyaluronidase solution and an 8% gelatin solution gave a satisfactory drop in viscosity. The pH of this water solution of the enzyme was about 5, and this proved to be satisfactory for the action of the enzyme.
Table XXIII

Time of Run - 7/10/48

Viscometers:
  C - water solution of hyaluronidase plus gelatin.
  B - blank.

On graph:
  Curve A - curve of enzymatic material.
  Curve B - blank.

<table>
<thead>
<tr>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
<th>Time</th>
<th>Sec.</th>
<th>K.V.</th>
</tr>
</thead>
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<tr>
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<td>31.6</td>
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<td>40.9</td>
<td>26.00</td>
<td>12:40</td>
<td>51.9</td>
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</tr>
<tr>
<td>1:10</td>
<td>40.1</td>
<td>25.70</td>
<td>12:55</td>
<td>51.7</td>
<td>31.10</td>
</tr>
</tbody>
</table>

% drop in K.V. = 17.00%

Run BB
Run BB

Conclusions

Urine contains two factors capable of decreasing the viscosity of a gelatin solution.

One of these factors is ether soluble and the other is not soluble in ether but does precipitate when mixed with alcohol.

The ability of urine to decrease the viscosity of a gelatin solution varies from day to day.

A sample of urine taken from a male eight to ten hours after meal showed the greatest capacity to reduce the viscosity of gelatin. This may be due to the presence of hyaluronidase.

A commercial sample of hyaluronidase did decrease the viscosity of gelatin solution. This fact leads to a tentative statement that hyaluronic acid may be present in gelatin. There are no authorities to quote for this statement.

Suggestions for Further Work

Determine if there is an increase in the rate of decrease of a gelatin solution after its viscosity has been decreased by hyaluronidase.

Determine if the viscosity change of a gelatin solution can be used as a quantitative determination of hyaluronidase.

Run BB can be extracted from the aqueous solution of other.

Time in Minutes
Conclusions

Urine contains two factors capable of decreasing the viscosity of a gelatin solution.

One of these factors is ether soluble and the other is not soluble in ether but does precipitate when mixed with acetone.

The ability of urine to decrease the viscosity of a gelatin solution varies from day to day.

The sample of urine taken from a male eight to ten hours after an emission showed the greatest ability to reduce the viscosity of gelatin. This may be due to the presence of hyaluronidase.

A commercial sample of hyaluronidase did decrease the viscosity of a gelatin solution. This fact leads to a tentative statement that hyaluronic acid may be present in gelatin. There are no authorities to quote for this statement.

Suggestions for Further Work

Determine if there is an increase in the reducing power of a gelatin solution after its viscosity has been decreased by hyaluronidase.

Determine if the viscosity change of a gelatin solution can be used as a quantitative determination of hyaluronidase.

Determine if hyaluronidase can be extracted from an aqueous solution by ether.
VI DISCUSSION

Discussion

is described in the procedure, measurement of the proteolytic activity is determined with the help of a blank. The blank is used to determine the kinematic viscosity of a solution which has no proteolytic activity. The water in the blank takes the place of the proteolytic material used in the other viscometer. Everything being identical, i.e., the amount of material used, pH, temperature and rate of efflux, an accurate kinematic viscosity of each solution can be determined. Then using the kinematic viscosities of the two viscometers, a per cent drop in kinematic viscosity can be determined to see whether any proteolytic activity is present.

In determining in kinematic viscosity for each solution, the first run on the blank is 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 having tested for proteolytic activity, is divided by the initial kinematic viscosity. The quotient is multiplied by 100. The number is the per cent drop in kinematic viscosity for the solution containing the solution and the proteolytically active material, i.e., the blank, by plotting the kinematic viscosity against the rate of efflux for both the blank and the proteolytic solution, we can see how much of a per cent drop there is in each run.

In using the water extractors, the other vapors when condensed and taken in the mixture of the flask containing.
Discussion

As described in the procedure, measurement of the proteolytic is determined with the help of a blank. The blank is used to determine the kinematic viscosity of a solution which has no proteolytic activity. The water in the blank takes the place of the proteolytic material used in the other viscometer. Everything being uniform, i.e., the amount of material used, pH, temperature and time of efflux, an accurate kinematic viscosity of each solution can be determined. Then using the kinematic viscosities of the two viscometers, a per cent drop in kinematic viscosity can be determined to see whether any proteolytic activity is present.

In determining the per cent drop in kinematic viscosity for each solution, the first run on the blank is 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 proteolytic activity, is divided by the initial kinematic viscosity. The quotient is multiplied by 100. The produce is the per cent drop in kinematic viscosity for the solution containing the gelatin and the proteolytically active material from the urine. By plotting the kinematic viscosity against the time of efflux for both the blank and the proteolytic material, one can see how much of a per cent crop there is in each run.

In using the Koch estrogen extractor, the ether vapors when condensed and taken to the bottom of the flask containing
the urine pick up any apparent proteolytic material in the urine while diffusing up through it. This ether has the advantage over acetone in that with ether only a small amount of material is left over after its evaporation which seems to be the apparent proteolytic material alone. With acetone, a large voluminous precipitate is formed which contains urea, phosphate salts and other material besides the proteolytic matter. The small residue from the ether extract seems to give better results than the acetone precipitate.

The residue left from the extraction method was reddish in color, and oily in nature. It had quite an offensive odor. Care had to be taken when driving off the ether from this material in the water bath. If the water was much above 40°C., the enzymatic material would be killed.

In regard to the hyaluronidase, the strength of it compared to the other enzymatic material the author worked with was not known. Therefore as a start, ten mg. in 50 mls. of water was used. It was found that taking 30 mls. of this water solution mixed with 30 mls. of 8% gelatin gave a good drop in kinematic viscosity, when 50 mls. of this mixture was run in a viscometer. 30 mls. of this water-enzyme mixture was the minimum amount of material needed to produce a kinematic viscosity drop.
VII CONCLUSIONS

SUGGESTIONS FOR FURTHER WORK

This book is centered around the structure, reactions and practical application of gelatin.


This book describes methods used for viscosity determination.


The author emphasizes the importance of each type of fluid and plastic and the conditions which affect viscosity.

8. ANNOTATED BIBLIOGRAPHY


This book presents a complete treatment of gelatin, much of it devoted to the effect of hydrogen ion concentration.


This book gives a lengthy discussion on gelatin and glue.


This book gives a good summary of enzymes. It describes in detail the properties of enzymes.

   This book is centered around the structure reactions and practical application of gelatin.


   This book describes methods used for viscometric determination.


   The author goes into the theory of each type of viscometry, showing various conditions which effect viscosity.


   This book presents a complete treatment of gelatin. Much of it is devoted to the effect of hydrogen ion concentration.


   This book gives a lengthy discussion on gelatin and glue.


   This book gives a good summary of enzymes. It describes in detail the properties of enzymes.

The chapter referred to was on viscosity. The limits of this book being wider than just viscosity, these authors give just the fundamental principles and definitions of viscosity.


This is a new journal. Dr. Harris describes his findings in the first issue of this journal.


"A Possible Prenatal Sex Determination and Viscometric Determination for the Proteolytic Activity of Various Urines Using Gelatin as a Substrate."


"A Viscometric Method for the Determination of the Proteolytic Effect of Normal Urine Using Gelatin as a Substrate."


Section of this book used for this thesis dealt with proteins and their linkages.


The section consulted dealt with the pH effecting enzyme.


Tauber gave a rather thorough discussion of the conditions effecting enzyme action in general. Most of the book is devoted to particular enzymes.


A small section of the book was used to obtain equation and definition of kinematic viscosity.


"A Measure of the Proteolytic Effect of Pepsin on Various Gelatins Using the New Digestion-Flask Viscometer.

APPROVED

John P. Koch  
Major Professor

DATE July 29, 1948.