STUDIES IN BENCE-JONES PROTEINURIA.

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The subject of these studies is an intelligent patient under the care of Dr. C. H. Schoff, of Media, Pa., who supplied the following summary of the history.¹

J. E. L. Age 52. White. Male. Childhood unimportant. When 26 he began to lose weight and after a short period of ill health had a hemorrhage from the lungs. After 2 years in the West he entirely regained weight and health. 4 years ago he commenced having attacks of bronchitis accompanied by severe shooting and aching pains in the back and legs. The last severe attack of these pains commenced in February, 1915, and continued for several months. In April of that year Bence-Jones protein was first discovered in the urine. There were at times cough and expectoration, but never tubercle bacilli in the sputum. A few of the latter were at one period said by another observer to be found in the urine, but this could never afterwards be confirmed. Marked anemia, red corpuscles 2,000,000, and hemoglobin 40 to 50 per cent. Great prostration. Weight reduced from a normal of about 150 pounds to 120 pounds. Considerable proteinuria. Since last autumn the patient has gradually improved, and at present, March 1, 1916, his weight has become practically normal; red corpuscles 4,000,000. He has now no acute pain in the back or legs, but sometimes slight aching and stiffness. He can walk some distance, but still has a feeling of weakness. Protein excretion still marked, hyaline and a few granular casts, not much improvement of vision (diagnosis by a specialist, paralysis of optic nerve). X-ray findings both of spine and long bones have always been entirely normal.

The urine has been uniform, of normal appearance except for the exaggerated foam formation upon slight shaking, and with marked acid reaction (litmus). The total protein was weighed from time to time and has also been rather constant, fluctuating between 23 and 30 gm. daily.

¹ A discussion of the clinical features of this case will be published shortly by Dr. Schoff.
Heat Reactions.—When the urine at natural reaction is heated coagulation commences at about $55^\circ$, and proceeds rapidly as the temperature passes this point. There is no apparent tendency to decrease until the boiling point is almost reached ($97^\circ$ or $98^\circ$) when a slight lightening of the density is sometimes to be noticed. As the liquid begins to boil, further clearing is observed, and when boiling has continued for a few seconds nearly all the protein is redissolved. Upon cooling, the protein commences to re-precipitate as soon as the temperature has been lowered a few degrees, and finally becomes as dense as before; this can be repeated indefinitely. It is clear that we have to do with the so-called Bence-Jones protein. In order to observe the above in typical form it is necessary to dilute the urine, which is best done by adding normal urine. If the dilution is made with water the protein will often redissolve badly unless a salt or, better, a few crystals of urea are added. If the urine is heated at its natural concentration some of the protein will be precipitated in elastic clumps (they can be drawn out to form bundles of coarse threads), which tend to adhere to the sides of the vessel or float on the surface and do not again dissolve. These clumps have also been described by Magnus-Levy. When the Bence-Jones urine is diluted the precipitate is fine and milky, going in and out of solution with ease.

The addition to the native urine of one drop of 50 per cent acetic acid to 10 cc. of urine, prevents the formation of the clumps and causes the solution at boiling and reprecipitation on cooling to be much more clearly manifested. That this may be associated with some such action as acid-albumin formation is suggested by the following experiment.

We heated in the water bath at $52^\circ$ for 10 minutes urine to which had been added one drop of 20 per cent acetic acid for each 10 cc. In order to obtain a maximum surface and so insure prompt heating, the urine was divided among a number of test-tubes. No apparent change was produced.

The temperature of the bath was then raised to $56^\circ$ for 10 minutes, with the result of a heavy white curdy precipitate. This was filtered off, and of this filtrate, A, one part was diluted with three parts normal urine and heated over the flame. This produced a heavy white opacity which largely disappears on boiling, and upon the addition of some urea almost entirely

disappears; in either case it reappears when the liquid cools, and again dis-
appears upon boiling.

The remainder of the filtrate A was put back in the water bath at 60° for
10 minutes. Result, heavy white curdy precipitate. It was filtered, and
of this filtrate, B, one part was diluted with three parts normal urine and
heated over the flame. This produced a moderate white opacity, which
disappeared almost entirely upon boiling and reappeared on cooling.

The remainder of the filtrate B was again heated in the water bath, this
time at 64° for 12 minutes. Result, moderately heavy white curdy precip-
itate. This was filtered, and of this filtrate, C, a portion diluted as before
was heated over the flame. This produced a slight opacity, which disap-
appeared almost entirely upon boiling, and reappeared on cooling.

The remainder of filtrate C was heated in the bath at 68° for 15 minutes.
Result, slight white curdy precipitate. This was filtered, and of this fil-
trate, D, a portion without dilution was heated over the flame. The result
was a slight opalescence, part of which disappeared upon boiling and re-
turned on cooling.3

The rest of filtrate D was heated in the bath at 72° for 15 minutes. Re-
sult, slight opalescence. The filtrate, which even after repeatedly passing
through the filter remained opalescent, was heated without dilution over
the flame. Result, no change.

Under the conditions of the above experiment there is a con-
tinued precipitation from the temperature at which the Bence-
Jones protein commences to come out up to the coagulation point
of serum protein. This may be explained either by assuming
that the Bence-Jones protein is composed of various fractions
with different coagulation points, at least with different rapidity
of precipitation; or, on the other hand, that the continued action
of the acid had resulted in gradual change of the order of acid-
albumin formation. We therefore made the following experiment.

Four tubes containing, respectively, 10 cc. of urine, 10 cc. of urine + 1
drop of 20 per cent acetic acid, 10 cc. of urine + 2 drops of acid, and 10 cc.
of urine + 3 drops of acid, and a control tube of 10 cc. of nephritic urine
(rich in albumin but apparently free from Bence-Jones protein) + 1 drop
of the above acid, were placed in a water bath at 60° for 1½ hours. The
Bence-Jones tubes commenced to precipitate in the order 2, 3, 1, 0 (referred
to by the acid content), following each other at intervals of 15 seconds to 1
minute. Soon all showed a heavy white curdy precipitate, the tubes appear-

3 In order to make a certain judgment with these slight degrees of opal-
escence, the tube of urine is brought to boiling and then, avoiding shaking,
the lower end (2 cm.) is held under the cold water tap, which permits of
easy comparison between the hot and cold fluid.
The control tube clouded slightly, and finally showed a very slight flocculent precipitate. At the end of the time the contents of all of the tubes were filtered and the respective filtrates heated over the flame. The filtrate from Tube 0 showed only slight opalescence, which was hardly affected by boiling. The same was true of Tube 1, although this was slightly decreased by boiling. These two filtrates also gave only moderate reactions with the Roberts test. The filtrate from Tube 2 showed marked opalescence, which nearly all disappeared upon boiling. The filtrate from Tube 3 gave a much heavier opalescence amounting almost to opacity, and this also was nearly all redissolved upon boiling. In both of these the original density returned on cooling. The filtrate from the control tube gave a considerable precipitate which showed no tendency to redissolve upon boiling.

It seems evident, therefore, that heating the Bence-Jones protein with even very moderate quantities of acetic acid is capable of effecting a change in the nature of the protein such that the temperature at which it coagulates is raised; but this in no way interferes with its characteristic behavior in regard to going into solution by boiling and reprecipitating on cooling.

*Salt Reactions.*—At the natural reaction of the urine one volume of saturated solution of ammonium sulfate added to one volume of urine causes incomplete precipitation, while two volumes cause complete precipitation.

Saturation with magnesium sulfate causes a considerable but not complete precipitation. This is opposed to the findings of most other writers, but Ville and Derrien found that saturation with magnesium sulfate precipitated all the protein. When the urine is strongly acidified with acetic acid all the protein is precipitated by adding three volumes of a saturated solution of magnesium sulfate to one volume of urine.

If the urine at natural reaction be saturated with sodium sulfate, a large but not complete precipitation occurs. If a twice cold saturated solution of sodium sulfate be made up with hot water and allowed to cool almost to room temperature, it will be some time before the excess crystallizes out; if before this takes place one volume of the solution is added to an equal volume of urine containing a considerable amount of acetic acid, the precipitation is complete. Ammonium sulfate has the tendency to remove the pigment from the urine along with the protein; but sodium sul-

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fate has little action on the pigments, and the protein when washed is pure white. The difficulty mentioned by Boggs and Guthrie\(^5\) in obtaining a pigment-free precipitate is thus avoided.

If the urine at natural reaction is saturated with sodium chloride a slight opalescence results, but half saturation produces no effect. However, if sufficient acetic acid is added, precipitation is complete. Thus:

<table>
<thead>
<tr>
<th>Urine</th>
<th>100 parts</th>
<th>50 per cent acetic acid</th>
<th>8 &quot;</th>
<th>Saturated solution NaCl</th>
<th>108 &quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gradual and incomplete precipitation.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Urine</th>
<th>100 &quot;</th>
<th>50 per cent acetic acid</th>
<th>20 &quot;</th>
<th>Saturated solution NaCl</th>
<th>120 &quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prompt and complete precipitation.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

When the clear filtrate from this last precipitation is added to an equal volume of Bence-Jones urine only a slight precipitate is caused; but if more acetic acid is added heavy precipitation occurs at once. It would seem that a large amount of acid is fixed in breaking up some alkaline combination in which the protein is normally found, and that most salts precipitate it quantitatively only after this decomposition has been effected.

Spiegler's solution, Roberts' reagent, and acetic acid and ferrocyanide all cause immediate heavy precipitation when used with the native urine; and all are capable of indicating minute traces of this protein. This is of course what would be expected, and is mentioned only because of the fact found by Folin and Denis\(^6\) that the Bence-Jones protein gave no precipitate with acetic acid and ferrocyanide except after long standing.

**Acids.**—Concentrated sulfuric, nitric, and hydrochloric acids added drop by drop cause a heavy white curdy precipitate, insoluble in moderate excess of the acid. A large excess causes solution by destruction, which is indicated by the change of color in the case of the sulfuric and nitric acids. Acetic acid added drop by drop may at first cause a very slight cloudiness (often entirely absent—nucleoprotein) which disappears upon adding a little more acid. Citric acid causes no cloudiness.

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\(^6\) Folin, O., and Denis, W., *J. Biol. Chem.*, 1914, xviii, 277.
Urine diluted with two parts of water and treated with a small amount of either of the three mineral acids mentioned gives a dense white precipitate which dissolves upon boiling and reprecipitates upon cooling.

This solvent power of hot acids furnishes a satisfactory means of distinguishing between normal serum- and Bence-Jones protein. To a test-tube containing a few cc. of the urine to be examined is added a drop of concentrated nitric acid. This produces a white cloud or white curdy precipitate according to the amount of protein present; if a heavy curd is formed the urine should be diluted with water. Upon shaking, the precipitate disappears. Continue adding the acid drop by drop, shaking between each addition, until the precipitate or cloud no longer dissolves, then add one or two drops more. The contents of the tube are now brought to boiling. If the precipitate is Bence-Jones protein it will quickly dissolve, and upon cooling reprecipitates with the same white appearance as before; and this solution and precipitation can be repeated. If, on the other hand, the precipitate is due to normal serum protein it does not dissolve on boiling, but assumes the form of discrete flocks of a dirty yellowish or brownish color, not affected by cooling and not resembling in any way the white Bence-Jones precipitate.

Precipitation with HCl has been recommended as a specific test for Bence-Jones protein in the urine by Bradshaw who states that albumin does not give this reaction unless present in very large amounts. Using strong HCl he assigns the limit of sensitiveness to Bence-Jones protein as 0.05 per cent, but apparently did not ascertain the percentage of serum protein that can be present without reacting. By a number of trials we found that the best differentiation occurs when: (1) the percentage of proteins is small; (2) the acid is comparatively weak, about 1 to 20 or 25; (3) when dilute acid is used, because if strong acid is added there is likely to be an immediate, more or less irreversible precipitation with either protein at the point of contact; (4) the mixture of urine and acid must be allowed to stand for a definite time, since eventually either protein will come down more or less completely.

In accordance with these principles the following method of testing was adopted. The dilute hydrochloric acid used consisted of concentrated acid one part, water three parts. In making the test the tube was well shaken after each addition, and after the acid was added was allowed to stand 1 minute before observing.

<table>
<thead>
<tr>
<th>Test Description</th>
<th>Volume</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal urine</td>
<td>5 cc</td>
<td>No reaction</td>
</tr>
<tr>
<td>Normal dog serum</td>
<td>0.25 cc</td>
<td>No reaction</td>
</tr>
<tr>
<td>HCl (diluted)</td>
<td>1 cc</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test Description</th>
<th>Volume</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal urine</td>
<td>5 cc</td>
<td>No reaction or doubtful.</td>
</tr>
<tr>
<td>Serum</td>
<td>0.5 cc</td>
<td></td>
</tr>
<tr>
<td>HCl (diluted)</td>
<td>1 cc</td>
<td>Doubtful or very faint.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test Description</th>
<th>Volume</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal urine</td>
<td>5 cc</td>
<td>Very faint opalescence.</td>
</tr>
<tr>
<td>Serum</td>
<td>2 cc</td>
<td></td>
</tr>
<tr>
<td>HCl (diluted)</td>
<td>1 cc</td>
<td></td>
</tr>
</tbody>
</table>

Even the last test was so faint that it would probably be rejected; but calling it positive, and assuming 6.5 per cent as the total protein content of the dog serum, the least amount of serum protein which will react with this test is 0.13 gm. protein or 1.86 per cent.

Testing now with Bence-Jones protein:

<table>
<thead>
<tr>
<th>Test Description</th>
<th>Volume</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal urine</td>
<td>5 cc</td>
<td>Faint opalescence.</td>
</tr>
<tr>
<td>Bence-Jones urine (2.7 per cent)</td>
<td>0.25 cc</td>
<td></td>
</tr>
<tr>
<td>HCl (diluted)</td>
<td>1 cc</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test Description</th>
<th>Volume</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal urine</td>
<td>5 cc</td>
<td>Well marked opalescence.</td>
</tr>
<tr>
<td>Bence-Jones urine (2.7 per cent)</td>
<td>0.5 cc</td>
<td></td>
</tr>
<tr>
<td>HCl (diluted)</td>
<td>1 cc</td>
<td></td>
</tr>
</tbody>
</table>

The first of these two tests is somewhat clearer than the last of the previous series. However, to be on the safe side, if the second test be taken as the limit then the content is 0.013 gm. protein; thus, urine with 0.23 per cent Bence-Jones protein will react in this test. It is evident that this reaction is at least eight
times more sensitive for the latter than for the blood proteins. The ring test as described by Bradshaw although far more sensitive is less specific.

Besides the above hydrochloric acid test, we have found that lead acetate and zinc sulfate each have in the presence of acetic acid and under certain conditions power of differential precipitation between Bence-Jones protein and serum proteins. However, this action seems to depend upon so many factors that we have not so far succeeded in working out a practicable method of using these salts to distinguish between the proteins.

From a review of these reactions, especially the conduct with heat, where there is nearly always failure to obtain a quite complete re-solution upon boiling, we are inclined to believe that associated with the Bence-Jones protein is a small amount of serum protein; this conclusion is supported by the fact that a few casts are usually to be found upon centrifugation.

Separation of the Protein.—To each 100 cc. of filtered urine are added 20 cc. of 50 per cent acetic acid, and 120 cc. of a twice cold saturated and still slightly warm solution of sodium sulfate. This is stirred, allowed to stand about 20 minutes, and filtered with suction, using hardened paper. When the surface is dry the precipitate is washed three times with a cold saturated solution of sodium sulfate well acidulated with acetic acid. As soon as dry from the last wash solution, but while still pasty, it is scraped off from the paper and rubbed up in a dish to a thin milky suspension with 95 per cent alcohol. This rubbing is lightly done and the suspension is kept very fluid so as to leave behind as much of the sulfate as possible. From time to time the milky fluid is poured off into centrifuge tubes, a fresh portion of alcohol added to the dish, and the rubbing continued, always pouring off and renewing the alcohol before the suspension becomes rich enough to give the suggestion of cream. In this way the protein is gradually removed from the dish, together with some sulfate. Toward the end of the process the contents of the dish will be found to feel distinctly gritty from the sulfate crystals, and the operation should then be interrupted and the remainder discarded. The tubes are then centrifuged, the supernatant liquid is poured off, the tubes are filled with absolute alcohol, thoroughly stirred, and again
centrifuged. This alcohol is poured off and the washing process repeated. Then the protein is washed in the same manner once with a mixture of equal parts of absolute alcohol and ether, then twice with ether; finally it is collected on a filter of hardened paper and allowed to dry. Upon slight rubbing this protein will crumble to a fine white mobile powder, non-hygroscopic, scarcely soluble in water, but readily soluble in water or Ringer’s solution when these are made slightly alkaline by dilute NaOH. In order to remove the remaining sulfate the dry protein is suspended in a large proportion of slightly warm distilled water in centrifuge tubes, stirred for a few minutes, and centrifuged. The wash water is then decanted from the protein, the alkaline water or other solvent poured in, and solution effected by stirring. Before dissolving, the washing with distilled water may be repeated, but this we usually omitted; if it be desired to obtain a strictly salt-free product this method should be replaced by dialysis.

When weak acetic acid is slowly added to a dilute slightly alkaline solution of the protein, some precipitation occurs at neutrality, which, however, readily redissolves upon the addition of a little more acid.

In alkaline solution the protein (like the native urine made alkaline) does not give the regular heat reaction. A solution of the protein with a very slight excess of acetic acid gives the reactions already described for native urine except that it does not coagulate upon heating. This is due to the absence of salts, for if a little sodium chloride is added, or especially if one or two volumes of normal urine are added to one volume of the protein solution, the typical heat behavior is fully restored. It is therefore safe to assume that no material denaturation has been caused by this method of preparation. This conclusion is fully confirmed by the biological results described later.

For the purpose of estimating the protein, the simple heat precipitation in centrifuge tubes as described by Folin and Denis (but with the use of only a trace of acetic acid) is very convenient.

For the purpose of demonstration the protein may simply be suspended in normal urine of acid reaction, which is then boiled; whereupon the suspended particles promptly go into solution, with, again, a reproduction of the native Bence-Jones urine.
Preparing a Protein-Free Urine.—For subsequent work we required, in addition to the protein, urine from the same subject from which the protein had been quantitatively removed. The filtrate from the sodium sulfate precipitation was not suitable because of the large content of acid and besides it often contains traces of protein. We were not successful in strictly quantitative removal of the protein by heat alone; even the method given by Magnus-Levy always allowed enough protein to remain in the filtrate to react with the Spiegler, Roberts, and the ferrocyanide tests. The following gave a strictly protein-free filtrate.

A mixture of 50 cc. of Bence-Jones urine, 50 cc. of water, and 100 cc. of sodium sulfate solution (100 gm. of sodium sulfate crystals in 200 cc. of water) is heated in the water bath; as soon as coagulation begins it is stirred occasionally with the thermometer until the temperature reaches 96-98°. Then it is taken from the bath, four drops of concentrated HCl are added, and the mixture is stirred well. After standing a minute or so it is rapidly cooled by immersing the vessel in cold water until the temperature has been reduced to about 40°, and filtered through 589 paper. The clear filtrate gives no reaction with any of the above reagents.

We also found that the protein can be quantitatively removed without heating, by the use of salt and acid. 2 cc. of concentrated HCl in 100 cc. of H₂O are saturated with sodium chloride. An equal volume of urine is added, and the mixture stirred for a minute, and filtered. The clear filtrate is entirely free from protein. This salt-acid solution is of course essentially the Roberts reagent for albumin.

Anaphylaxis.—This protein is capable of causing active anaphylaxis, as the following experiments show.
**Anaphylaxis Series I.**

**Sensitization.**—Nov. 27. Twelve guinea pigs were injected subcutaneously. Nos. 1 to 6 each received 1 cc. of Bence-Jones urine (0.027 gm. of protein). Nos. 7 to 12 each received 1 cc. of a 2 per cent solution of protein in Ringer's solution (0.020 gm. of protein).

Dec. 23. All were injected; the injection was into the jugular vein except for No. 2 which was injected directly into the heart.

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Intoxication. Dec. 23 (26 days).</th>
<th>Results.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Diluted Bence-Jones urine, 0.012 gm. protein.</td>
<td>Moderate anaphylaxis with complete recovery at end of 15 min.</td>
</tr>
<tr>
<td>2</td>
<td>&quot; urine, 0.003 gm. protein.</td>
<td>Very slight anaphylaxis with recovery in 6 min.</td>
</tr>
<tr>
<td>3</td>
<td>&quot; &quot; 0.004 &quot; &quot;</td>
<td>Slight anaphylaxis with recovery in 11 min.</td>
</tr>
<tr>
<td>4</td>
<td>1 cc. Ringer's solution, 0.002 gm. protein.</td>
<td>No symptoms.</td>
</tr>
<tr>
<td>5</td>
<td>4 cc. Ringer's solution, 0.008 gm. protein.</td>
<td>Very slight anaphylaxis with recovery in 5 min.</td>
</tr>
<tr>
<td>6</td>
<td>1 cc. urine, 0.024 gm. protein.</td>
<td>Same as No. 5.</td>
</tr>
<tr>
<td>7</td>
<td>1 cc. diluted urine, 0.004 gm. protein.</td>
<td>Marked anaphylaxis with death in 7 min.</td>
</tr>
<tr>
<td>8</td>
<td>1 cc. diluted urine, 0.002 gm. protein.</td>
<td>Marked anaphylaxis with death in 9 min.</td>
</tr>
<tr>
<td>9</td>
<td>1 cc. Ringer's solution, 0.002 gm. protein.</td>
<td>Marked anaphylaxis with death in 8 min.</td>
</tr>
<tr>
<td>10</td>
<td>*</td>
<td>Marked anaphylaxis with death in 8 min.</td>
</tr>
<tr>
<td>11</td>
<td>1 cc. Ringer's solution, 0.002 gm. protein.</td>
<td>Moderate anaphylaxis with recovery in 13 min.</td>
</tr>
<tr>
<td>12</td>
<td>2 cc. Ringer's solution, 0.004 gm. protein.</td>
<td>Marked anaphylaxis with death in 5 min.</td>
</tr>
</tbody>
</table>

* Lost.

From Series I it will be seen that: (1) the native Bence-Jones urine is capable of provoking only slight sensitization, whether the intoxicant is more of the urine or whether it is isolated protein; (2) the isolated protein is capable of causing very much higher sensitization against both native urine and isolated protein; (3) the native urine will intoxicate a sensitized guinea pig; (4) the isolated protein will also do this. The intoxicating power of the
native urine and of the isolated protein is about the same, so obviously the protein as prepared by us has suffered no material denaturation.

An interesting fact developed by this series is the weak sensitizing action of the native urine. Apparently some factor exists which prevents the protein from exerting its normal power; yet does not interfere with the subsequent intoxication of an animal properly sensitized by isolated protein. The experiments described in Series II were undertaken to study this inhibiting factor.

**Anaphylaxis Series II.**

*Sensitization.*—Jan. 12. Six guinea pigs were injected subcutaneously. Nos. 1, 2, and 3 each received 1 cc. of Bence-Jones urine (0.026 gm. of protein). This urine had previously been heated at 55° for 1 hour. Nos. 4, 5, and 6 each received 4 cc. of the following solution: 0.1 gm. of Bence-Jones protein dissolved in 20 cc. of urine from which the protein had been removed as described, and then made slightly alkaline. Nos. 4, 5, and 6 therefore each received 0.020 gm. of protein.

Feb. 4. All were injected into the jugular.

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Intoxication. Feb. 4 (23 days)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 cc. of diluted Bence-Jones urine, 0.001 gm. protein.</td>
<td>No symptoms.</td>
</tr>
<tr>
<td>2</td>
<td>1 cc. of undiluted urine, 0.025 gm. protein.</td>
<td>Well marked anaphylaxis with death in 10 min.</td>
</tr>
<tr>
<td>3</td>
<td>0.5 cc. urine, 0.013 gm. protein.</td>
<td>Moderate anaphylaxis with recovery in ½ hr.</td>
</tr>
<tr>
<td>4</td>
<td>1 cc. diluted urine, 0.001 gm. protein.</td>
<td>Marked anaphylaxis with death in 5 min.</td>
</tr>
<tr>
<td>5</td>
<td>1 cc. diluted urine, 0.0005 gm. protein.</td>
<td>Same as No. 4.</td>
</tr>
<tr>
<td>6</td>
<td>1 cc. diluted urine, 0.00025 gm. protein.</td>
<td>Moderate anaphylaxis with recovery in ½ hr.</td>
</tr>
</tbody>
</table>

From Series II it is clear that the inhibiting factor is thermostable at 55°; for the guinea pigs sensitized with the urine which had been heated at this temperature showed about the same sen-

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*The urine became slightly opalescent from beginning precipitation of protein, and was not filtered.
siveness, allowing for the larger intoxicating doses, as did the animals of Series I which were sensitized with unheated urine.

On the other hand, it is clear that the power of sensitization of the isolated protein when dissolved in the deproteinized urine is even greater than when the same protein is dissolved in Ringer's solution. The removal of the protein from the urine was effected by the first (sulfate and heat) method, described on page 290, and was undoubtedly quantitative within the limits of the tests there mentioned; therefore the greater sensitization cannot be attributed to the presence of protein unaccounted for. Evidently the inhibiting factor is present neither in the isolated protein nor in this protein-free filtrate; it is apparently destroyed by chemical manipulation or by heating to near the boiling point. We are at present unable to make any further statement regarding this matter.

The marked anaphylactic activity of this protein confirms the belief that we are dealing with a higher protein of individual biological stamp and not with a degradation stage in the hydrolysis of any body protein.

Direct Toxicity. While it has generally been assumed that Bence-Jones protein is not directly toxic, there seems to be in the literature no definite experimental evidence bearing on this question. We therefore tried the effect of a large dose. A dog was etherized, and received into the femoral vein 2 gm. per kilo of body weight of protein dissolved in slightly alkaline Ringer's solution. The dog remained etherized for about 2 hours, and during this entire time the blood pressure taken by cannula from the carotid artery and the respirations from the trachea were recorded by the continuous paper kymograph. From the femoral artery blood was taken at short intervals to test its coagulability. In no direction was there the slightest indication of toxic action; and the normal clotting of the blood speaks strongly against the presence of proteoses, which would have been in sufficient concentration to interfere with coagulation. This is quite in accord with the anaphylactic activity already described.

Digestibility of Bence-Jones Protein.—This protein in the circulation of the patient is, like the plasma proteins, protected from digestion; but the isolated substance, evidently undenatured, is readily attacked by either pepsin or trypsin.
In one of two flasks, each containing a little less than 100 cc. of water, were placed 1 gm. of protein and 0.1 gm. of Gruebler trypsin; in the other flask 1 gm. of protein and 0.1 gm. of pepsin. The flasks were then brought to the proper respective reactions, made up to 100 cc. volume, a small quantity of toluene was added, well shaken, and placed in the incubator at 39°. After 48 hours in the incubator 25 cc. were withdrawn from each flask, 225 cc. of absolute alcohol added to each, and the whole was allowed to stand about an hour. The pepsin digestion gave at once strong opalescence followed by a flocculent precipitate, which weighed 0.042 gm. The filtrate from this precipitate was well diluted with water, boiled down to drive off the alcohol, and then gave a strong biuret reaction, showing that substantial digestion had occurred. The trypsin digestion was treated in the same way. Weight of precipitate, 0.031 gm. filtrate as above.

The flasks were returned to the incubator for another 48 hours, when 25 cc. portions were taken as before. The precipitate from each of these portions was found to be only 3 mg. less than after the first period, and the digestions were not carried any further. It would appear therefore that while nearly all the protein yields readily, a small fraction resists digestion with considerable tenacity.

As control a 1 per cent solution of protein was precipitated without any digestion; the filtrate did not give a biuret reaction; so that at least within the limits of this test all undigested protein is thrown down by alcohol. As further control another trypsin digestion was set up with the same proportions as before, but was boiled 15 minutes before placing in the incubator. After 48 hours the filtrate from the alcohol precipitate gave no biuret reaction.

If the Bence-Jones protein is to be regarded as foreign to the tissues of the subject, we should by the Abderhalden hypothesis expect that a special ferment would soon be aroused to destroy it in the body; and certainly there would seem to be nothing in the nature of the substance itself to resist such a fate. Yet instead of being digested large quantities of it are excreted in its native condition, apparently free from any hydrolysis; and this can continue indefinitely. Under such circumstances we must dismiss the idea of a protective ferment.

Abderhalden and Rostoski produced by several injections of Bence-Jones protein in rabbits precipitins which reacted not only with this protein but also with human serum and the proteins prepared from it. Hopkins and Savory did not obtain precipitins (which they explain as probably due to injecting insufficient amounts of protein), but they state that

anti-human serum gave a slight but definite reaction with solutions of Bence-Jones protein. Boggs and Guthrie, by injecting solutions of Bence-Jones protein, produced in rabbits weak precipitins which reacted both to this protein and to blood proteins. In this connection it is interesting to note that Magnus-Levy states that the character of the excretion of his patient slowly changed, becoming much less soluble on boiling than in the early stages of the disease, and finally behaved like ordinary blood protein. Yet he was satisfied that it was really the same substance because by changing the physical conditions of the test, as by the addition of salts or urea, the solubility at 100° returned.

From every point of view it is clear that this substance in our case as in others is a higher protein, a human product closely related to the normal blood proteins.

Our next paper will consider more fully the protein in the body and its elimination.

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STUDIES IN BENCE-JONES PROTEINURIA
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