SOME OBSERVATIONS ON THE NATURE OF THE 'BENCE-JONES' PROTEIN

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The peculiar protein found in the urine in cases of multiple myeloma has, since the time when Bence-Jones first described it,[1] been found on but few occasions, owing to the rarity of the disease with which it is associated. Notwithstanding this, a great deal of work has been done towards elucidating its characters. The result has been that the various workers have placed it in different classes, and little harmony can be found in the results obtained. This contribution, based upon the study of the protein obtained from an undoubted case, tends to show that the protein bodies found in the urine differ from time to time even in the same case, and probably the true explanation of the varying results found by different observers lies in the fact that, in the disintegration of the bones and tendons, chondro-mucins are liberated which are more or less broken up, and thus excreted differently according to the stage of the disease.

The case from which the protein was obtained was that of a miner, aged 30 years, who was first seen on January 11th of this year, when a small protrusion on the right fifth rib was found. He then complained of great weakness and a very poor appetite. His doctor, who had known the man for some time, found that he had wasted very considerably. He gradually became weaker, and on February 8th there was some rigidity in the upper dorsal spinal region. An examination of the nervous system pointed to some involvement of the spinal cord in this region, and on pressing between the spinous processes of the last cervical and first

1. *Philosoph. Trans. of the Royal Soc.*, 1848.

dorsal, and between the latter and second dorsal, the spinous ligaments were felt to be much less resistant than in any other part of the spinal column. Later, there was marked rigidity of the neck muscles, the head being thrown backwards and held stiff.

On February 12th, on examining the ribs, the first on the right side and the second and third on the left side fractured. On February 18th the sternum fractured spontaneously just below the manubrio-gladiolar junction, giving rise to marked deformity in the chest.

On the 19th the left sterno-clavicular joint became disorganised, and later the inner end of the clavicle was drawn up into the neck by the sterno-mastoid muscle. Before death, on the fifth of March, the whole of the ribs were soft and easily gave way under pressure.

A piece of rib was allowed for examination, and Dr. E. E. Glynn examined it and found that it was characteristic of the disease multiple myeloma, and analogous in all respects with the specimens prepared from a case described by Sir James Barr.

The Urine

On February 11th the urine gave the specific reactions of the Bence-Jones protein.

On warming after the addition of acetic acid, there developed at 52° C. a milky, then heavy, precipitate which disappeared almost completely at 95° C. The acetic acid gave no precipitate in the cold.

On adding nitric acid a heavy precipitate formed which was soluble on warming, and reappeared on cooling. This could be repeated several times before the protein failed to disappear on warming.

On repeated saturation with ammonium sulphate, and washing with the same fluid, the precipitate obtained, dissolved in either dilute sodium chloride or water, gave the biuret test.

It gave the test for loosely combined sulphur, and the potassium ferrocyanide and acetic acid test.

The hydrochloric acid test (Bradshaw's test) was negative.

The dialysed urine gave the same reactions.

For the first few days the urine was passed quite clear, but later it became turbid and then milky.

The nature of this protein has given rise to a great deal of controversy. Bradshaw, as the result of examination of the urine of ten

cases, states that it is an albumose. Magnus Levy, who isolated the protein and obtained it in a crystalline form, states that it is nearly related to a genuine albumin. Cohnheim considers it a coagulated albumin. Lindeman concludes that while it cannot without objections be put in any group of proteins, it is nearest the true albumins.

In the different cases recorded, the properties of the substance found have differed so much that either they were different bodies or were not tested pure.[1] That the proteins found can differ even from day to day is shown by the fact that the second specimen examined from the case under consideration (on February 13th) gave a well marked precipitate with cold acetic acid. A specimen of this precipitate was obtained from 500 c.c. of urine, purified and found to contain a carbohydrate radicle and no phosphorus; it therefore is shown to be a mucin.

The reactions varied slightly from time to time, though on most days the nitric acid test, coagulation temperature, loose sulphur, and 'salting out,' were quite typical.

On February 19th a dialysed specimen gave the hydrochloric acid reaction quite typically, and no precipitate with acetic acid in the cold.

It was clear from the outset, after finding different reactions on two successive days, that there was probably a mixture of proteins in the urine, or that a common body was being split up differently from day to day.

The dialysed specimen which gave the hydrochloric acid reaction was kept in an open vessel for two days, after which the reaction was much less marked. It then gave a precipitate with cold acetic acid (2%) which it had failed to do before. This precipitate was phosphorus free, and contained a carbohydrate radicle. The probability was, therefore, that in this case there was a conjugated protein present which under certain conditions resolved into other bodies, one of which was a mucin.

Mucin and allied compounds belong to the group of glyco-proteins. Such proteins occur in the tissues undergoing degeneration in such cases, viz., cartilage, tendon, and bone, conjugated with chondroitin sulphuric acid.

In the table there are the results of two days' complete analyses of the urine, which were performed with a view of obtaining some indications on which to work. It will be observed that there was an increased excretion of calcium, and diminished chloride excretion.

1. Emerson. *Clinical Diagnosis*.

CASE OF MULTIPLE MYELOMA

Urine	February 17th, 1800 c.c.	February 18th, 2100 c.c.
Total Nitrogen	13·5 grams	15·96 grams
Urea	27·0 ,,	29·61 ,,
Uric acid...	2·07 ,,	0·504 ,,
Ammonia (as NH_3)	0·252 ,,	0·336 ,,
Phosphates (as P_2O_5)	2·16 ,,	2·31 ,,
Chlorides (as Cl)	1·728 ,,	1·55 ,,
Calcium	0·594 ,,	0·714 ,,
Sulphates (as $BaSO_4$)—		
Organic	0·025 per cent.	0·002 per cent.
Inorganic	0·28 ,,	0·28 ,,
Ratio	1 : 11 ,,	1 : 160 ,,

Creatinin (by Jaffe's Test) was present.

	Feb. 17	Feb. 18	Feb. 22	Feb. 28	Mar. 3
Sulphate ratio (ethereal to inorganic)	$\frac{1}{11}$	$\frac{1}{160}$	$\frac{1}{10}$	Ethereal practically nil	$\frac{1}{4}$

The most characteristic feature observed was the marked change in ethereal sulphate excretion, it being normal on the first occasion and markedly diminished on the second. This observation, coupled with the fact that the Bence-Jones body contains loosely combined sulphur, suggested that the body might have some relation to the proteins which have recently been isolated from bone containing a high sulphur content.

Loebisch, Gies,[1] and others have isolated a substance from tendons which does not differ from true mucins in its properties, and Gies has prepared an identical substance from bones which he calls ' osseo-mucoid.' It also possesses a high sulphur content and contains likewise a paired sulphuric acid, thus resembling a chondro-mucoid.

It is obvious from the history that in this case both tendon and bone were being rapidly broken down, and the probability of their derivatives being found in the urine is not unlikely.

The marked variation in the excretion of ethereal sulphates with a protein in the urine containing loosely combined sulphur led us to study the relationship between these two conditions, but unfortunately before this could be completed the patient died.

1. Mann. *Chemistry of the Proteids*, 1906, p. 541.

On February 18th, from 500 c.c. of urine 0·4 grams of the protein were obtained in a pure condition. It contained 10·5 per cent. of sulphur.

On March 2nd, 0·3 grams of protein were obtained, and it contained only 0·55 per cent. of sulphur.

The variability in the nature of the protein found is thus obvious.

It is of striking interest to observe that with this change in the nature of the protein there was a variation in the output of ethereal sulphate, on the first occasion there being practically no ethereal sulphates in the urine, and on the second occasion there being an excess.

In view of the fact that the case terminated before these observations could be completed, it would be unwise to draw any fixed conclusions from these observations, but it is of extreme interest to find that in a disease where the bones and tendons containing chondro-mucoid are being rapidly broken down, proteins of a similar nature—in that they contain a carbohydrate radicle and a high sulphur content—are found in the urine.

Two other cases of bone disease which are being investigated reveal the presence of the mucin-like body, but they do not show any of the other reactions.

Another unusual feature of the case was the constant occurrence of a crystalline deposit in the urine which was found to be sodium urate, a point of interest in view of the marked increase in calcium katabolism. Professor Benjamin Moore, in the last number of this Journal, calls attention to the importance of altered calcium metabolism in gout, and it is thus a striking feature that in a case where there is obviously a marked increase of calcium katabolism, a crystalline form of sodium urate is excreted. So far, I have been unable to find any reference to a similar crystalline sodium urate in urine.

My thanks are due to Professor Benjamin Moore for his valuable suggestions as to the method of approaching the problem and his constant help; also to Dr. T. R. Bradshaw for the interest which he took in the case, and information concerning his previous investigations of 'Bence-Jones' protein.