59. IMMUNOLOGICAL DIFFERENCES OF CRYSTALLINE BENCE-JONES PROTEINS

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ONE of the first suggestions that the protein appearing in the urine that gives the peculiar Bence-Jones reaction might not necessarily be the same protein in all cases was made by Ville & Derrieu [1907]. Later Hopkins & Savory [1911], on the basis of analyses of the amino-acid composition of the proteins from two cases, concluded that they were identical in chemical constitution. Bayne-Jones & Wilson [1922], in a study of the immunological properties of Bence-Jones proteins by means of the precipitin reaction and the anaphylactic reaction, concluded that there were at least two and possibly three groups of Bence-Jones proteins recognizable by the tests used. In their study they used proteins from 5 cases, three diagnosed as multiple myeloma, one carcinoma with metastases to the bones and one without demonstrable lesions in the bones. Only one of the proteins used was reasonably pure. This protein crystallized spontaneously from the urine and was further purified by recrystallization. It could be argued that the differences observed might have been due to the impurities of all but one of the protein preparations used. Robinson [1927] studied the antigenic properties of four preparations of Bence-Jones protein from four cases of myelomatosis by means of the precipitin reaction and came to the conclusion that at least two of these protein preparations were different. These preparations were crude and the comments on the work of Bayne-Jones & Wilson [1922] are applicable here also.

From our previous work on Bence-Jones protein we had available four crystalline proteins from four different patients. We also had some of the crystallized protein furnished us by Dr D. Wright Wilson [1923] and some of the first crystallized protein ever isolated from urine prepared by Noel Paton [1892]. Antisera were prepared for our own four crystalline proteins. Table 1 shows the results of the titration of the six crystalline proteins against these antisera.

Table 1. *Precipitin reactions with the antisera for the crystalline proteins*

	Antisera			
	Bence-Jones 1	Hogan	Mahle	Nelson
Bence-Jones 1 [Hektoen & Welker, 1924]	4	0	0	0
Hogan [Hektoen *et al.* 1924]	0	4+	3	3
Mahle *et al.* [1938]	0	4	4	3
Nelson [Mahle *et al.* 1938]	0	4	3	3
Wilson [1923]	0	3	3	3
Paton [1892]	0	2		

0 = No reaction.
1 = Titre in dilution 1:1000.
2 = Titre in dilution 1:10,000.
3 = Titre in dilution 1:100,000.
4 = Titre in dilution 1:1,000,000.

These results show that the crystalline proteins fall into two distinct immunological groups. It is striking that five out of the six belong to the same group. This is probably due to the fact that the larger group can under certain conditions be obtained readily in crystalline form, while the other group can only be obtained in crystalline form with great difficulty.

From our previous work we had also available non-crystalline Bence-Jones proteins from five other cases. Dr Wilson furnished us with Bence-Jones from five patients. Dr H. O. Calvery & Dr R. H. Freyberg [1935] furnished us with one Bence-Jones protein which they had analysed for the amino-acid composition. Dr Grace Medes [Berglund & Medes, 1935] furnished us with Bence-Jones protein from five patients which she had analysed for their amino-acid composition. Table 2 shows the results of the titration of solutions of the non-crystalline proteins against the antisera for our crystalline proteins.

Table 2. *Precipitin reactions with non-crystalline Bence-Jones proteins*

	Antisera			
	Bence-Jones 1	Hogan	Mahle	Nelson
Wilson:				
1–M.	2	0	±	0
2–T.	1	0	0	0
4–S.	±	0	0	0
3–R.	0	3	3	3
5–2	3	1	2	1
Medes:				
G.G.	3	0	0	0
C.K.	3	0	0	0
A.B.	1	0	0	0
A.G.	0	3	4	3
M.G.	0	3	2	3
Calvery-Freyberg:	0	3	2	3
Hektoen-Welker:				
1–R.	0	3	3	3
2–F.	0	3	3	3
3–S	3	0	0	0
4–D.R.	3	0	0	0
5–M.C.	3	3	4	3

From these results it is clear that these Bence-Jones proteins fall primarily into two main groups. In two of the cases it appears that the patients were eliminating both types (5–2 and 5 M.C.). Some of the samples were relatively insoluble and the low titres with some of the antigens may have been due to the small amount that went into solution. An antiserum was prepared against protein A.B. and its reactions with our antigens placed it into the Bence-Jones 1 group.

The analyses for nitrogen distribution made by Medes on the samples furnished us showed that all of them with the exception of C.K. gave approximately the same results, while C.K. showed marked differences. The results in Table 2 show that three of these proteins belong to Bence-Jones 1 group, among them C.K., and two to group 2. These results are difficult to reconcile unless we assume that not all of a given protein molecule is involved in its antigenic properties and that the chemical configuration of the portion of the molecule involved in its antigenicity determines the character of its antigenicity. The analytical results obtained by Calvery & Freyberg on the protein they isolated differ markedly from those obtained by Medes. The immunological properties of this protein indicate that it belongs to group 2. This result further emphasizes that differences in composition do not necessarily mean different antigenicity.

SUMMARY

With antisera for four different crystalline Bence-Jones proteins, two groups of these proteins have been demonstrated as immunologically distinct. This observation confirms the work of Bayne-Jones & Wilson [1922] and that of Robinson [1927].

Chemical composition as determined by the percentages of the various amino-acids present in the protein is not the sole determining factor of its antigenic properties.

An individual may eliminate both types of these immunologically distinct Bence-Jones proteins at the same time as indicated by two of the cases in this study.

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