CXXIII. BENCE-JONES PROTEINS.

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MYELOMATOSIS or Kahler's disease is characterised by alterations in the bone marrow and by the occurrence in the urine of a protein substance which coagulates when warmed and redissolves on boiling [Bence-Jones, 1848].

The earlier workers concluded from its heat-solubility that the substance in the urine was an albumose [Kühne, 1883, 1884; Huppert, 1897; Mathes, 1896; Ellinger, 1898]. Magnus-Levy [1900], Abderhalden and Rostosky [1905], and Grutternink and de Graaf [1901] conclude from its behaviour as an antigen and from its hydrolysis products that the compound is a protein. Although unable to prepare antibodies by intraperitoneal injection Hopkins and Savory [1910] give analyses similar to those of other proteins, and they emphasise the importance of salts and acid for the re-resolution of the coagulated protein. Their suggestion is that the protein combines more readily with ions at the boiling-point than at lower temperatures and the protein is thus re-dissolved through formation of soluble salts.

Ville and Derrien [1907] conclude that different Bence-Jones proteins with the common property of heat-solubility may occur. The same conclusion is reached by Hektoen [1921], Bayne-Jones and Wilson [1922] and Hektoen and Welker [1924] as the result of precipitin, complement fixation and anaphylaxis tests. Miss Robinson [1927] applied the precipitin technique to some of the proteins described in this paper and found differences between them. Willheim [1927] also postulates the existence of different Bence-Jones proteins.

In this paper Bence-Jones proteins obtained from five cases of myelomatosis are described. Notes on the cases have been reported elsewhere [Hewitt, 1929]. In one as much as 36 g. *per diem* of the protein were excreted without appreciable albuminuria. The protein was detected in the blood but not in the cerebrospinal fluid.

THE PROTEIN.

Preparation. Specimens of urine collected over a 24-hour period and preserved with toluene were treated with an equal volume of saturated ammonium sulphate solution and sufficient 10 % acetic acid to bring to p_H 5. After standing, the precipitate was filtered off, dissolved in water, and the solution was neutralised. The protein was re-precipitated by addition of

saturated ammonium sulphate solution and then redissolved in a small volume of water.

Lipins were extracted from the protein by pouring the solution into alcohol-ether mixture at low temperature [see Hewitt, 1927]. The white powdery extracted proteins may be kept indefinitely at room temperature. The solid was dissolved in water and dialysed in collodion sacs against distilled water in the ice-chest for 10 days. The solutions were filtered through sterile Seitz filters and were much less coloured than those of urinary albumin prepared in the same way.

Salting-out. The proteins required 40–55 % saturation with ammonium sulphate for complete precipitation from neutral solutions, the precipitation limits being very sharp. In Case I, 40 % saturation produced no precipitate whilst precipitation was complete at 44 % saturation. With the protein from Case III the solution remained clear at 33 % saturation but the protein was completely precipitated at 42 % saturation. The hydrogen ion concentration has a marked effect on the precipitation. With protein from Case I 10 % saturation is sufficient for salting-out at p_H 3.9. The salting-out limits are not dissimilar to those of pseudoglobulin.

Solubility. The protein from four of the cases dissolved in water quite readily but that from Case I was less soluble. In this case the temperature coefficient of solution was high, a waxy solid separating on cooling a solution made at room temperature. A sample of this protein (not extracted with alcohol) was dissolved in water at room temperature and dialysed against distilled water in a collodion sac in the ice-chest; a white solid was soon precipitated. The supernatant fluid was poured off and the solid was found to dissolve readily in water. Both the supernatant fluid and the dissolved solid had $[\alpha]_{5461} - 57.8^\circ$. This precludes the possibility of denaturation or fractionation of different proteins.

Heat coagulation and re-dissolution. Each of the proteins coagulated at a low temperature when warmed but not all redissolved when the suspension was boiled. A slightly acid solution and the presence of salts is necessary for re-dissolution on boiling and re-precipitation on cooling [Hopkins and Savory, 1910].

		Cases				
		I	II	III	IV	V
Opalescent at	...	42°	44°	52°	56°	50°
Coagulated at	...	50°	46°	55°	58°	57°
Clear soln. at	...	—	80°	—	80°	80°
Filter, cool	...	sl. ppt.	ppt.	—	ppt.	ppt.

It was found that proteins from Cases I and III could not be made to re-dissolve when boiled unless some 40 % of alcohol were present. Under these conditions all the proteins coagulated when warmed and redissolved when boiled. This, however, is not a specific property of Bence-Jones proteins for it was found that serum, serum-albumin and serum-pseudoglobulin behaved in the same way in slightly acid solution in the presence of salts and 40 %

alcohol. The possible reasons for this phenomenon are (a) a shift of the isoelectric point of the protein in hot alcohol and the formation of soluble salts, or (b) a pure solvent effect of the hot alcoholic solution. The latter does not seem probable.

Isoelectric point. The method used was that of adding alcohol to buffered solutions of the protein at different p_H . Those tubes in which precipitation most readily occurs are deemed to be in the region of the isoelectric point, the assumption being made that isoelectric protein is less soluble than its salts. It is certain that this assumption is not universally applicable; for example, protein phthalates are so insoluble that phthalate buffers cannot be used in this method, and in the presence of considerable amounts of sodium chloride pseudoglobulin is not precipitated completely until the acidity is increased to the neighbourhood of p_H 4. Acetate buffers were used in the present series of experiments and maximum precipitation appeared to be at p_H 3.6–4.4, but on warming for a few seconds maximum precipitation occurred at p_H 5.5–6.0 in Cases I and III. When boiled the protein dissolved in each case, and on cooling maximum deposition of solid was in the p_H range 5.5–6.0. The latter range appears more probably correct than p_H 3.6–4.4. Cataphoretic migration was slow so that the actual isoelectric point is doubtful, but some evidence was obtained that Protein I still wandered to the cathode at p_H 5.5. The isoelectric point of Protein V appeared to be in a more acid region.

Optical rotatory powers. Rotations were determined in 0.2 or 1.0 dm. tubes, using a Schmidt and Haensch polarimeter with Hilger direct-vision spectroscopic eye-piece, a mercury vapour lamp and a Nernst lamp and colour filter.

Case No.	$[\alpha]_\lambda$			
	$\lambda=6660$	5780	5461	4358 Å.
I	-39.7°	-55.3°	-64.3°	-111.6°
II	-31.0	-43.2	-48.5	-83.6
III	-35.9	-50.8	-58.1	-106.2
IV	-43.1	-59.6	-67.5	-113.9
V	-34.4	-46.0	-52.9	-97.8

The very greatly differing rotatory powers for the different proteins will be noticed. The limits for $[\alpha]_{5461}$ are from -48.5° to -67.5°; these are all rather less than that for serum-pseudoglobulin, *i.e.* -68.7° [Hewitt, 1927]. The rotatory dispersions are similar to that of pseudoglobulin.

Ultraviolet absorption spectra. The ultraviolet absorption spectra were determined in the Department of Physiology by kind permission of Prof. Roaf and Mr Smith. The same peak and trough were obtained in each case with differences in intensity. The spectrum of protein from Case I is given in Fig. 1 with that of serum-pseudoglobulin and that of albumin all prepared in the same way [Smith, 1929]. The Bence-Jones protein has a similar absorption curve to that of serum-pseudoglobulin but the extinction coefficient is greater.

Refractive indices (Abbé refractometer).

Case	Protein concn. %	n_D	n_D calc. for 100 % protein
II	6.46	1.3458	1.575
III	4.73	1.3432	1.594
IV	4.92	1.3441	1.605
V	9.45	1.3524	1.587
Serum-albumin [Hewitt, 1929]	7.45	1.3484	1.586

When allowance is made for the considerable experimental errors and method of calculation, the refractive index of Bence-Jones protein is approximately the same as that of serum-albumin.

Fig. 1. Absorption spectra of 0.1 % protein solutions.

Aromatic amino-acids. The Bence-Jones proteins give a more intense colour with the phenol reagent [Wu, 1922] than serum-proteins. Some 24 mg. serum-proteins, and an average value of 18 mg. of Bence-Jones protein, gave the same intensity of colour as 1 mg. of tyrosine. This indicates a higher concentration of available aromatic amino-acids. Protein from Case I was coagulated and hydrolysed and amino-acids were determined by the method of Folin and Ciocalteu [1927]. The results were tyrosine 6.14 %, tryptophan 1.73 % (higher concentrations than in serum-proteins). The same sample of protein contained 14.8 % of nitrogen and less than 0.01 % of phosphorus (all the results are uncorrected for ash content).

DISCUSSION.

From their antigenic powers, hydrolytic products and general physico-chemical behaviour it seems clear that these Bence-Jones compounds are true proteins. They possess properties in common with serum-proteins, especially pseudoglobulin. They are soluble in water, precipitated by about 50 % saturation with ammonium sulphate and they have similar refractive indices and absorption spectra to those of the serum-proteins. The phosphorus content is low and that of aromatic amino-acids is high. It seems possible therefore that Bence-Jones proteins and serum-proteins have a common origin, *e.g.* in the bone marrow.

Proteins from different cases have different properties; those examined differed in optical rotatory powers, in the amount of salt required to precipitate them, and in solubility. The urinary protein from one case was less soluble in water than the others and separated from solution when cooled, thus behaving similarly to that described by Paton [1892]. Each of the proteins coagulated at a low temperature when warmed but not all redissolved on boiling. Solubility when boiled is a general criterion for Bence-Jones proteins, but the urinary proteins from two cases of typical myelomatosis did not redissolve when boiled. It was found that in the presence of about 40 % of alcohol all the Bence-Jones proteins coagulated when warmed and redissolved when boiled. This, however, is not a specific property of the Bence-Jones protein since serum-proteins behave similarly. The effect of salts such as those of cerium and lanthanum, ferrocyanides and thiocyanates in increasing the solubility of proteins may be explained by assuming the formation of molecular salts which shift the apparent isoelectric point of the protein (Loeb, 1921-22), but the action of alcohol seems obscure. That the hot alcohol exerts a purely solvent effect seems unlikely.

SUMMARY.

Bence-Jones proteins from different patients differ in properties. Not all are redissolved in boiling salt solutions. In the presence of alcohol both Bence-Jones proteins and serum-proteins are dissolved when the coagulated suspensions are boiled.

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