μg/ml), folic acid (0.00017*M*), and coenzyme 1 (0.0001*M*). It was found that such supplementation of the simpler medium did not result in increased virus production by the chorioallantoic membrane. Furthermore, no significant blocking of the inhibitory action of 0.0000065*M* TRB by the supplements used was noted.

Previous studies (*1*) showed that DRB is capable of inhibiting Lee-virus multiplication in embryonated eggs and in mice without causing significant signs of toxicity in either host. In view of these results and the results described here, extension of the animal studies to other viruses appeared indicated. The effect of DRB and of TRB on the multiplication of mumps virus in 8-day-old embryonated eggs was determined. As can be seen in Table 2, both compounds caused marked inhibition of multiplication of mumps virus in the allantoic sac of embryonated eggs. Precise quantitative comparison of the effects of DRB and TRB *in vivo* is vitiated by the low solubility of these compounds. In the *in vivo* experiments, both DRB and TRB were used in suspension form. Under the experimental conditions employed, neither compound caused obvious slackening of the spontaneous activity of the embryos. No deaths attributable to DRB or TRB occurred.

The results reported here lend further support to the contention (*1*) that an unnatural benzimidazole nucleus, particularly with respect to the benzenoid ring, is of great importance in relation to the virus-inhibitory activity of the ribofuranosides of benzimidazole. Previous observations (*1, 3*) that the inhibitory activity cannot be blocked by certain suspected metabolites have been extended. The finding that DRB and TRB inhibit mumps-virus multiplication *in vivo* without causing apparent damage to the host indicates that these compounds are selective in their action. However, it is doubtful (*1, 6*) that these compounds interfere with chemical reactions that are specific for the viruses and do not occur in the uninfected host. It appears that the explanation for the selective action may concern the relative quantitative importance of certain metabolic processes for the virus as compared with the host and intracellular factors of accessibility.

Table 2. Inhibition of mumps-virus multiplication by benzimidazole derivatives *in ovo*.*

Compound	Incubation (hr)	Hemagglutination titer† of allantoic fluid
None	96	128
DRB	96	< 2
TRB	96	< 2
None	120	512
DRB	120	16
TRB	120	22

* Inoculum : mumps virus, $10^{2 \cdot 9}$ EID_{50}. Thirty minutes after inoculation of virus each egg received, by allantoic injection, 0.25 ml of saline, or compound suspended in saline. One milligram of either DRB or TRB was injected per egg.

† Expressed as the reciprocal of dilution at end-point. Six eggs were used per group, and aliquots of infected allantoic fluid were pooled for titration. Final concentration of chicken RBC was 0.045 percent.

References and Notes

1. I. Tamm *et al., J. Exptl. Med.* **99**, 227 (1954).
2. This and other benzimidazole derivatives were obtained through the kindness of Karl Folkers and Clifford H. Shunk of the Research Laboratories, Chemical Division, Merck & Co., Inc., Rahway, N.J.
3. C. H. Shunk *et al.*, data in preparation.
4. I. Tamm *et al., J. Exptl. Med.* **98**, 245 (1953).
5. J. T. Syverton, W. F. Scherer, and P. M. Elwood, *J. Lab. Clin. Med.* **43**, 286 (1954).
6. I. Tamm and D. A. J. Tyrrell, *J. Exptl. Med.*, in press.

27 July 1954.

On the Nonidentity of Bence-Jones Proteins

Frank W. Putnam and Aiko Miyake

Department of Biochemistry and
Argonne Cancer Research Hospital,
University of Chicago, Chicago, Illinois

A century ago Bence-Jones first described urinary proteins that now bear his name and are identified by the property of coagulation at low temperature (45° to 55°C) with dissolution on boiling. These proteins occur rarely, always in association with some pathology and are most often, but not invariably, found in the urine of patients with multiple myeloma. Some workers have concluded that Bence-Jones proteins are chemically identical, although it has been shown by immunological and physicochemical analysis that different patients may excrete biologically and molecularly dissimilar proteins. Recently, in a study of Bence-Jones proteins obtained from nine cases none were found to be identical in all the physical properties studied—that is, sedimentation constant (s_{20}), diffusion constant, isoelectric point *pI*, *p*H-mobility curve, and stability in dilute acid or alkali (*1*).

In further characterization of normal plasma proteins and of the pathological proteins in multiple myeloma by means of N-terminal amino acid analysis, Bence-Jones proteins from eight cases have thus far been studied. The fluorodinitrobenzene method of Sanger (*2*) was used to detect and estimate the N-terminal amino acids (that is, terminal residues having a free amino group). Buffered silica gel or celite was employed for chromatographic separation of the dinitrophenyl (DNP) derivatives, and paper chromatography for their identification (*3–5*). As is shown in Table 1, six of the eight proteins were similar in s_{20} (about 3.3 Svedberg units) but were distinguishable by their electrophoretic properties. Proteins B, E, and G migrated electrophoretically with skewed patterns indicative of heterogeneity but unlike Ma did not separate into two components within the *p*H stability range (*p*H 5 to *p*H 9). A, D, F, and Ag con-

Table 1. N-terminal groups of Bence-Jones proteins.*

Protein	s_{20}	Mobility (pH 8.6)	pI	Aspartic acid	Glutamic acid	Serine	Threonine	Leucine	Tyrosine
				(moles per 44,000 g)					
A	3.41	−4.7	4.75	1.50	0.07	0.12	0.05		
F	3.08	−3.4	4.9	1.62	0.13	0.08	0.05		
Ag	2.14	−2.2		1.71					
Ma	2.25	−4.0		1.17	0.07	0.03	0.16		
B	3.14	−4.2	4.6	trace	trace	0.52			
D	3.44	−2.4	5.5	0.06	0.04	0.04			
E	3.36	−1.4	6.7	< 0.01		0.05			
G	3.28	−2.6	5.6					0.12	> 0.74

* s_{20} (in Svedberg units) and mobility (in units of 10^{-5} cm^2 sec^{-1} v^{-1}) were determined in pH 8.6 veronal buffer, 0.1 ionic strength (*1*). Calculations assume literature values for hydrolytic destruction of DNP-amino acids and a protein content of 75 percent for the DNP-protein. For further physical data on A, B, D, E, F and G, see (*1*).

tained about 3 percent of a faster moving component upon electrophoresis at pH 8.6.

Of the eight proteins, four had essentially only aspartic acid in the N-terminal position, whereas this amino acid was either undetected as an end-group or present as such only in trace amounts in the other proteins. The latter, in fact, fell into three types according to the nature of their amino end-groups. The N-terminal amino acid content per 44,000 g (the molecular weight of A, D, and proteins of similar s_{20}) was as follows: A, F, Ag, and Ma, 1.2 to 1.7 moles of aspartic acid; B, 0.5 mole serine; G, about 1 mole of tyrosine plus some leucine(or isoleucine). In several attempts only traces of ether-soluble DNP-amino acids were obtained from E, a crystalline protein having the highest pI or from D with a pI of 5.5. Integral values are to be expected for a chemically homogeneous protein that does not have a cyclic structure. Fractional values for the minor end-groups indicate chemical heterogeneity. Protein B, which contained a nonstoichiometric amount of N-terminal serine, was physicochemically the most heterogeneous protein. No correlation has yet appeared between the nature of the end-group and physical properties such as pI or s_{20}, with electrophoretic patterns of the serum or with hematological or clinical findings.

Thus, although the eight subjects each excreted predominantly only one type of Bence-Jones protein, there were at least four kinds on the basis of the N-terminal amino acid, two groups from s_{20}, three from the isoelectric point, and up to five according to mobility. Differences in the amino acid composition of Bence-Jones proteins probably exist, but reliable analyses of physically homogeneous specimens from different patients have not yet been reported. Hence, this is the first evidence that Bence-Jones proteins excreted by various individuals differ in chemical structure as well as in physical constants. Similar differences in end-groups have been reported for the pathological globulins of multiple myeloma serums (*6*). Isotopic investigation, however, has yielded evidence that the Bence-Jones proteins are not derived by degradation of serum or tissue proteins (*7*), and the origin and function of these unique urinary proteins remain unknown.

Note added in proof. Since the submission of this manuscript, G. Biserte of the Laboratoire de Chimie Biologique, University of Lille, France, has informed us of work now in progress in collaboration with P. Burtin of the Institut Pasteur, Paris. Of four cases of Bence-Jones proteins investigated by these workers, three contained N-terminal aspartic acid, and one was devoid of ether-soluble DNP-amino acids. We, ourselves, have recently studied a crystalline Bence-Jones protein that differed in N-terminal groups from all those listed in Table 1.

References and Notes

1. F. W. Putnam and P. Stelos, *J. Biol. Chem.* **203**, 347 (1953).
2. F. Sanger, *Biochem. J. (London)* **39**, 507 (1945).
3. S. Blackburn and A. G. Lowther, *ibid.* **48**, 126 (1951).
4. G. Biserte and R. Osteux, *Bull. soc. chim. biol.* **33**, 50 (1951).

5. The end-group analyses were begun in the Department of Biochemistry, University of Cambridge, with the aid and encouragement of Fred Sanger. Support was received from the Lasdon Foundation and from the National Cancer Institute, National Institutes of Health (grant C-1331-C3).

6. F. W. Putnam, *J. Am. Chem. Soc.* **75**, 2785 (1953).
7. F. W. Putnam and S. Hardy, *J. Biol. Chem.*, in press.

29 July 1954.

Blood Serum Protein of the Marine Elasmobranchii

Hirosi Irisawa and Aya Funaishi Irisawa

Department of Physiology, School of Medicine, Hiroshima University, Kure, Japan

Species differences of serum protein fractions have been shown electrophoretically by Deutsch and Goodloe (*1*), Deutsch and McShan (*2*), Moore (*3*), Gleason and Friedberg (*4*), and Janssen (*5*). Janssen claimed that although the relative amount of serum components may vary considerably, all components (albumin, α-, β-, γ-globulin) are present in each animal; thus, he concluded that all animals have a common plan of producing serum protein. But the afore-mentioned experimenters did not study the serum pattern of the Elasmobranchii.