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.

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Blood Serum Protein of the Marine Elasmobranchii

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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.

In our experiments we gathered data on the blood serum protein of the skate, *Raja kenojéi* Müller et Henle, and the shark, *Heterodontus japonicus* Duméril.

Blood samples were obtained directly from the aorta. Japanese hand-worked paper and Whatman filter paper No. 1 (*6*) were used for the carrier of the protein. Compared with the latter, the former is very thin, but its fibers are almost parallel and its strength was sufficient for our purpose (*7*). A rectified 220-v with a current of 0.5 ma was used throughout the experiment; M/20 veronal-buffer solution was adjusted to *p*H 8.6. The paper was stained with 1-percent bromphenol blue (B.P.B.) solution containing $HgCl_2$, as previously described (*8*).

The relative mobility of the fastest component of these Elasmobranchii serums was not electrophoretically the same as that of other animals. Figure 1 shows the comparison of the pattern of shark serum and that of human serum. Both serums were run parallel in the same apparatus at the same time.

From these data, it appears that the Elasmobranchii serum does not contain albumin fraction. In another experiment, we used a shark serum that was mixed with horse serum albumin (Fig. 2). It is interesting to note that in the mixture, the horse serum albumin moves faster than any other components of the shark serum.

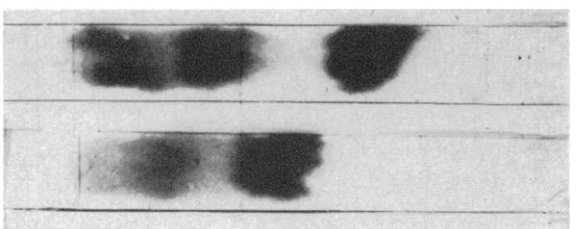

Fig. 1. (Top) Human serum; (bottom) shark serum, showing lack of albumin component.

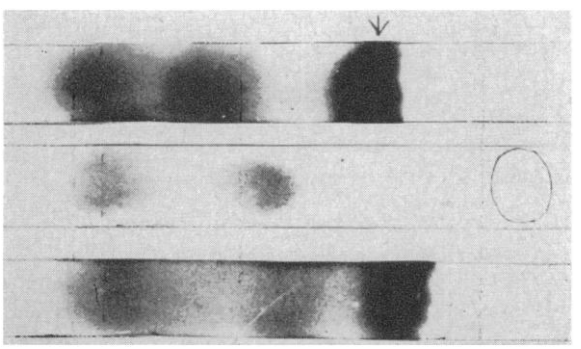

Fig. 2. (Top) Human serum; (middle) shark serum; (bottom) shark serum plus horse serum albumin, showing that the fastest component of shark serum differs from mobility of albumin. Arrow or circle shows the place that B.P.B. moved. In the top paper B.P.B. moved with the fastest component (albumin), but in the middle paper B.P.B. moved independently with the shark serum. In the bottom paper B.P.B. moved with the horse serum albumin. These papers indicate lack of albumin component in shark serum.

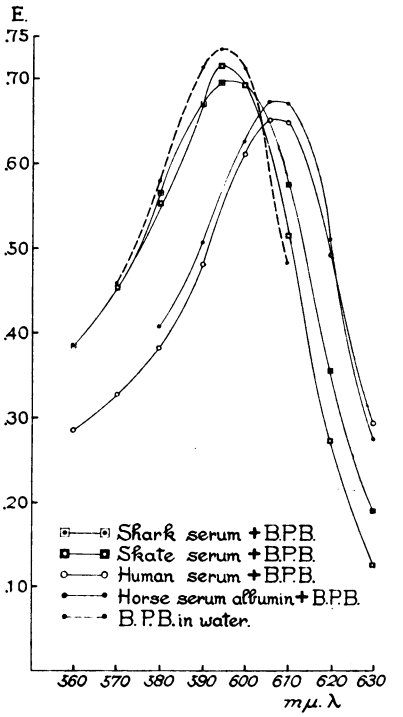

Fig. 3. Optical density of B.P.B. solution in serums of shark and skate, compared with that in human serum and horse serum albumin. Abscissa is the wavelength; ordinate is optical density. In each case, 0.1 ml of 0.05 percent B.P.B. was added to 2.0 ml of M/15 phosphate buffer (*p*H 7.2) and 0.2 ml of serum was used.

For another proof of this fact, 0.05-percent B.P.B. solution was added to each serum in the experiment. Usually, B.P.B. moves with horse serum albumin, but in the shark serum B.P.B. moved independently from the serum fraction (Fig. 2). From this observation it seems that the fastest component of the shark serum is different from horse serum albumin, since the dye combines with native albumin (*9*).

This difference of dye combination was also demonstrated spectrophotometrically (Beckman, Type DU). Bromphenol blue has an absorption maximum at 595 mu, but when it was combined with albumin the absorption maximum changed to 605 mu for its metachromasia (Fig. 3). When Elasmobranchii serums were added, it stopped at 595 mu. That is, these serums do not show metachromasia.

These facts suggest that the fastest component of Elasmobranchii serum differs from that of other animal serums and suggest that the lower vertebrates do not have the same plan of producing serum protein as do the higher vertebrates.

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Glucose-6-Phosphatase Studies in Fasting

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Glucose-6-phosphatase, an enzyme, which hydrolyzes glucose-6-phosphate to glucose and inorganic phosphate, was first demonstrated by Fantl *et al.* (*1*) and has been studied by Swanson (*2*) and De Duve *et al.* (*3*). The enzyme has been demonstrated in different organs, but it is the most active in liver and kidney. It can be distinguished from other nonspecific phosphatases by its *p*H optimum (6.5 to 6.8), its thermal instability, its sensitivity to acids, and its substrate specificity on glucose-6-phosphate, with negligible or no activity toward glycerophosphate, glucose-1-phosphate, or fructose-6-phosphate. The liver enzyme is associated mainly with the microsome fraction. Chiquoine (*4*) demonstrated with histochemical method that the enzyme is more abundant in the peripheral third of the hepatic lobule than in the inner two-thirds. Within the hepatic cell glucose-6-phosphatase is concentrated about the nuclear membrane.

However, there is very little known about the physiological and pathological behavior of this enzyme. Cori and Cori (*5*) demonstrated its importance in the pathology of carbohydrate metabolism. They reported the almost complete absence of the enzyme from liver in human cases of glycogen storage disease.

A well-established fact is that liver is virtually the sole source of the blood sugar in the fasting animal (*6*). Since the blood sugar level is maintained throughout long periods of fasting, the glucose secreted by the liver into the blood, therefore, must be derived from stored carbohydrate or noncarbohydrate precursors. It seemed to be of interest to study the behavior of liver glucose-6-phosphatase in fasting (*7*).

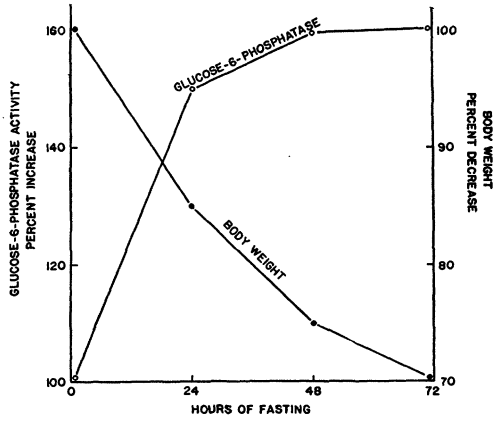

Fig. 1. Effect of fasting on body weight and liver glucose-6-phosphatase activity in mice.

Table 1. Effect of 48-hr fasting on the glucose-6-phosphatase activity in rat liver.

Rat	Body weight (g)	Liver glycogen (g percent)	Liver glucose-6-phosphatase activity*
Controls: fed chow			
1	210	4.80	292
2	210	4.96	292
3	210	2.91	185
4	210	4.26	321
5	210	3.00	250
6	210	4.20	281
Mean	210	4.02	270
Percentage	100	100	100
Fasted: for 48 hr			
7	180	0.12	415
8	185	.06	467
9	190	.69	395
10	190	.09	421
11	178	.01	410
12	180	.32	381
Mean	184	0.22	432
Percentage of control values	88	5.5	160

* Expressed in micrograms of phosphorus liberated in 15 min at 31°C per 100 mg liver wet weight.

Twenty-eight male albino mice (31 to 33 g) were divided into four groups, each containing seven mice. The three fasting groups received no Purina Fox Chow for 24, 48, and 72 hr. Water was given *ad libitum* to all groups. Animals were sacrificed by a blow on the head, decapitated, and bled. Livers were quickly excised and pooled on ice and 5-percent homogenate was prepared in 0.25*M* sucrose. The glucose-6-phosphatase was assayed according to the method of Cori and Cori (*5*). Incubation time was 30 min at 31°C. Figure 1 shows the increase in glucose-6-phosphatase activity as expressed in percentage increase when the control value is taken as 100 percent.

The increased liver glucose-6-phosphatase activity was also demonstrated in fasting rats. The following experiment is a representative example. Twelve male Wistar rats of weight 210 g were separated into two groups. The control group had a free access to Purina Fox Chow and water, while the fasting group received only water for 48 hr. Animals were sacrificed, and the enzyme was determined as described in the foregoing paragraph, except that 10-percent homogenates were prepared from each liver, and they were assayed in separated, instead of pooled, livers. Incubation time