Preliminary Observations on Difference in Carbohydrate Binding between Abnormal Serum and Urine Proteins of Multiple Myeloma

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Multiple myeloma is unique among neoplastic diseases by virtue of the fact that the cells of this particular neoplasm elaborate abnormal proteins which can be found in the patient's serum and/or urine. Since the initial demonstration of the unusual solubility properties of the urinary proteins in many cases of this disease by Bence Jones in 1848 (*1*), and the subsequent finding of Ellinger (*2*) that the serum may contain large amounts of an abnormal protein, extensive study has been made of the physicochemical properties of these proteins. These studies have documented differences in molecular weight (*3–8*), immunochemical characteristics (*4, 9*), amino acid constituents (*10–12*), solubility properties (*5, 7, 8, 13*), and rates of turnover of N^{15}-labeled glycine (*14*) among these abnormal proteins in each individual case. It would thus appear that the abnormal proteins are almost never identical with respect to *all* of their physicochemical properties in any two cases of this disease.

Another aspect of this problem that has received considerable attention is the nature of the interrelationship between the abnormal serum and urine proteins when both are present in a particular case. Again, study of the immunological and physicochemical properties of the myeloma serum and urine proteins from a given patient reveals marked differences in these characteristics, but it is still undetermined whether the two proteins are independently elaborated by the myeloma cells, or whether the urinary (Bence Jones) constituent is derived as a fragment from the serum protein. Although the observations (*15*) reported here still do not provide an answer to this question, the results suggest that differences in carbohydrate content between the serum and urine constituents may represent one of the principal areas of dissimilarity between these proteins.

It has repeatedly been observed that, in a given case, the urinary protein usually exhibits significantly greater electrophoretic mobility than the abnormal serum protein in that patient. One possible mechanism for this difference in mobility is that a relatively "electroneutral" (uncharged) portion of the serum protein

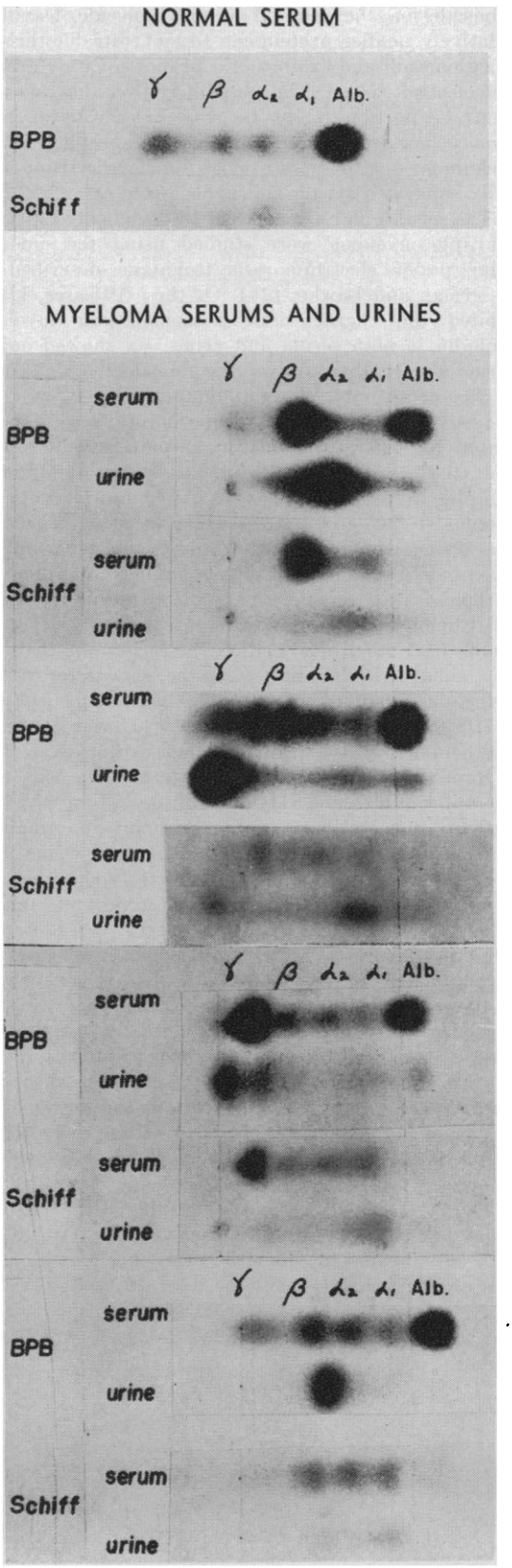

Fig. 1. Filter paper electrophoretic patterns of the serum and urine proteins from four cases of multiple myeloma, with a normal serum, shown at the top to demonstrate the normal distribution of Schiff-positive constituents in serum. Duplicate sets of the myeloma serums and urines are stained with bromphenol blue (BPB) and periodic acid-Schiff. As shown, the myeloma serum proteins are Schiff-positive, whereas the urine protein constituents fail to stain with the Schiff reagent.

molecule may be split off of this molecule, leaving a relatively smaller protein constituent, filtrable through the glomeruli and exhibiting a proportionately greater net electric charge. The possibility that this "electroneutral" portion might be a carbohydrate or lipid moiety has been explored using the specific staining techniques for these substances on the electrophoretically separated serum and urine proteins.

The serums and urines from 19 documented cases of multiple myeloma were studied using the modified filter paper electrophoresis technique described by Osserman and Lawlor (*16*). Of these 19 cases, 11 exhibited the characteristic homomolecular myeloma globulin in both serum and urine; six showed only a serum abnormality; in two, the abnormality was found in the urine only. Veronal buffer, *p*H 8.6, was employed throughout. All urine samples were concentrated by dialysis against a 25-percent solution of polyvinyl-pyrollidone. Triplicate serum and urine samples were run on a single paper, and at the completion of the separation, one set (serum plus urine) was stained for protein with bromphenol blue, and the two other sets were respectively stained for lipids and carbohydrates.

Utilizing both the Sudan *IV* (*17*) and the oil red O stains (*18*) for lipids, neither the serum nor the urinary abnormal globulins could be shown to contain detectable amounts of fat. The periodic-acid Schiff (HIO_4-Schiff) staining technique (*19*) was employed for demonstrating "protein-bound carbohydrate."

Representative duplicate patterns, stained with bromphenol blue and HIO_4-Schiff, from four myeloma patients that had both serum and urine abnormalities are shown in Fig. 1, along with a normal serum sample, stained with both dyes for direct comparison. It is apparent that the abnormal *serum* proteins stained intensely with HIO_4-Schiff, whereas the abnormal urine proteins were HIO_4-Schiff negative. The normal serum pattern shows HIO_4-Schiff positive constituents in the alpha- and beta-globulin regions, indicating the presence of the normal muco- and glycoproteins in these areas. In all four myeloma urine patterns of Fig. 1, the small amounts of alpha-globulin that frequently accompany the considerably larger amounts of myeloma protein in the urine retain their HIO_4-Schiff positivity, whereas the myeloma proteins are completely HIO_4-Schiff negative.

The HIO_4-Schiff staining results were uniform (that is, serum, positive; urine, negative) in all except one instance in which the urine myeloma globulin was distinctly HIO_4-Schiff positive. In this particular case, the serum showed no characteristic abnormality but rather displayed a marked decrease in gamma-globulin content.

To be sure, the well-recognized nonspecificity (*20*) of the HIO_4-Schiff reaction limits the conclusions that may be drawn from these observations. HIO_4-oxidation is considered to indicate the presence of vicinal OH^- or NH_2^+ groups. Esteric, glycosidic, or polymeric linkage of these groups will block aldehyde formation with HIO_4. Theoretically, proteins containing hydroxyamino acids (—CHOH—$CHNH_2$—) would be HIO_4-Schiff positive, but engagement of these groupings in peptide linkages blocks the reaction except where they might occupy a terminal position in the polypeptide chain. Thus it is generally agreed that the most important HIO_4-Schiff positive substances are glycogen, starch, cellulose, glycolipids, and mucopolysaccharides such as mucin, mucoproteins, and glycoproteins.

Quite obviously, these results still do not provide an answer to the question of whether the urine and serum proteins are independently elaborated, or whether the urine constituent represents a fragment of the parent serum molecule. The demonstration of this systematic difference in apparent chemical composition between these proteins could be interpreted as further evidence in favor of independent elaboration. The alternate hypothesis, however—that an HIO_4-Schiff positive carbohydrate moiety may be removed from the parent serum globulin, possibly by an enzyme in the kidney itself—is deemed worthy of further investigation.

References and Notes

1. H. B. Jones, *Trans. Roy. Soc. (London)* **138**, 55 (1848).
2. A. Ellinger, *Deut. Arch. Klin. Med.* **62**, 255 (1899).
3. R. A. Kekwick, *Biochem. J. (London)* **34**, 1248 (1940).
4. D. H. Moore, E. A. Kabat, and A. B. Gutman, *J. Clin. Invest.* **22**, 67 (1943).
5. A. B. Gutman, *Advances in Protein Chemistry*, vol. IV, M. L. Anson and J. T. Edsall, Eds. (Academic Press, New York, 1948).
6. R. W. Rundles, G. R. Cooper, and R. W. Willett, *J. Clin. Invest.* **30**, 1125 (1950).
7. F. W. Putnam and B. Udin, *J. Biol. Chem.* **202**, 727 (1953).
8. F. W. Putnam and P. Stelos, *ibid.* **203**, 347 (1953).
9. H. G. Kunkel, R. J. Slater, and R. A. Good, *Proc. Soc. Exptl. Biol. Med.* **76**, 190 (1951).
10. G. Ågren, *Acta Chem. Scand.* **3**, 301 (1949).
11. C. E. Dent and G. A. Rose, *Biochem. J.* **44**, 610 (1949).
12. F. T. Grisolia and P. P. Cohen, *Cancer Research* **13**, 851 (1953).
13. A. B. Gutman *et al.*, *J. Clin. Invest.* **20**, 765 (1941).
14. F. W. Putnam and S. Hardy, *Federation Proc.* **13**, 277 (1951).

15. This work was supported in part by an institutional grant from the American Cancer Society.

16. E. F. Osserman and D. P. Lawlor, *Am. J. Med.*, in press.
17. I. N. Rosenberg, *Proc. Soc. Exptl. Biol. Med.* **80**, 751 (1952).
18. E. L. Durrum, M. H. Paul, and E. R. B. Smith, *Science* **116**, 428 (1952).
19. E. Köiw and A. Grönwall, *Scand. J. Clin. & Lab. Invest.* **4**, 244 (1952).
20. G. Gomori, *Am. J. Clin. Pathol.* **22**, 277 (1952).

18 June 1954.

Science increases our power in proportion as it lowers our pride.—CLAUDE BERNARD, 1813–1878.