crystals in the renal tubules.

Rats given 4-APP also showed a significant decrease in the content of nucleated cells in the bone marrow. In intoxicated dogs, though bone marrow aspirated from the iliac crest contained decreased numbers of nucleated elements, microscopic study of decalcified sections revealed marked congestion in the marrow capillaries but neither decreases nor abnormalities in hematopoietic elements. It is possible that the aspirated marrow appeared depleted due to dilution with blood from the congested capillaries.

Adrenal cortical hemorrhages were found in several of the rats and dogs which had been sacrificed for pathological study. The significance of these lesions is obscure; but their presence is interesting in light of the finding that 4-APP also induces hypochloremia and polyuria in dogs.

Summary and conclusions. The toxic effects of 4-aminopyrazolo-(3,4-d)-pyrimidine (4-APP) have been studied in mice, rats, cats, and dogs. The agent was found to produce a fatty change in liver which was associated neither with necrosis nor with inflammation. Hepatic insufficiency was evidenced by hyperbilirubinemia, increases in one-stage prothrombin time and decreases in plasma fibrinogen. In rats fat nephrosis and depletion of hematopoietic elements in bone marrow were also seen. Hepatic insufficiency should be anticipated as a possible hazard in clinical trials with 4-APP.

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Demonstration of a Marked Bence-Jones Proteinemia.* (22770)

JANE I. MORTON AND H. F. DEUTSCH

Department of Physiological Chemistry, University of Wisconsin, Madison

Previous reports of Bence-Jones proteinemia have been founded upon a variety of observations: precipitate formation in multiple myeloma sera when heated to 55-60°C(1-3); aberrant serum protein solubility characteristics on salt fractionation(4); undemonstrated but postulated identity of a crystalline substance from a myeloma serum with crystals in the corresponding Bence-Jones protein containing urine(5); appearance of a new or elevated serum peak in electrophoretic pattern which closely corresponds in mobility to that of urinary Bence-Jones protein(4); immunological cross reaction with and blocking of antibody to Bence-Jones protein by the myeloma serum(6); and demonstration of slow sedimenting serum components which are electrophoretically in the γ- and β-globulin areas(6).

Most of these criteria can not be considered

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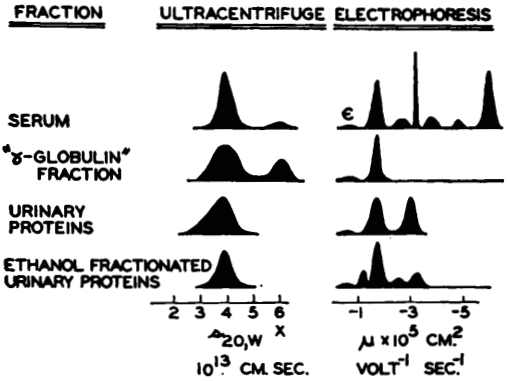

FIG. 1. Ultracentrifuge and electrophoresis diagrams of the serum and urinary proteins and fractions thereof from a case of multiple myeloma with Bence-Jones proteinemia.

in themselves adequate evidence of serum Bence-Jones protein, although the above experiments strongly suggest its presence. In this study, analysis of a multiple myeloma serum and fractions thereof by a combination of electrophoretic and ultracentrifugal technics has clearly demonstrated a considerable amount of Bence-Jones protein. This serum entity has properties in common with one of the major protein components of the urine.

Experimental and results. A patient with clinically diagnosed multiple myeloma had a total serum protein of 8.9% and an A/G ratio of 0.94 as determined by Howe fractionation. Electrophoretic analysis of the serum in pH 8.6, 0.1 ionic strength diethylbarbiturate buffer showed an increased γ-globulin area comprising 26% of total protein. The electrophoretic and ultracentrifuge diagrams of this serum and other protein systems studied, are presented in Fig. 1. The ultracentrifuge analysis of the serum carried out in the Spinco apparatus at 59,780 r.p.m. in pH 7.4, 0.2 ionic strength potassium phosphate buffer evidenced three components. These consisted of the usual small amount of an 18 S component and 2 other slower sedimenting boundaries. Only the latter 2 are seen in Fig. 1. The minor of these had an $s_{20,w}$ near 6 S and comprised 16% of the total. The slower peak had an average sedimentation constant of 3.87 S. The small amount of the 6 S component of this serum was surprising in view of the enhanced γ-globulin level (26% as compared to normal serum level of near 12%). This result suggested that the elevated γ-globulin area might also contain considerable amounts of a low molecular weight protein.

The serum was fractionated by cold ethanol technics(7) to remove the γ-globulin fraction. Electrophoretic examination of the "purified" γ-globulin fraction (see Fig. 1) revealed a single component with a mobility of -1.8×10^{-5} cm^2 volts^{-1} sec.$^{-1}$ at pH 8.6. It is represented by a single symmetrical electrophoretic peak at pH values from 4.6 to 8.6. The isoelectric point was 5.9.

Ultracentrifugal analysis of this material, however, showed that 65% of it consisted of protein sedimenting with the velocity of a Bence-Jones protein (8,10). Its $s_{20,w}$ was 3.87 S and showed no concentration dependency from 0.47 to 3.0%. The faster component sedimented with a velocity of 6.02 S and thus appears to be a γ-globulin. The yield data indicated that the slower sedimenting material was present in the serum at a level near 1.4% and thus comprised near 16% of the total serum proteins.

The patient evidenced an "albuminuria" of about 10 g/day. Electrophoretic examination of the dialyzed urine and of the urinary proteins insoluble in 50% saturated ammonium sulfate revealed 2 main components. One of these components had electrophoretic mobility of a β-globulin while the other was near that of serum γ-globulin peak. Data for electrophoretic mobility of the latter urinary component and serum γ-globulin fraction as a function of pH is plotted to give Fig. 2. It is apparent that the charge relationships of the

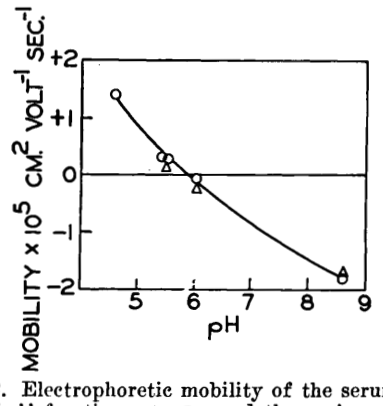

FIG. 2. Electrophoretic mobility of the serum "γ-globulin" fraction –○–○– and the major urinary component –△–△– as a function of pH.

protein from blood and urine are quite similar.

Ultracentrifuge analysis of the urinary proteins revealed a broad peak, with about 35% of the material sedimenting as a slow shoulder of the main component. The latter sedimented near 3.8 S.

The urinary proteins insoluble in 50% saturated ammonium sulfate were precipitated twice with ethanol (pH 7.0, 20% ethanol, 0.01 ionic strength, −6°C) in an attempt to purify the component with electrophoretic mobility of a γ-globulin. The resultant material when examined in the ultracentrifuge showed none of the slow shoulder characteristic of the crude urinary protein (Fig. 1). The sedimentation constant was 3.87 S, which is the same as that obtained for the low molecular weight portion of the serum γ-globulin fraction. The electrophoretic pattern (Fig. 1) shows that the minor urinary protein components had been concentrated.

Neither urinary nor serum proteins nor fractions thereof evidenced the typical Bence-Jones protein heat precipitation reaction. They coagulated on heating to near 70°C and failed to redissolve at 100°C. Filtrates of the boiled material did not give any precipitate on cooling. Nevertheless, their size and charge properties, their association with clinically diagnosed myeloma and their presence in the urine indicate that these are Bence-Jones proteins. Analogous heat reactions of such proteins have been encountered previously(9,10).

Discussion. The above results offer conclusive evidence that a protein corresponding to urinary Bence-Jones proteins may be present in certain myeloma sera. Attention should be called to the fact that a combination of fractionation, electrophoretic, and ultracentrifuge experiments on both serum and urine are required to clearly show this. Previous work using various single methods of study have not been entirely convincing. Shortcomings are present in conclusions based on electrophoretic similarity of serum components to Bence-Jones proteins without concurrent sedimentation studies being carried out. Immunological cross-reaction and blocking between the lower molecular weight Bence-Jones protein and the serum globulin components may be so strong that distinction by this means is not possible(11).

The serum protein studied here showed no concentration dependency of its sedimentation constant and would thus appear to be a relatively symmetrical molecule possessing a molecular weight of 35,000 to 40,000(9,10). The ability of this small molecule to accumulate in the serum is surprising. Previous studies in the dog have indicated that molecules of this size are cleared through the kidney quite rapidly(12). Furthermore, most myeloma patients with Bence-Jones proteinuria do not appear to show a significant Bence-Jones proteinemia(3,6).

It might be pointed out that the relatively high incidence of "albuminuria" reported in the condition of multiple myeloma is likely due to Bence-Jones proteins of the heat coagulable type described in the present and in other reports(9,10).

Summary. Simultaneous electrophoretic and ultracentrifugal studies of the serum proteins of a multiple myeloma patient have conclusively demonstrated a considerable Bence-Jones proteinemia. The properties of the serum component correspond to those of one of the major urinary components.

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