PROTEINS AND OTHER BIOCOLLOIDS OF URINE IN HEALTH AND IN CALCULOUS DISEASE. I. ELECTROPHORETIC STUDIES AT pH 4.5 AND 8.6 OF THOSE COMPONENTS SOLUBLE IN MOLAR SODIUM CHLORIDE [1]

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In approximately 92 per cent of all primary urinary calculi, more than 95 per cent of the total cation content is composed of calcium (1–3). Concepts concerning the mechanism of biologic calcification have undergone a marked revision in recent years (4). Rubin and Howard (5) have presented histochemical evidence that an acid mucopolysaccharide of cartilage and bone matrix may act as a "target substance" for the deposition of calcium and the eventual formation of bone salts. The structure of urinary concretions suggests that the initial matrix may be composed of ion-binding mucopolysaccharides or other "substrates," which not only provide the architectonic framework but may actually "extract" specific inorganic components of urine from solution. The separation and identification of the organic components of urine are essential prerequisites to an exploration of this possibility.

A series of experiments, in which some subjects have been repeatedly examined over a period of two years, has demonstrated that the biocolloids of urine may be separated into three groups of substances, on the basis of their solubilities in different buffer systems. It is the purpose of this report to present the results of studies on one of these groups—those components which are soluble in molar sodium chloride and in two buffers which have been used in the electrophoretic study of serum proteins (6–8).

METHODS

Three female and 10 adult male subjects from the staff of the North Carolina Baptist Hospital and the Bowman Gray School of Medicine were selected as a control group; all were known to have normal renal function. The test group was made up of 17 patients (7 females and 10 males) selected over a period of 20 months because they were demonstrated roentgenographically to have progressively developing calculi. Later analysis of these stones showed them to contain pure calcium phosphate or a mixture of calcium phosphate and calcium oxalate. These patients were studied prior to operation, and no individual with signs or symptoms of acute infection was included. In addition, two patients, ages 7 and 11 years, with the nephrotic syndrome and two patients, ages 16 and 18 years, with lupus erythematosus were studied as examples of altered capillary permeability. Four additional patients with bilateral obstructive urinary tract disease and bacterial pyelonephritis were studied before operation, but after the signs of pyelonephritis had subsided.

Clean voided specimens of urine were collected into chemically clean containers without preservatives, and immediately refrigerated at 0° C. Hospitalized subjects were provided with bedside ice chests to insure prompt refrigeration. Each 24-hour specimen was centrifuged at 800 times gravity to remove formed elements, and then dialyzed against distilled water at 3° C. until the electrical conductivity approached 100×10^{-6} and the pH was the same as the distilled water.

The dialyzed specimens were subjected to ultrafiltration in stainless steel filters through a modification of the collodion membranes described by Gorbman (9). Variations in porosity of the membranes were minimized by preparing 30 individual membranes from the same collodion mixture. These membranes, prepared on glass plates floated on mercury with exact control of reagents, temperature and time, were of a reproducible porosity, as judged by the filtration rates for distilled water and retention of Bence-Jones protein. The membranes were stored in distilled water to which a few thymol crystals were added, and urine specimens from at least one normal subject were examined with membranes from each batch.

Ultrafiltration was conducted in the cold room at 3 to 5° C. Forty-eight hours was required for filtration of a normal 24-hour specimen through a single membrane under increasing pressures of 5 to 125 pounds per square inch. The large quantity of colloidal material contained

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in the urine of patients with calculi frequently required three to six membranes for filtration of a 24-hour specimen.

The colloidal residues were removed from the membranes as a gel, immediately frozen, and stored until a total of 72 to 240 hours of continuous urinary output per subject had been processed. To insure removal of all soluble colloids from the membranes, each was shredded and immersed in 30 ml. of Veronal buffer, ionic strength 0.1, pH 8.6, and stored at 1.0° C. In a preliminary series of experiments the membranes were dissolved in 15 ml. of ethanol-ether at 0.5° C.; the precepitate was then extracted three times with ethanol-ether, dried with ether, and added to the solid gel obtained above. This procedure did not appear to have any advantage over buffer extraction of the membranes.

After completion of the collection period, each 24-hour colloidal residue was extracted three times with 10 ml. of the buffer used to extract the collodion membrane, and finally with 10 ml. of fresh buffer. Extraction with molar sodium chloride gave results which were quantitatively and qualitatively similar to the buffer extracts from the same individuals. All of the soluble proteins contained in the gel seemed to carry a high concentration of adsorbed or bound urinary pigments. These pigments served as a convenient guide to the completeness of extraction. The pooled extracts of normal urine usually totaled 120 to 200 ml. per 72 to 120 hours of urinary excretion. These were concentrated to a volume of 8 ml. by perevaporation—that is, suspension of the solution in cellophane bags 15/16 inch wide in a cold air stream at 5° C. Similar results have been obtained by pressure dialysis against 2 liters of Veronal buffer, and this technique has been routinely used for subsequent experiments.

The total protein content of a small sample of the dialyzed extract was determined by the biuret method, and the solution was diluted with Veronal buffer to give a resulting protein content of 1 per cent. A final equilibration dialysis against Veronal buffer of pH 8.6 was made prior to electrophoretic studies with the Aminco electrophoresis unit and calibrated cell of 8 ml. capacity. The proteins were allowed to migrate for 7200 seconds under a potential gradient of 7.4 volts per cm. at 0.5° C. in Veronal buffer of ionic strength 0.1 and pH 8.6 (10). Photographs were made with the slit diaphragm, cylindrical lens system, using a red filter and high contrast panchromatic film.

Samples of urinary proteins from the same patients were also studied electrophoretically at pH 4.5, by the method others (8, 11–14) have used for serum proteins. After the proteins had been collected and concentrated in Veronal buffer, they were dialyzed against an acetic acid, sodium acetate buffer of pH 4.5, ionic strength 0.1 (15) for 72 hours. Intermediate dialysis against phosphate buffers to prevent precipitation was found to be unnecessary. Electrophoretic migration was studied in the same cell for three to five hours under a potential gradient of 6.0 volts per cm. at 0.5° C.

A very small precipitate which formed during the acid dialysis was removed by centrifugation. This precipitate was much larger in the group of patients with calculous disease than in the control group. It could not be completely redissolved in Veronal buffer of pH 8.6, but the soluble portions from five patients and from three normal subjects were studied electrophoretically in Veronal buffer, pH 8.6, ionic strength 0.1, at a potential gradient of 7.4 volts per cm. for two hours. The insoluble portion gave a positive Molisch test and was soluble in glycine buffer of pH 10.

RESULTS

The colloidal gel obtained from normal 24-hour specimens measured 0.90 to 1.25 ml. in volume. The dry weights of these lyophilized residues from five normal subjects averaged 0.0902 Gm. per 24 hours, with a range of 0.0865 to 0.0975. In the patients with calculous disease the lyophilized residue averaged 0.496 Gm. per 24 hours, with a range of 0.296 to 1.253.

Following extraction with Veronal buffer there was a considerable residue of gel-like material, which occupied a volume almost as great as the original material. After lyophilization, the dry weights of these residues from five normal subjects were in the range of 0.0439 to 0.0448 Gm. per 24 hours. On the basis of dry weights it appears that approximately 42.6 to 52.7 mg. of the original gel was extracted by Veronal buffer. The ratio of the insoluble residue to the total colloids was much higher in the group of patients with calculi. Since no component of this gel was soluble in Veronal buffer of pH 8.6 or in acetate buffer of pH 4.5, without procedures which modify its molecular structure, further studies of this material will not be considered in the present report. The largest single component of this residue is a conjugate of carbohydrate and protein, which has been subjected to electrophoretic, spectroscopic, and biochemical analysis. The resulting data are being prepared for publication.

Electrophoretic studies at pH 8.6

Figure 1 illustrates the most typical Schlieren pattern obtained from normal urine, as compared with the maximal variations encountered in 18 determinations from 13 normal subjects. These variations are in accord with the observations of Rigas and Heller (16), who noted minor variations in the patterns obtained from different individuals, whereas the pattern of a given individual tended to remain constant over several months.

In Figure 2 are shown tracings of patterns obtained from normal plasma, as compared with tracings of the patterns made by urinary proteins subjected to electrophoretic studies at pH 8.6. A comparison of Figures 2-A and 2-B reveals that all the electrophoretically separable protein fractions found in normal serum are also present in normal urine. This observation was originally made by Rigas and Heller (16), who identified as a mucoprotein the component with a mobility greater than that of albumin. These investigators also identified an immobile polysaccharide as the cause of the unusually large delta and epsilon boundaries. We have observed a tendency for these "salt plus polysaccharide" boundaries to show a very slight migration toward the cathode. Since the mobilities of the albumin fractions have been consistently reproducible in all of these studies, the projected patterns were aligned on the albumin peaks rather than on the delta and epsilon boundaries. The illustration is thus more nearly representative of the calculated mobilities as measured from the initial boundary. There is evidence that fibrinogen does not occur even in nephrotic urine (7, 17–19), and the small boundary near the position of theta may be properly designated gamma-1 globulin.

The pattern illustrated in Figure 2-C was obtained from the same subject and during the same 10-day period of collection as the pattern seen in Figure 2-B. Alternate 24-hour specimens were subjected to the technique of recovery already described, with the result shown in Figure 2-B. The remaining five days' collection was concentrated by the alcohol-ether precipitation method described by Rigas and Heller (16), with the result shown in Figure 2-C. Two components with mobilities greater than albumin (fc-1 and fc-2) are present in both. Figures 2-D, E and F illustrate the proteins present in the urine of patients with various diseases of the urinary tract.

Comparable patterns obtained by the electrophoretic study of proteins in the serum and urine of a patient with lupus erythematosus are shown in Figure 3. All of the Veronal-soluble proteins in a single 24-hour specimen of urine, 420 ml. in volume, were concentrated into 10 ml. of buffer, and the resulting protein solution of 3.5 per cent was subjected to electrophoresis.

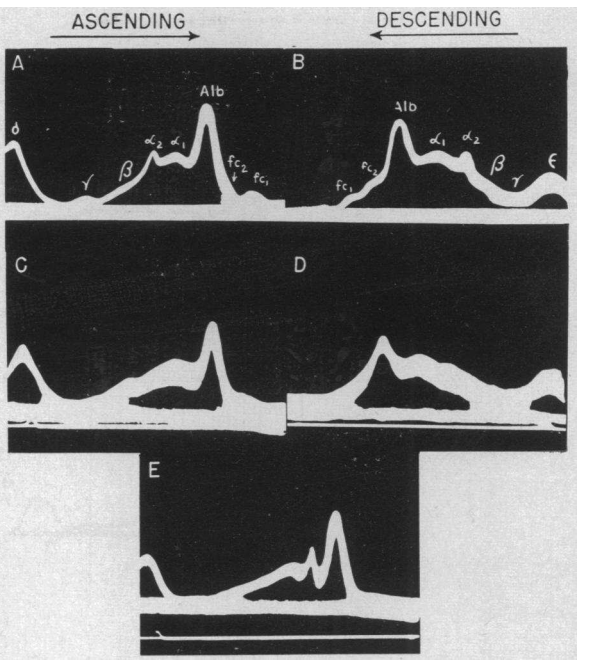

FIG. 1. SCHLIEREN DIAGRAMS OF URINARY PROTEINS FROM NORMAL SUBJECTS

A and B are the most typical patterns encountered in 13 subjects. C, D and E represent the greatest variations from the typical normal pattern encountered in 18 determinations.

Figure 4 shows the electrophoretic patterns obtained from normal urine and from the urine of five patients with calculous disease. These five patterns are typical of the variations from the normal which were observed in all of the 17 patients with renal stones. As measured by the biuret test, the average protein excretion in this group was 0.204 Gm. per 24 hours, with a range of 0.086 to 0.312. The patterns of all of these patients were markedly similar, and were characterized by a separation of the albumin from the adjacent large boundaries.

As determined by calculations after the method of Dole (6), the mobilities of the albumin and of the beta and gamma globulin fractions in the urine of these patients were comparable to those in normal urine and normal serum. The mobilities of the two boundaries between the albumin and beta globulin peaks were essentially the same for each of the patients with calculous disease, but varied significantly from those of the alpha-1 and alpha-2 fractions of normal urine. These two boundaries

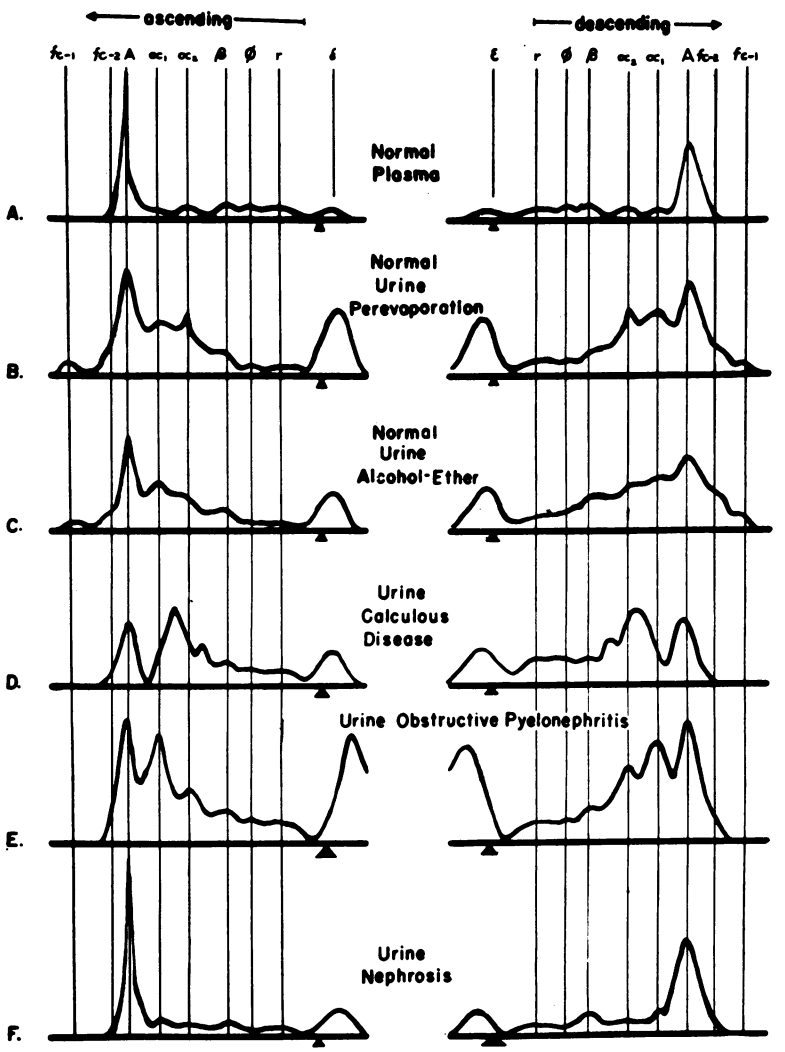

FIG. 2. TRACINGS OF ELECTROPHORETIC MIGRATION PATTERNS OF NORMAL HUMAN PLASMA AND OF URINARY PROTEINS IN HEALTH AND IN THREE DISEASE STATES ACCOMPANIED BY INCREASED PROTEINURIA

Veronal buffer, pH 8.6, ionic strength 0.1, potential gradient 7.4 volts per cm. for 7,200 seconds.

Tracings of the projected patterns are utilized to eliminate inequalities of exposure which the presence of urinary pigments introduces into the original photographs. These inequalities do not impair the usefulness of any single pattern, but preclude the simultaneous copying of the large number of patterns used in this illustration.

together comprised 46.7 per cent of the total amount of protein in the urine of patients with calculous disease (Table I). The larger peak had an average mobility of $-4.81\ (\pm 0.11) \times 10^{-5}$ cm.^{2}/volt sec., and the smaller an average of $-3.92\ (\pm 0.02) \times 10^{-5}$ cm.^{2}/volt sec. Dole (6) gives a mobility of -5.07×10^{-5} cm.^{2}/volt sec. for alpha-1 globulin and -4.08×10^{-5} cm.^{2}/volt sec. for alpha-2 globulin.

In Figures 4-B, 4-C and 6-B a small peak can be seen between those representing albumin and the large components of calculous disease urine just discussed. These small peaks have a calculated mobility of the same value as alpha-1 glob-

ulin of the serum. This fact suggests that the alpha globulins are present in calculous disease urine, but may be "overshadowed" by the large volume of those components which are thought to be mucoproteins.

In Table I the components of the urinary proteins in normal subjects and in 25 patients with various diseases, as measured in planimeter units by the method of Longsworth, are averaged and expressed as percentages of the total. In Table II the approximate average quantities of these components are expressed as milligrams per 24 hours of urinary excretion.

Electrophoretic studies at pH 4.5

Figure 5 shows the patterns obtained when urinary proteins and serum proteins from the same normal subject were studied by electrophoresis at pH 4.5. Under these conditions the albumin forms a stationary boundary, while the globulins are positively charged and migrate toward the cathode. The serum globulins separate into three components—A, B and C—which have been demonstrated to represent, respectively, the alpha, beta and gamma globulin fractions observed at pH 8.6 (12, 13). The acid mucoprotein MP-1 (orosomucoid) seen in normal serum has not been conclusively demonstrated in normal urine, but has seemed to be present in minute concentrations in some specimens. An acid component with the same mobility as MP-2 was always present (Figure 5).

A precipitate formed during the acid dialysis of the proteins, and only a very small portion could be redissolved in Veronal buffer of pH 8.6. The Veronal-soluble portion gave a positive biuret tests. When it was subjected to electrophoretic studies, only albumin could be detected, although the electrophoretic patterns in Figure 4 indicate a possible loss of globulin also.

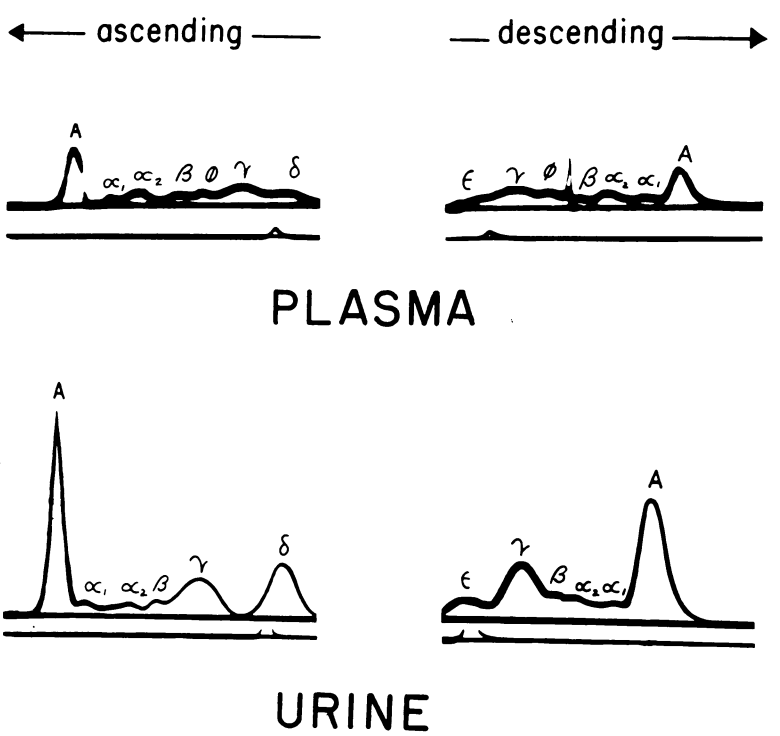

FIG. 3. ELECTROPHORETIC MIGRATION PATTERNS OF PROTEINS IN THE PLASMA AND URINE OF A PATIENT WITH LUPUS ERYTHEMATOSUS

Veronal buffer, pH 8.6, ionic strength 0.1, potential gradient 7.4 volts per cm. for 7,200 seconds.

The albumin peak of the ascending pattern of the urinary proteins extended beyond the photographic plate, and has been drawn into the print.

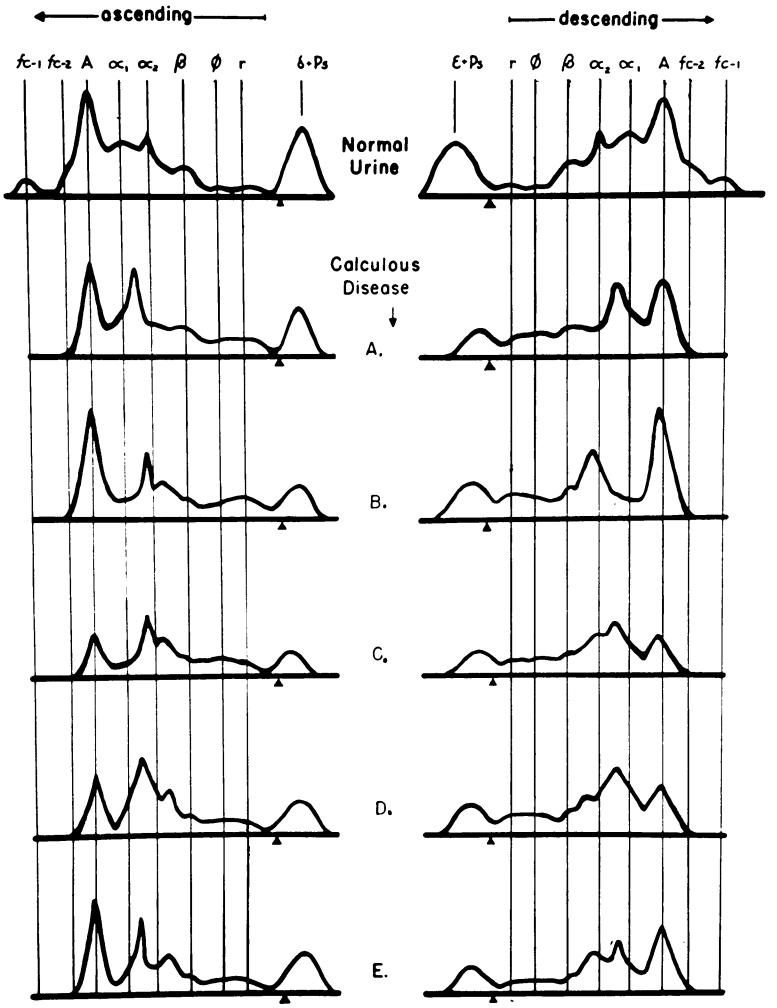

FIG. 4. TRACINGS OF ELECTROPHORETIC MIGRATION PATTERNS OF URINARY PROTEINS IN HEALTH AND IN CALCULOUS DISEASE

Veronal buffer, pH 8.6, ionic strength 0.1, potential gradient 7.4 volts per cm. for 7,200 seconds.

In a preoperative study of the patient with a functioning adenoma of the parathyroid gland (4-C), a 24-hour specimen of urine contained 180 mg. of proteins.

In Figure 6 the electrophoretic patterns from normal urine and from the urine of patients with calculous disease and chronic pyelonephritis are compared at pH 8.6 and 4.5. For the purpose of this illustration the proteins were recovered from the cell following studies at pH 8.6, concentrated to the original volume, and dialyzed to a pH of 4.5. The precipitate which occurred in every case was composed primarily of a polysaccharide (Molisch test) which was immobile at pH 8.6. In the normal and pyelonephritic urines the only other detectable component of the precipitate was a very small amount of albumin. In the urine of patients with calculous disease the precipitate contained a relatively greater concentration of the mucoprotein than of albumin (Figure 6-G). Note that, although the precipitated albumin retains the normal mobility for albumin at pH 8.6, the mobility of the precipitated mucoprotein is even less than it was in the original determination at pH 8.6

TABLE I

Relative concentrations of proteins in normal serum, normal urine, and the urine of patients with renal calculi and other pathologic conditions

Subjects	Determinations	Quantity mg./24 hrs.	A/G Ratio	Area of each boundary determined by planimetry and expressed as % of total						
				fc-1	fc-2	Albumin	Alpha-1	Alpha-2	Beta	Gamma
Normal serum*		7.23 Gm./100 cc.	1.33			56.8	7.2	8.7	12.8	14.4
Normal urine (13)	18	42.6 to 52.7	0.64	2.2	?	37.9	27.3	19.5	8.8	3.3
Calculous disease urine (17)	36	86 to 312	0.45			28.8	28.4	18.3	7.3	17.2
Obstructive pyelonephritis urine (4)	6	263	0.37			26.9	36.2	18.1	8.8	10.1
Nephrosis urine (2)	4	800 to 1,610	1.94			66.0	7.5	8.5	10.4	7.5
Lupus erythematosus urine (2)	3	350	1.15			53.6	1.5	6.0	5.7	33.2

* Reiner, M., Fenichel, R. L., and Stern, K. G., Electrophoretic studies on the protein distribution in normal human serum. Acta Haematologica, 1950, **3**, 202.

(Figure 6-B). This material was recovered from the cell and gave both a positive Molisch test and a positive biuret test.

The unprecipitated mucoprotein of calculous disease urine contributes to the stationary albumin boundary, but in both the normal and the pyelonephritic urines the mucoprotein migrates toward the anode. This finding has been generally true in patients with calculous disease, although the presence of a small quantity of negatively charged mucoprotein in the urine of some of these patients has been demonstrated at pH 4.5.

DISCUSSION

The Schlieren diagrams are of necessity illustrated on the same scale. For this reason, anyone not thoroughly familiar with the techniques involved may be deceived regarding the concentration of protein in the individual urine specimens. For example, the pattern for normal urine in Figure 4 represents the total proteins from 8.1 liters of urine (120 hours' output), whereas the pattern shown as Figure 4-A represents the total proteins from only 1.6 liters of urine (20 hours' output). The purpose of Tables I and II is to show the relative quantities of the urinary proteins per 24 hours of collection time. The figures in these tables are not presented as absolute values, but they are of considerable importance in the interpretation of the Schlieren diagrams.

These figures were obtained by determining the lyophilized dry weight of the total colloidal residue of one 24-hour urine specimen from each subject. The soluble proteins were extracted from this residue with 0.1 molar Veronal buffer of pH 8.6, by the method used in preparing samples for the electrophoretic studies. The lyophilized dry weight of the remaining insoluble residue was then determined, and the difference in weights was taken as the value for the soluble proteins. The individual components were determined by planimetry, and the area of each component was translated into milligrams on the basis of the difference in dry weights.

TABLE II

Average quantity of urinary proteins obtained from normal subjects and from patients with renal calculi and other pathologic conditions

Subjects	Determinations	Milligrams per 24 hours of urinary excretion						
		fc-1	fc-2 Albumin	Alpha-1	Alpha-2	Beta	Gamma	Total
Normal (13)	5	0.9	17	12	9	4	2	45
Calculous disease (17)	17		59	58	37	15	35	204
Obstructive pyelonephritis (4)	2		70	95	48	23	27	263
Nephrosis (2)	2		620	71	80	98	71	940
Lupus erythematosus (2)	2		187	5	24	19	115	350

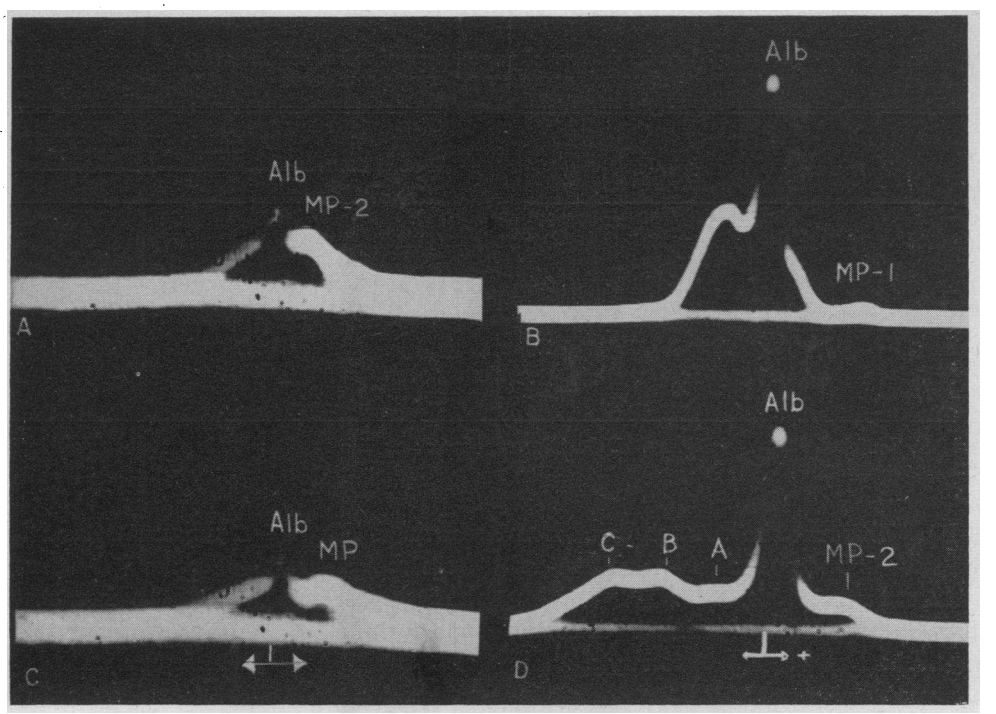

FIG. 5. ELECTROPHORETIC MIGRATION PATTERNS OF NORMAL SERUM AND OF NORMAL URINARY PROTEINS AT pH 4.5

Slit diaphragm, cylindrical lens patterns of boundaries ascending toward the anode. Acetate buffer, pH 4.5, ionic strength 0.1, potential gradient 6.2 volts per cm.

A. Urinary proteins at 3,600 seconds.

B. Serum proteins at 3,600 seconds.

C. Urinary proteins at 10,800 seconds.

D. Serum proteins at 10,800 seconds. MP-1 has migrated beyond the photographic range.

Certain errors are obviously inherent in this technique. The polysaccharide, which contributed to the total weight of the soluble material, is immobile at pH 8.6 and is not included in the planimetric studies, since a variable portion of this refractive gradient (E + Ps in Figure 4) is also due to the epsilon "salt" boundary. Inspection of the patterns will demonstrate that planimetric separation of the individual gradients is much more arbitrary in studies of these urinary proteins than in comparable studies of serum proteins. Furthermore, the overlap of fc-2 and albumin gradients made it desirable to express these values as a single sum (Tables I and II).

There are two components of normal urine whose electrophoretic mobilities at pH 8.6 are greater than that of albumin. The urinary mucoprotein recovered by Tamm and Horsfall (20) is electrophoretically homogeneous at pH 6.8 and 8.6 (21), and has a mobility slightly greater than that of human serum albumin (22). This mucoprotein is insoluble in molar sodium chloride, but it is possible that it may have a limited solubility in the 0.1 molar Veronal buffer used for extraction in our experiments. The refractive gradient fc-2 may therefore represent the mucoprotein of Tamm and Horsfall. Since these authors obtained a yield of 0.025 Gm. of mucoprotein per liter of urine, it is obvious that fc-2 (Figures 1, 2, 6) is only a portion of the total quantity of this mucoprotein present in the 72- to 120-hour urine specimens represented by these Schlieren patterns. This finding would be in accord with the limited solubility of this material in 0.1 and 0.025 molar buffers (20).

The large alpha globulin boundaries in normal urine at pH 8.6 are composed not only of the alpha globulins of serum but also of an acid protein. This protein has a mobility at pH 4.5 and

8.6 similar to that of the mucoprotein MP-2 previously observed in normal and pathologic human sera (8, 12–14). The greater portion of the alpha globulin boundaries of the urinary proteins is made up of this acid mucoprotein; and if it is taken into consideration in calculations of the albumin-globulin ratio of the urinary proteins, the result is a urinary A/G ratio even greater than that of normal serum.

Nephrosis and lupus erythematosus are diseases characterized by increased capillary permeability and increased proteinuria. The additional protein in the urine is composed almost entirely of proteins found in the serum of these patients. The lipoproteins found in the serum of nephrotic patients do not appear in the same relative proportions in the urine (19), but beta globulin is present in the urine (7). The gamma globulin fractions found in the blood of patients with lupus erythematosus do appear in the urine of these patients in increased concentrations. In these patients the urinary proteins have an A/G ratio more nearly similar to that of normal serum than to that of their own serum; this finding indicates a relatively greater urinary loss of albumin than of globulin.

Renal calculous disease is accompanied by increased proteinuria, presumably the result of inflammatory or exudative processes within the collecting portions of the urinary system. The urinary A/G ratio is reversed from that of normal

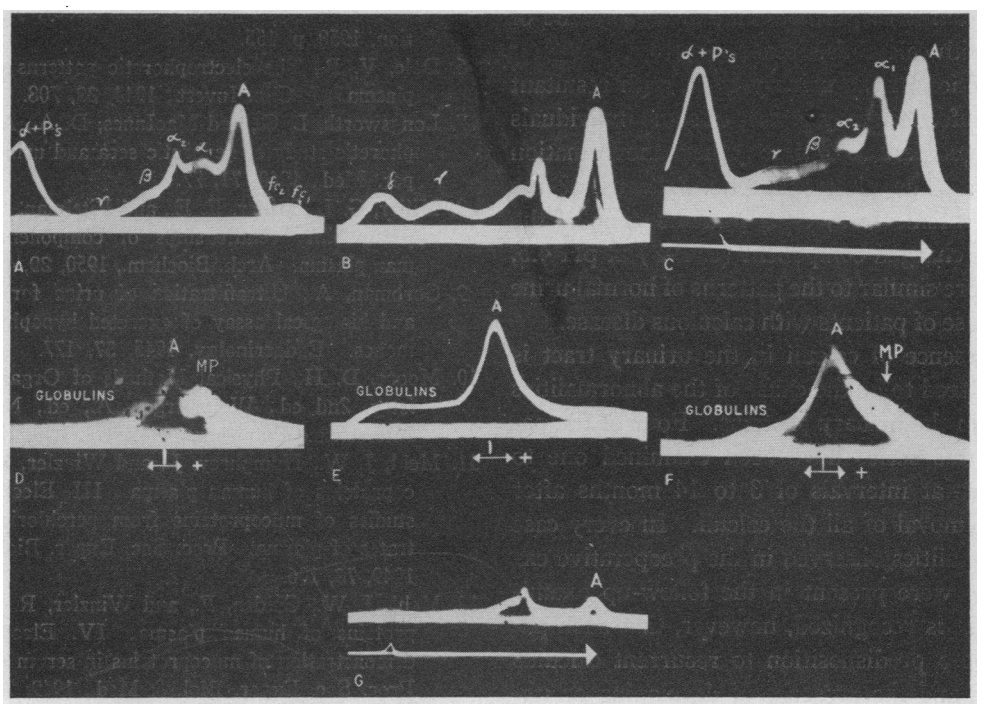

FIG. 6. ELECTROPHORETIC MIGRATION PATTERNS OF URINARY PROTEINS AT pH 8.6 AND pH 4.5

Patterns at pH 8.6 were taken after 7,200 seconds migration in Veronal buffer, pH 8.6, ionic strength 0.1, potential gradient 7.4 volts per cm. Patterns at pH 4.5 were taken after 10,800 seconds migration in acetate buffer, pH 4.5, ionic strength 0.1, potential gradient 6.0 volts per cm. All patterns are ascending toward the anode.

A. Normal urinary proteins at pH 8.6.
B. Calculous disease urinary proteins at pH 8.6.
C. Pyelonephritis urinary proteins at pH 8.6.
D. Normal urinary proteins at pH 4.5.
E. Calculous disease urinary proteins at pH 4.5.
F. Pyelonephritis urinary proteins at pH 4.5.
G. Precipitate from acid (pH 4.5) dialysis of calculous disease urinary proteins redissolved in Veronal buffer of pH 8.6.

serum, because the urine contains two components which have mobilities slightly less than the alpha globulins. These two components are isoelectric at pH 4.5, where they tend to precipitate spontaneously and to contribute to the stationary albumin boundary. Whether these are structural modifications of the alpha fractions of normal urine or are entirely different substances peculiar to the urine in calculous disease remains at present unknown. The observed alteration in the mobility of these alpha boundaries (Figures 2 and 4) may be due to the fact that, in patients with calculous disease, the relative concentrations of these and other components of urine differ from those found in normal subjects (Table II). Studies are now in progress to isolate and identify further these components from both normal urine and the urine of patients with calculous disease.

Obstruction of the urinary tract, with resultant bacterial infection, may persist in some individuals for prolonged periods of time without the formation of calculi. The electrophoretic patterns of the urinary proteins from such patients contain the negatively charged component (MP-2) at pH 4.5, and are more similar to the patterns of normal urine than to those of patients with calculous disease.

The presence of calculi in the urinary tract is not considered to be the cause of the abnormalities observed in the urinary proteins. Four of the patients in this series have been examined one to three times at intervals of 3 to 14 months after surgical removal of all the calculi. In every case the abnormalities observed in the preoperative examinations were present in the follow-up examinations. It is recognized, however, that such patients have a predisposition to recurrent calculus formation, and the presence of developing or unrecognized stones cannot be excluded at the present time.

SUMMARY

The total quantity of proteins and other particles of colloidal size in the urine of patients with calculous disease is 3 to 13 times greater than that in normal urine. Electrophoretic studies of those components soluble in buffers of pH 4.5 and 8.6 have demonstrated consistent variations in the proteins from the urine of patients with calculous disease as compared with normal urine. These variations have been most apparent in the "alpha" fractions.

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ERRATUM

A Possible Fourth Plasma Thromboplastin Component, by Theodore H. Spaet, Paul M. Aggeler, and Beverly G. Kinsell, J. Clin. Invest., 1954, **33**, 1095. Table V.

The item, "5% normal plasma + PTA-deficient blood" should read "5% normal plasma + PTC-deficient blood."

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