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Research Article

OBSERVATIONS ON DOUBLE ALBUMIN: A GENETICALLY TRANSMITTED SERUM PROTEIN ANOMALY * †

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Knedel (3, 4) has published observations on "double albumin" noted on electrophoresis of the serum proteins of eight members of two families in Germany. The anomaly appeared in three generations of one of the families. Immunochemical studies demonstrated that both fractions in the albumin region, designated A_1 and A_2 by Knedel, were indeed albumin.

The present paper is a report of a similar anomaly in 25 of 43 members of a family of Norwegian descent living in Illinois. A number of immuno- and physicochemical observations on the anomalous protein are described.

In our preliminary report (1) we designated the two fractions A and B, being unaware at the time of Knedel's prior work and designations of fractions A_1 and A_2. Despite Knedel's priority, we have decided to retain our terminology of albumin A and albumin B. Albumin A corresponds to Knedel's fraction A_1 and probably is normal albumin, as will be shown. Albumin B corresponds to Knedel's fraction A_2, and is an anomalous albumin. No evidence exists that the anomalous albumin fractions in the several unrelated families are chemically identical. Paralbumin, therefore, might be a more acceptable general designation for the anomalous protein.

OBSERVATIONS

1. Discovery of anomaly, clinical and genetic data. A 22 year old white male complained of pain in the right costo-vertebral angle area. The urine contained 2 plus protein, rare red blood cells, 6 to 8 white blood cells per high power field and rare hyaline casts. The blood urea nitrogen was 42 mg. per 100 ml.; the urea clearance, 17 per cent of normal; and the phenolsulphonphthalein excretion, 5 per cent in 15 minutes. Total serum proteins were 7.2 Gm. per 100 ml.; the albumin, 5.2; and the globulin, 2.0 (by precipitation). The serum cholesterol was 222 mg. per 100 ml. The right kidney appeared to be normal on intravenous pyelograms but the left kidney and ureter were not visualized. On cystoscopy the right trigone was prominent but neither a left trigone nor a left ureteral orifice could be visualized.

We were very surprised to observe on the paper electrophoretic pattern of his serum proteins what appeared to be a double albumin (Figure 1). The persistence of this pattern has been demonstrated in four samples of serum obtained from this patient over a period of one year. A similar anomaly was demonstrated in his urinary proteins after concentration by dialysis (Figure 1).

On the thought that the protein anomaly and the absence of a kidney might be associated and transmitted genetically, we instituted a study of the patient's family. Sera from his mother and four of her siblings did not have the anomaly. However, study of the patient's father and his family was very rewarding, as shown by the

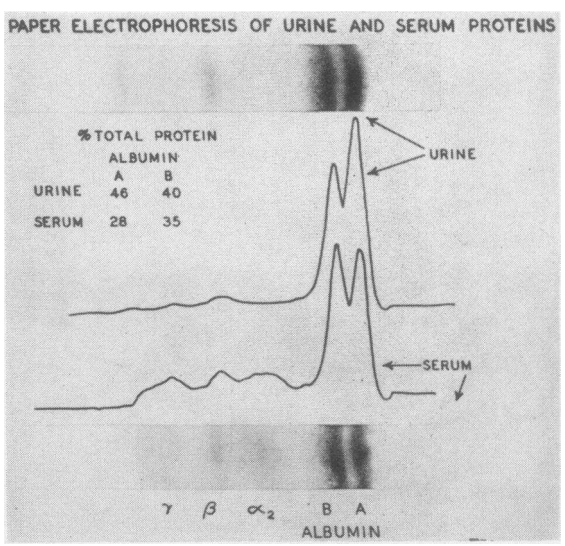

FIG. 1. PAPER ELECTROPHORETIC PATTERNS OF SERUM AND URINARY PROTEINS OF ORIGINAL SUBJECT WITH DOUBLE ALBUMIN

* Some of these data were presented at the annual meeting of the Association of American Physicians, May, 1958 (1), and at the International Biochemistry Congress of Vienna, September, 1958 (2).

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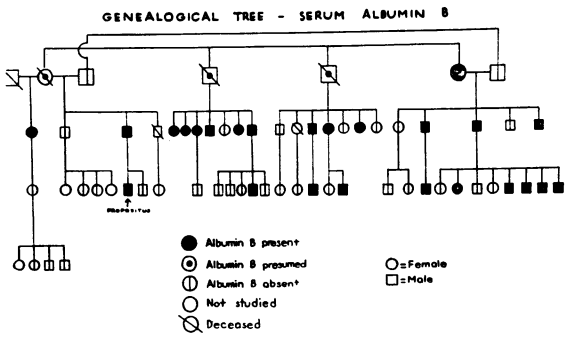

FIG. 2. GENEALOGICAL TREE OF FAMILY WITH DOUBLE SERUM ALBUMIN

genealogical tree (Figure 2). The anomaly, as indicated by solid dots (females) and squares (males), undoubtedly resides in his paternal grandmother's family. The deceased paternal grandmother had affected offspring by two different mates. Her sister, still alive, has the double peak, while her two deceased brothers presumably had the anomaly since it appeared among their descendents. Finally, neither the paternal grandfather nor his brother (brothers married sisters) have the double peak (Figure 2). The paternal grandmother and her sister were the children of immigrants to Illinois from Norway.

So far as can be determined, the anomalous albumin in this family is not associated with disease except incidentally. Intravenous pyelograms were obtained in six affected members of the family; the original subject is the only one with congenital absence of a kidney. Furthermore, no other affected member of the family has significant proteinuria. A fairly high incidence of arteriosclerotic heart disease was noted in the family, but individuals without the protein anomaly did not differ in this respect from members with the anomaly.

The fact that eight offspring of known negative parents are not affected supports the assumption that unaffected members of the family cannot pass the anomaly to their children. The latter, therefore, are excluded from the "genetic risk" group in Table I. On the other hand, three deceased members of the first generation whose offspring had the anomaly are assumed to have had the anomaly and, therefore, they and all their offspring are included in the "genetic risk" group. Two offspring of parents not studied and without known anomalous offspring, as well as 11 collateral relatives, are not affected. On the basis of these assumptions, 28 of 46 exposed to the genetic risk are affected (Table I).

TABLE I

Incidence of double albumin in family

	Total subjects	With double albumin
Exposed to genetic risk	46	28
Offspring of negative parents	8	0
Offspring of parents not studied and without other children	2	0
Collateral relatives	11	0

TABLE II

Serum proteins and cholesterol in family with double albumin

	28 Normal subjects	25 Affected subjects
$\frac{\text{Albumin B}}{\text{Total albumin}} \times 100$	0	57±4.4*
$\frac{\text{Total albumin}}{\text{Total protein}} \times 100$	49	50
Total protein (Gm./100 ml.)	7.5	7.6
Total serum cholesterol (mg./100 ml.)	259	268

* If alpha 1 globulin (which is obscured by albumin B) were deducted, this figure would approximate 50 per cent.

The relative amounts of albumins A and B are remarkably constant (Table II). Albumin B makes up on the average 57 per cent of the total serum albumin, with a standard deviation of 4.4 per cent. If the usual normal value for alpha 1 globulin (which usually is obscured on paper electrophoresis by albumin B) were deducted, albumin B would account for approximately half the total serum albumin of the anomalous subjects. The sum of albumins A and B is equal to the normal serum albumin value, while the total serum protein value in the anomalous subjects likewise is normal. In anomalous subjects, therefore, the extra albumin fraction apparently replaces an equivalent amount of normal albumin.

Total serum cholesterol values are slightly greater in the anomalous subjects than in the normal members of the family, but the difference is not statistically significant.

2. Immunochemical observations. Materials and methods: Sera from five individuals with the anomalous serum protein, from two of their siblings without the anomaly, and from three normal persons unrelated to the pedigree were selected for immunochemical studies.[1] The proteins of the sera were first separated by zonal electrophoresis in both powdered starch (5) and agar gel (6) at pH 8.6 in diethylbarbiturate-citrate buffer having an ionic strength of 0.1. Electrophoresis was performed at 4° C. using 8 to 10 volts per cm. of length of starch or agar for 24 hours. In some instances, a crystal or two of bromphenol blue was dissolved in a serum before placing the serum in the starch or agar. Under these circumstances the separation of the albumins, identified by means of the bound blue dye, could be observed during electrophoresis (Figure 3). On other occasions, the localization of the proteins in the starch or agar was determined by

[1] We are greatly indebted to Dr. Dale Learned of Kankakee, Ill., for his generous assistance in obtaining large amounts of blood for these studies.

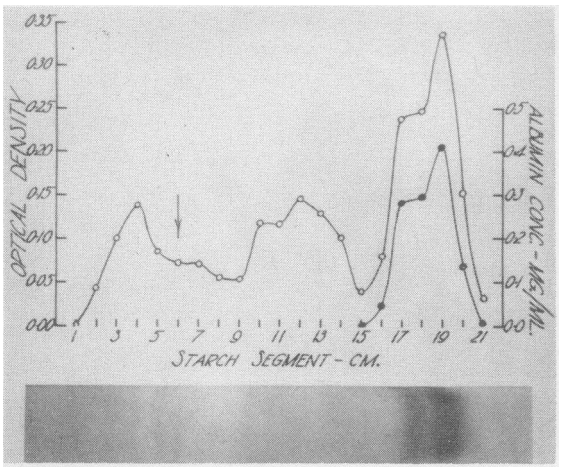

FIG. 3. STARCH ELECTROPHORESIS (○—○—○) OF SERUM FROM PROPOSITUS, AND ALBUMIN CONCENTRATIONS (●—●—●) ESTIMATED WITH RABBIT ANTI-ALBUMIN ANTISERUM

Arrow indicates origin. Optical density = O.D. $\frac{1\text{ cm.}}{280\ m\mu}$. Below graph is a photograph of the starch block showing binding of bromphenol blue by both albumins. Anode is toward right side of figure.

pressing a dry strip of Whatman No. 1 filter paper against the block. The wet paper strips were then stained in 1 per cent bromphenol blue (in 95 per cent ethanol saturated with mercuric chloride) and finally washed in tap water, the result being a "print" of the protein distribution in the block.

For electrophoresis, two different starch preparations were used from which little or no ultraviolet light absorbing material was released with pH 8.6 barbiturate-citrate buffer or with 0.15 M NaCl. One batch was prepared by repeated washing of potato starch (Fischer, iodometry grade) with distilled water followed by several washes each of pH 8.6 barbiturate-citrate buffer, 0.1 M pH 8.6 borate buffer, distilled water and finally acetone. The starch was dried in air at room temperature. The other starch preparation was obtained from Connaught Laboratories (Toronto) as a hydrolyzed derivative for use in forming starch gels (7). In this study, however, the starch was used only as a powder and proved admirably suited for this purpose. The starch blocks measured 4 by 30 cm., were covered with wax paper and supported on individual one-fourth inch thick plate glass strips. After electrophoresis, the starch blocks were cut into 0.5 or 1.0 cm. segments. The serum proteins were eluted with 0.15 M NaCl. The optical densities of the eluates were measured in a Beckman spectrophotometer at a wave length of 280 mμ. When bromphenol blue had been dissolved in the sera, the eluates were measured at 600 mμ as well. At the latter wave length, the blue color of the dye complex was the principal absorbant. Where applicable, as for the eluted albumins from sera without added dye, the extinction coefficient for albumin $E\,\frac{1\text{ per cent}}{1\text{ cm., }280\ m\mu}$ was taken as 5.32 (8).

Aliquots of those eluates representing the central starch segment for each albumin peak were appropriately diluted with 0.15 M NaCl so that a series of concentrations were obtained for each albumin. Antigen-antibody reaction mixtures were prepared by adding 1.0 ml. of a given albumin dilution to 1.0 ml. aliquots of rabbit antiserum *vs.* normal human serum albumin prepared as described elsewhere (9). The mixtures were incubated at 37° C. for one hour and then at 4° C. for 20 hours. The resulting specific precipitates were collected by centrifugation at 3,000 rpm at 0° C. for 15 minutes and washed twice with cold 0.15 M NaCl. The amount of specific precipitate was measured by a spectrophotometric method (10). The quantitative precipitation curves obtained by plotting the amount of specific precipitate *vs.* the relative amount of albumin added to the antiserum were compared as described previously (9). The concentration of albumin in all the eluates was estimated by the same precipitation method, the rabbit antiserum being standardized with crystallized pooled human serum albumin, preparation Decanol 10 (8), the antigen against which the antiserum was made.

The immunochemical characteristics of the albumins separated by starch electrophoresis were compared with each other and also with pooled normal albumin by means of the two-dimensional agar diffusion method of Ouchterlony (11). For this purpose, 2 per cent agar in 0.1 M, pH 8.6 borate buffer was poured into Petri dishes to a depth of 4 to 5 mm. Seven holes, each with a diameter of approximately 7 mm., were cut through the agar with a cork borer. The bottoms of the holes were sealed with fresh agar. Six of the wells were situated in a circle approximately equidistant from each other and from the seventh central well (Figures 4 and 6). The central well was filled with rabbit or horse antiserum *vs.* normal human serum albumin. One of the peripheral wells was

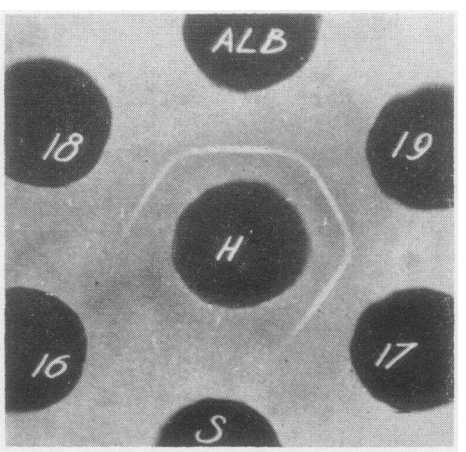

FIG. 4. AGAR GEL ANTIGEN-ANTIBODY DIFFUSION REACTIONS USING HORSE ANTI-ALBUMIN IN THE CENTRAL WELL (H) AND NORMAL HUMAN SERUM ALBUMIN (ALB) AND 0.15 M NaCl (S) AS CONTROLS

Numbers in wells refer to the number (cm.) of segment in Figure 3 from which eluates were obtained.

filled with 0.1 per cent crystallized pooled normal human serum albumin in 0.15 M NaCl and the opposite well with 0.15 M NaCl. Each of the remaining wells was then filled with an eluate from the starch segments. The horse antiserum used has been described elsewhere and is that preparation designated as Bleeding No. 3 (12). The Petri dishes were kept in moist chambers at room temperature and examined daily. The reader is reminded that in such agar diffusion systems, a band of antigen-antibody precipitate forms in the agar gel between the wells of antigen and antiserum. If a band of specific precipitate forms between the antiserum well and one antigen well and this crosses but does not merge with that formed between the same antiserum and a second antigen well, forming an X, then the reacting antigens in the two wells are immunochemically different. On the other hand, if the *end* of one precipitation band merges into the *side* of the other, forming a Y, then the two antigens are considered to have some haptenic groups in common. If one end of each precipitation band merges completely into the other to form a V, the two antigens under proper conditions are at least qualitatively identical as regards to their haptenic groups; the latter reaction is termed an "identity" reaction in this report.

The agar gels used for electrophoresis were prepared on $\frac{1}{4} \times 8 \times 10$ inch or $\frac{1}{8} \times 6 \times 8$ inch pieces of plate glass, the gel block being divided into four or five channels of equal width by longitudinal troughs or wells. The details of this preparation of agar plates have been presented elsewhere (13). After electrophoresis, horse antiserum *vs.* normal human serum albumin was placed in the wells and the plates kept in moist chambers at room temperature. In 24 to 48 hours, curvilinear bands of specific precipitate appeared where anti-albumin antibodies diffusing into the agar reacted with human serum albumin.

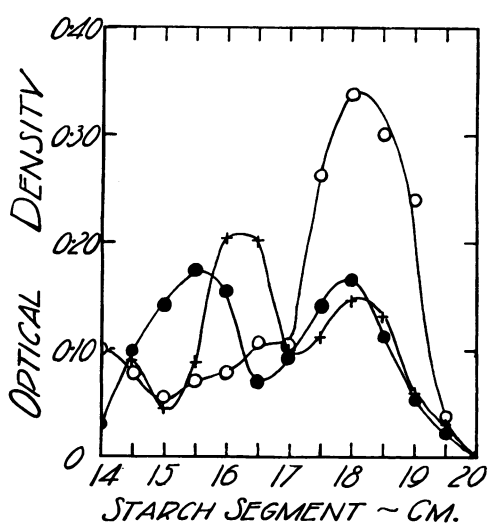

FIG. 5. STARCH ELECTROPHORESIS OF SERA FROM TWO PERSONS WITH DOUBLE ALBUMIN (F = ●—●—●, V = +—+—+) AND OF SERUM FROM A NORMAL RELATIVE (C = ○—○—○)

Only a part of each electrophoretic pattern is shown.

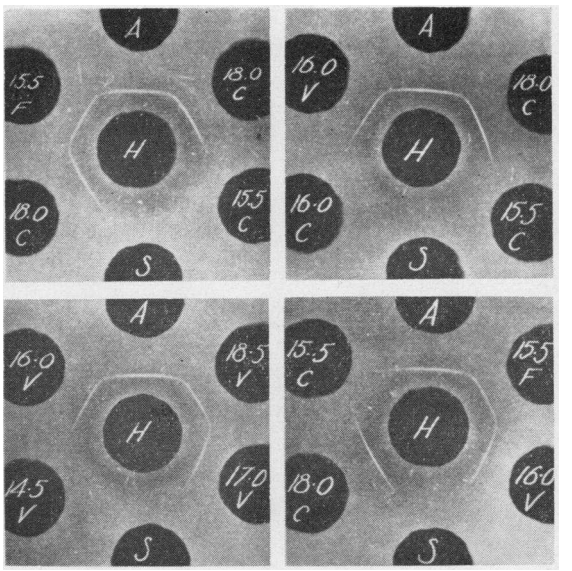

FIG. 6. AGER GEL ANTIGEN-ANTIBODY DIFFUSION REACTIONS USING HORSE ANTI-ALBUMIN (H), WITH NORMAL ALBUMIN (A) AND 0.15 M NaCl (S) AS CONTROLS

Numbers in wells refer to the number (cm.) of segment in Figure 5 from which eluates were obtained. The letters below the numbers refer to the subjects in Figure 5 (C = normal relative, V and F = individuals with double albumin).

RESULTS

The extent of separation of albumin A and B, obtained by starch electrophoresis, is shown in Figures 3 and 5. The precipitation curves obtained by reacting rabbit anti-albumin antiserum with various dilutions of these albumins in the zones of antibody excess and equivalence were indistinguishable from each other and from that obtained by reacting the antiserum with normal human serum albumin. The immunochemical estimations of albumin in the eluates from the starch segments paralleled the optical densities for the albumin peaks as shown in Figure 3. When bromphenol blue was omitted from the sera, the concentration of albumin in eluates from the central portion of the albumin peaks as estimated from optical density was within ±5 per cent of the concentration determined with rabbit antiserum.

The results obtained when eluted proteins from starch electrophoresis were reacted with horse anti-albumin antiserum in agar gels are indicated

in Figures 4 and 6. It may be noted that: *1*) No albumin was detected in segments from normal human sera analogous to those in which albumin B was found in anomalous sera (Figure 6, top left, Wells 15.5-C and 15.5-F; top right, Wells 16.0-C and 16.0-V). *2*) The precipitation bands of albumin A and B merged into each other without crossing, representing an "identity" reaction (Figure 4, Wells 17 and 19). *3*) Albumin B gave an "identity" reaction with serum albumin from a normal sibling (Figure 6, top left, 15.5-F and 18.0-C). *4*) Albumin B from two different individuals gave "identity" reactions (Figure 6, bottom right, Wells 15.5-F and 16.0-V). *5*) All albumins gave "identity" reactions with pooled normal human serum albumin. Similar results were obtained with rabbit anti-albumin antiserum.

Agar gel electrophoresis of normal human serum followed by diffusion of horse anti-albumin antiserum into the gel resulted in a curved precipitation band with a single vertex (Figure 7, A and D). Sera containing albumins A and B gave a single continuous curvilinear precipitation band with *two* vertices (Figure 7, B, C and E).

Free electrophoresis

Free electrophoresis in the Tiselius apparatus of serum and plasma from anomalous subjects (Figure 8) confirmed the paper electrophoretic data. The double peak in the albumin region is apparent at pH 8.6. Albumin A mobility is similar to that of normal human serum albumin.

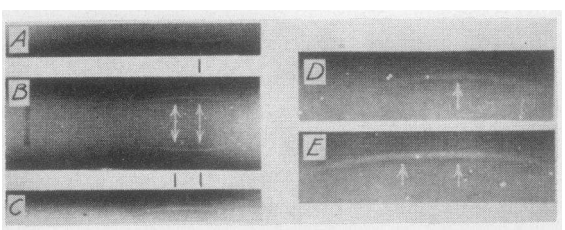

FIG. 7. AGAR GEL ELECTROPHORESIS OF SERUM FROM A NORMAL PERSON (SECTION A) AND OF SERA FROM TWO PERSONS WITH DOUBLE ALBUMINS (SECTIONS B AND C)

Only the lower half of agar Section A and the upper-half of agar Section C are shown. Horse antiserum had been placed in wells between Sections A and B, and B and C after electrophoresis. The precipitation band seen in C is enlarged in E and that from the upper half of A is enlarged in D. The lines between the agar sections and the white arrows indicate vertices of the precipitation bands. Anode is to the right of the figures.

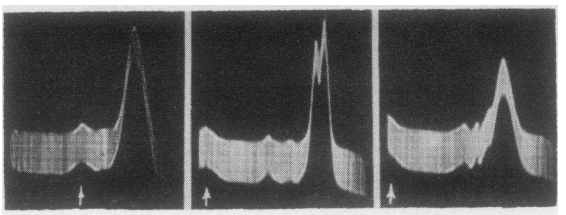

FIG. 8. MOVING BOUNDARY ELECTROPHORESIS AT VARIOUS pH'S OF FRACTION IV + V OBTAINED FROM SERUM OF THE PROPOSITUS

Left: pH 3.5, acetate buffer; middle: pH 8.6, diethylbarbiturate-citrate buffer; right: pH 12.2 glycine-NaOH buffer. Descending patterns; anode to left at pH 3.5 and to right at pH 8.6 and 12.3. Arrows indicate buffer anomaly.

Comparison of migration of I^{131} labeled human serum albumin with albumins A and B

I^{131} labeled human serum albumin mixed with sera of anomalous subjects migrated upon paper electrophoresis with fraction A rather than fraction B.

Ultracentrifugation

The two albumin fractions could not be separated by ultracentrifugation of plasmas from affected individuals. Likewise, Cohn Fraction IV + V from two abnormal sera did not reveal any variations from normal when studied by ultracentrifugation in a Spinco ultracentrifuge, Model E, at approximately 250,000 times gravity in 0.15 M saline. Albumin B, therefore, is not a polymer of albumin A nor does it represent a fraction of it.

Solubility

When an anomalous serum was fractionated according to Cohn's method 10 (14) three fractions resulted: Fractions I + II + III, IV + V and VI. Fraction IV + V contained the major part of the albumin. However, Fractions I + II + III and VI, respectively, contained small amounts of albumin too, which split into A and B in approximately equal amounts as judged by paper electrophoresis at pH 8.6 in barbiturate-citrate buffer. It can be inferred from these data that the solubilities of albumin A and B in this system must be almost identical. Thus, the substitution of amino acid residues containing carboxyl groups in albumin A by other residues to form albumin B (*vide infra*) does not affect solubility.

Starch column electrophoresis and subfractionation with cationic detergent [2]

Subfractionation by the cationic detergent method of Jacox (15) revealed no differences between albumins A and B separated by starch column electrophoresis.

3. Electrophoretic studies at several pH's. Methods: The relative electrophoretic mobilities of albumins A and B were studied at various pH's using paper and moving boundary electrophoresis. When paper electrophoresis was employed, I^{131} labeled normal human serum albumin, prepared as described elsewhere (16), was mixed with each serum before applying the serum to the paper. This facilitated the identification of albumin A since albumin A and radioiodinated normal serum albumin migrated together. The weight ratio of radioiodinated albumin to the total albumin of the serum was less than 1:100 and the amount of radioactivity in the volume of serum sample used for electrophoresis, 5 to 10 μl., was approximately 0.1 μc. After electrophoresis the paper strips were stained with bromphenol blue, dried and scanned in a photometer for color intensity. The strips were then cut into 2.5 or 5 mm. wide segments perpendicular to the axis of migration and the segments assayed for radioactivity in a well-type Na I-crystal scintillation counter which gave approximately 1×10^6 counts per μc. of I^{131}. Normal sera and crystallized human serum albumin also were electrophoresed under the same conditions as the controls.

For moving boundary electrophoresis, the Perkin-Elmer instrument, Model No. 38, was employed. The sera were first fractionated by the low temperature ethanol-water method of Lever and co-workers (14). Fraction IV + V, after lyophilization, was used. With this fractionation method, over 90 per cent of the total albumin of each serum, as determined electrophoretically and immunochemically, appeared in Fraction IV + V. In the case of anomalous sera, the ratio of the two albumins as determined electrophoretically was the same in Fraction IV + V as in the original whole serum. The total albumin in Fraction IV + V represented approximately 80 to 85 per cent of the proteins in the fraction.

Electrophoresis was carried out over a pH range from 3.0 to 12.3. All buffers were of ionic strength 0.1. The following were used: phosphate, pH 3.0; acetate, pH 3.5 to 5.8; cacodylate, pH 7.0; phosphate, pH 7.0 to 12.0; barbiturate-citrate, 8.6 to 10.1; and glycine-NaOH, pH 9.6 to 12.3.

RESULTS

Between pH 3.5 and 12.2, albumin A had the same electrophoretic mobility as normal serum albumin. Between pH 4.5 and 10.1 inclusive the net charge on albumin A was always negative relative to the net charge on albumin B. Within this pH range, when both albumins had net positive charges, albumin B migrated toward the cathode more rapidly than albumin A, and when both albumins had net negative charges, albumin A migrated toward the anode more rapidly than albumin B. Between pH 11.6 and 12.2 and at pH 3.5, albumins A and B had the same mobility as evidenced by a single albumin peak in the anomalous sera, or in Fraction IV + V obtained from the anomalous sera. In Figure 8 are shown representative descending patterns obtained with moving boundary electrophoresis of Fraction IV + V from a single anomalous serum. The small peak on the Σ-anomaly side of the albumin peak at pH 12.2 (Figure 8) was not a specific characteristic of albumins A or B alone, since crystallized normal serum albumin acquired an identical irregularity at this pH. The other small peaks in Figure 8, aside from the Σ-anomaly, were attributable to proteins other than albumin in Fraction IV + V (14).

The changes in the *relative* mobilities of albumins A and B observed were *not* attributable to the use of different buffers at different pH's. Thus, in phosphate or glycine buffer below pH 11, albumins A and B had different mobilities, but at pH 11.6 in the *same* buffer systems, both albumins migrated together. Similarly, in acetate buffer at pH 4.5 the two albumins had different mobilities, but at pH 3.5 in the same buffer the albumins had the same mobility.

After electrophoresis at those pH's where both albumins migrated together, it was possible when using the moving boundary method to recover the sample of Fraction IV + V from the electrophoresis cell, redialyze it and then re-electrophorese it at pH 8.6. This was done to demonstrate that any effect of the previous high or low pH on the mobilities of the albumins was reversible. In each case, the double albumin peaks reappeared.

DISCUSSION

Our observations on "double albumin" in 25 of 43 members of a family of Norwegian extraction confirm and extend those of Knedel (3, 4), who described this anomaly in two families, and Nennstiel and Becht (17), who described one family. Dr. Knedel (18) informs us that he has found

[2] We are greatly indebted to Dr. Ralph F. Jacox for performing these studies.

a similar serum protein anomaly in another family as have Bennhold, Ott and Scheurlen more recently (19). All the families reside in the same area in Germany but are not related so far as is known. Weiner (20) has discovered a similar double albumin in six members of a large family of Italian (Salerno) extraction living in New York City. In all, therefore, seven families with double albumin have been discovered to date.

The data from the family described in this report leave little doubt that the anomaly is transmitted genetically. Each anomalous individual is heterozygous for the characteristic, having received a normal gene from one parent and an anomalous gene from the other. All unaffected persons of the family probably have received a normal gene from each of their parents. Penetrance appears to be complete since double albumin has been found in individuals ranging from one and one-half to 68 years of age.

No known marriages between two persons having the anomaly have occurred, so there has not been an opportunity to observe the effect of the anomalous gene in the homozygous condition. However, our observations of heterozygous individuals suggest the use of the term "codominant" because the effect of each gene, the normal and the anomalous, is seen in the heterozygotes.[3] Further, in the anomalous subjects albumins A and B each make up approximately half the total serum albumin and their sum is equal to the normal serum albumin level. Knedel (18), however, reports in his families that ratios of A to B of 3 : 2, 2 : 3, 2 : 1, 1 : 2 and 1 : 1 have been found. Nennstiel and Becht (17) report more B and A. The differences of the relative proportions of albumins A and B in various families probably can be explained by the relative effectiveness of the mutant gene in different families. Various other anomalies are inherited in a similar manner, as has been demonstrated particularly well in the inheritance of abnormal hemoglobin types (21). The extensive observations in our family, and those of the other families with this anomaly, indicate that double serum albumin is not associated with disease.

In view of the immunochemical data, little doubt exists that the protein designated as B in this report is indeed an albumin. The reactivity of fraction B with rabbit and horse antisera was the same quantitatively and qualitatively as that of albumin A and of normal serum albumin. Consequently, both the nature and number of haptenic groups of these albumins appear to be identical. Knedel (3, 4) reports that his fraction A_2 (corresponding to albumin B) on immunochemical and immunoelectrophoretic study appears to be an albumin. Albumin B behaved exactly as did normal serum albumin electrophoretically, immunochemically and ultracentrifugally. While the evidence available to date is not conclusive, it strongly suggests that albumin A *is* normal serum albumin. In accord with this, preliminary studies indicate that albumin A and normal serum albumin are indistinguishable as regards chromatography on carboxymethyl cellulose columns and as regards the binding of certain dyes. Albumin B, however, is quite unlike normal serum albumin or albumin A under these conditions. From this evidence, albumin B appears to be an anomalous serum protein. Its detection in certain sera is *not* simply due to an increase in the concentration of a protein normally present in the serum in small amounts. By sensitive immunochemical methods, albumin could not be found in normal serum in the albumin B electrophoretic position.

The change in the electrophoretic mobility of a protein that occurs with a change in pH is due to an alteration in the net charge on the protein molecules and is the result of the titration of the various hydrogen-ion binding groups of the molecules of protein at different pH values (22). In the pH range from 10 to 11.3, where the net charge on albumin B becomes equal to that on A, the groups that are titrated, and hence change in charge, are (23) : *1*) the ε-amino groups of lysine residues, *2*) the free sulfhydryl groups, and *3*) the phenolic hydroxyl groups of tyrosine residues.[4] As titration proceeds towards increasingly alkaline pH, the lysine residues lose their positive charge and the sulfhydryl and tyrosine residues gain a negative charge. In the acid pH range the free carboxyl groups lose their negative charge but the ε-amino group of lysine remains positively charged. The

[3] We are greatly indebted to Dr. Herman M. Slatis of Argonne National Laboratory for his helpful advice on our genetic data.

[4] Although the guanidinium groups of arginine residues may begin to titrate at the alkaline end of this range, their titration continues well beyond this range.

TABLE III

*Charge contributed to serum albumin by certain titratable groups in various pH ranges**

Group	Charge at pH 3.0 to 3.5	Charge at pH 4.5 to 10	Charge at pH 11.3 to 12.3
ΣNH_2 (lysine)	+	+	0
Guanidinium (arginine)	+	+	+–0†
Imidazolium (histidine) and α-NH_2	+	+–0†	0
Phenolic hydroxyl (tryosine) Sulfhydryl (cysteine)	0	0	
Carboxyl (aspartic, glutamic acids and α)	0		

* Adapted from Reference 23.
† Charge changes within range given.

observed changes might suggest a substitution of tyrosine, cysteine or lysine residues in albumin B for an equal number of carboxyl residues in albumin A. Reference to Table III suggests that such substitutions would result in the same net charge on the two albumins above pH 11.3, where they would have the same electrophoretic mobility. Between pH 4.5 and 10, albumin A would be negatively charged relative to albumin B. These substitutions could explain the observed difference in electrophoretic mobility between albumins A and B. The question of whether or not additional structural differences exist between albumins A and B (such as additional substitution of uncharged amino acids in B for other uncharged amino acids in A) can only be answered by analysis of these fractions by methods other than those herein described. On the other hand, it can also be noted from Table III that simple substitution of free carboxyl residues in A by lysine residues in B would not give equal charges and mobilities at pH 3.5 and 11.6.

SUMMARY AND CONCLUSIONS

1. A serum protein anomaly, double albumin, has been found on electrophoresis of the serum proteins in 25 of 43 members of a family, and is presumed to have been present in three deceased members of the family.

2. The anomaly has been observed in the heterozygous state. It is transmitted as a codominant characteristic with complete penetrance.

3. Anomalous albumin B is assumed to be the result of a mutation of a gene responsible for the synthesis of normal serum albumin.

4. The anomaly is not associated with disease.

5. The anomalous fraction replaces one half of normal serum albumin, and by immunochemical evidence is found to be an albumin.

6. Data obtained by electrophoresis at several pH's suggest that anomalous albumin B contains tyrosine, cysteine or lysine residues substituted for an equal member of carboxyl residues in normal albumin A.

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