A New Type of Inherited Serum Albumin Anomaly *

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After Scheurlen's (1) finding of a serum with two separate albumin bands on paper electrophoresis (pH 7.8) Knedel (2) reported the familial occurrence of this type of bisalbuminemia with one normal and one electrophoretically retarded albumin fraction in roughly equal concentrations. Mutation in an autosomal gene responsible for albumin synthesis is assumed to cause the abnormal albumin fraction, and bisalbuminemia is the manifestation in heterozygotes (3). The condition is rare and has been observed so far only in Caucasians (4). Wieme (5) described a second and Tárnoky and Lestas (6) a third familial type of bisalbuminemia characterized by one normal albumin fraction and one that migrates faster. The resolving power of paper electrophoresis was not high enough to give two distinct fractions, but agar gel electrophoresis revealed two bands in Wieme's family, and in the other the abnormality was revealed by electrophoresis on cellulose acetate strips. Paralbuminemia has been proposed as a blanket name (3) instead of bisalbuminemia, since different mutations may cause a number of more or less easily recognizable changes of the albumin fraction. No disease seems to be linked with the types of paralbuminemia described.

This paper reports a new type of familial electrophoretic albumin heterogeneity, which may have connection with diseases of the supporting tissue.

Methods

Electrophoretic screening was carried out on 1,550 sera from patients admitted to the Department of Orthopedic Surgery. Sera from five of the patients contained an electrophoretically anomalous albumin fraction. Sera

were collected from 23 relatives of one of them. Two thousand sera from healthy subjects (793 factory workers and 1,207 registered blood donors) and 1,200 sera from randomly selected hospitalized subjects served as controls.[1]

Agarose gel screen electrophoresis

The agarose gels were 1.0 mm thick and were supported on cooled glass plate during the runs at 20 v per cm. A barbital buffer (0.075 mole per L, pH 8.4) containing 2 mM calcium lactate was used. The migration rate of the albumin front was roughly 7 cm per hour. This fast agarose gel electrophoresis was introduced as a routine screening method to detect serum protein abnormalities.

Serum protein was estimated with a biuret method, albumin with methyl orange (7). The albumin, orosomucoid, α_1-antitrypsin, and ceruloplasmin contents of serum were estimated with an immunochemical method based on electrophoresis of the antigens into agarose gels containing the corresponding rabbit antibody (8). Highly purified neuraminidase from pneumococci was utilized to release neuraminic acid.

The degree of electrophoretic heterogeneity of albumin was studied by antigen-antibody crossed agarose gel electrophoresis (9) with rabbit antihuman albumin. After vertical starch gel electrophoresis with borate buffer (10) the gels were, in some experiments, sliced, and a 3 mm broad 2 mm thick gel strip was transferred to a trough in an agarose gel-containing antialbumin. The two types of gel were firmly united by sealing the slits with agarose. Electrophoresis was continued, but we then turned the electric field 90° to the earlier direction of migration of the proteins to obtain precipitates in the electrophoretic zones containing albumin.

Two sera with anomalous albumin were fractionated on DEAE-cellulose with a phosphate gradient of decreasing pH and increasing ionic strength (11) and by filtration through Sephadex G-200 at pH 7 with a phosphate buffer (0.05 mole per L) and NaCl (0.5 mole per L) (12).

Ultracentrifugation was done at 59,780 rpm (Spinco model E). The same partial specific volume of the solute (0.733) was used for both determinations, which were run at the same temperature.

Clinical material

Patient 1. M.N. is a 61-year-old man with known systemic lupus erythematosus (LE cells, positive antinu-
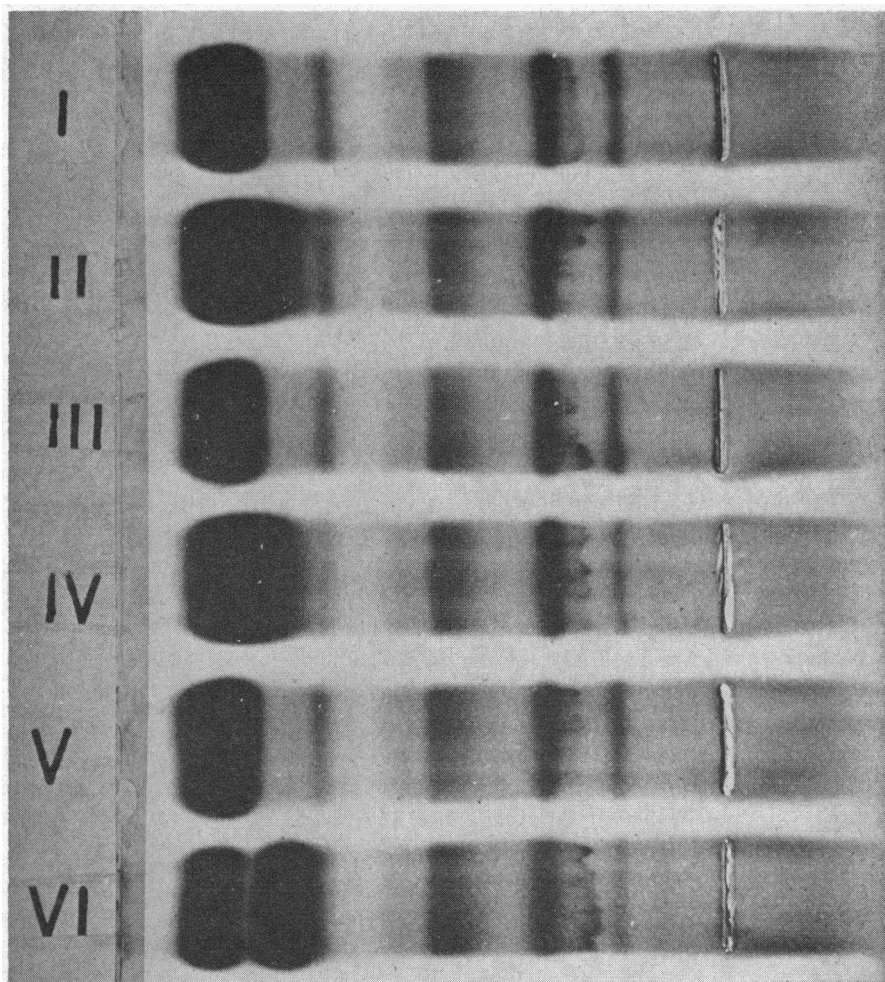
FIG. 1. PATTERNS AFTER ELECTROPHORETIC SEPARATION OF SERA IN AGAROSE GEL. Sera with normal albumin (I, III, and V), with anomalous breadth of albumin (II and IV), and with bisalbuminemia (VI).

clear factor, and hypergammaglobulinemia). He had arthralgias, especially of the back and left hand. He was admitted to the orthopedic department because of a tumor of the left upper arm; the tumor was excised, and the histological diagnosis was rheumatoid bursitis.

Patient 2. B.A. is a 29-year-old fireman. He was a member of a local soccer team. For many years his right knee had been painful, and he was admitted to the orthopedic department during a severe attack of such pain; he was unable to extend his right knee. A ruptured internal semilunar cartilage in the right knee joint was removed. Three months after the operation he returned to work and even continued to play football (goalkeeper). Now the contralateral knee is painful.

Patient 3. K.-A.A. is a 30-year-old male engineer. At the age of 25 he sustained a slight blow against the right knee, with fracture of the patella. Half of the patella was removed. He afterwards had slight intermittent pain in both knees. Once, after turning, he could

no longer extend his left leg; he was admitted to the hospital, where a ruptured internal semilunar cartilage was removed. Three months after the operation he was symptom-free.

Patient 4. S.A. is a 35-year-old clerk. For many years he has had recurrent dislocation of his left shoulder joint. The joint was surgically corrected.

Patient 5. H.O. is a 51-year-old factory worker. When young, he was an athlete. At the age of 42 he had a bicycle accident and fractured the right trochanter major. He returned to work after 3 weeks. Eight years later, he visited a doctor because of protracted pain over the cervical and thoracic spine. X ray revealed nothing remarkable. A year later he was admitted to the orthopedic department after a blow against his left knee with consequent rupture of the lateral meniscus and a comminuted fracture of the lateral condyle of the tibia. At operation the lateral meniscus and a large amount of Hoffa's fat tissue were excised.
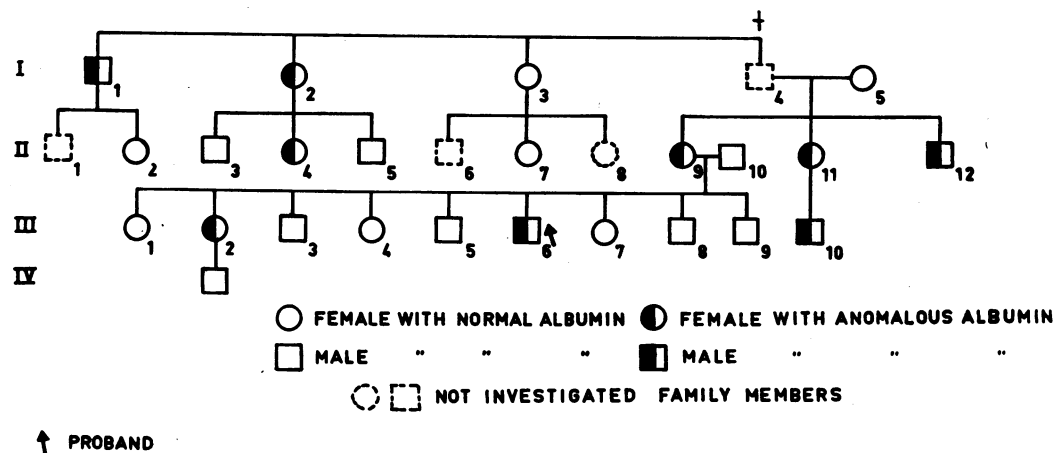
FEMALE WITH NORMAL ALBUMIN ● FEMALE WITH ANOMALOUS ALBUMIN

☐ MALE " " " ■ MALE " " "

NOT INVESTIGATED FAMILY MEMBERS

↑ PROBAND

FIG. 2. THE PEDIGREE OF PATIENT 2.

A blood donor with anomalous albumin

S.-E.S. is a 45-year-old factory worker with 20 years' history of pain in the lower back and legs. He had avoided heavy work and tried to cure himself by sleeping on a hard bed.

Results

Recognition of sera with electrophoretically anomalous serum albumin. Figure 1 shows the appearance of sera separated on agarose gel and stained with bromophenol blue. There is no difference between the serum albumin frontiers. Sera I, III, and V derive from normal subjects. Sera II and IV show a pattern with an albumin zone extending further towards the α_1 band than normally, whereas the pattern of serum VI shows one normally located albumin band and one to-

TABLE I

The amount of albumin, orosomucoid, α_1-antitrypsin, and ceruloplasmin in the family investigated

| No. in pedigree* | Initials | Age | Sex | Albumin concentration | | | Oroso-mucoid | α_1-Anti-trypsin | Cerulo-plasmin |
				Paper electro-phoresis	Methyl orange	Immuno-chemi-cally			
		years			*g/100 ml*		*% mean normal value*		
I: 1	U.L.†	79	M	4.1	4.3	3.7	88	105	104
2	K.J.†	77	F	4.4	4.6	4.1	104	73	119
3	A.J.	68	F	4.3	4.5	4.1	96	67	131
5	H.L.	84	F	4.3	4.2	4.0	108	103	107
II: 2	U.M.L.	43	F	4.4	3.9		102	113	102
3	K.G.	45	M	5.0	5.0		115	102	94
4	K.G.†	43	F	4.9	5.0		87	116	101
5	G.J.	39	M	5.4	5.1	4.6	112	90	88
7	B.G.	41	F	4.6	4.5	4.2	115	93	119
9	S.A.†	56	F	4.9	4.7	4.1	126	60	132
10	J.A.	61	M	4.8	4.7	4.0	120	38	119
11	H.A.†	53	F	4.7	4.6	4.0	104	51	110
12	H.L.	49	M	4.6	4.9	4.2	80	57	107
III: 1	S.P.	37	F	5.0	4.9	4.9	77	53	110
2	B.S.†	36	F	4.7	4.5	4.3	77	48	101
3	A.A.	34	M	5.2	5.1	4.6	92	15	100
4	I.A.	33	F	4.5	4.2	4.8	72	110	94
5	K.E.A.	31	M	5.1	4.9	4.6	96	90	95
6	B.A.†	30	M	3.7	4.5	3.9	200	72	
				4.3			110	52	106
7	G.A.	29	F	5.2	4.7	4.5	96	42	104
8	L.A.	28	F	5.0	4.9	4.3	78	39	83
9	T.A.	26	M	4.9	4.9	4.5	67	93	81
10	R.A.†	29	M	5.1	4.8	4.4	80	108	83
IV: 1	K.S.	17	M	5.0		4.5	122	63	100

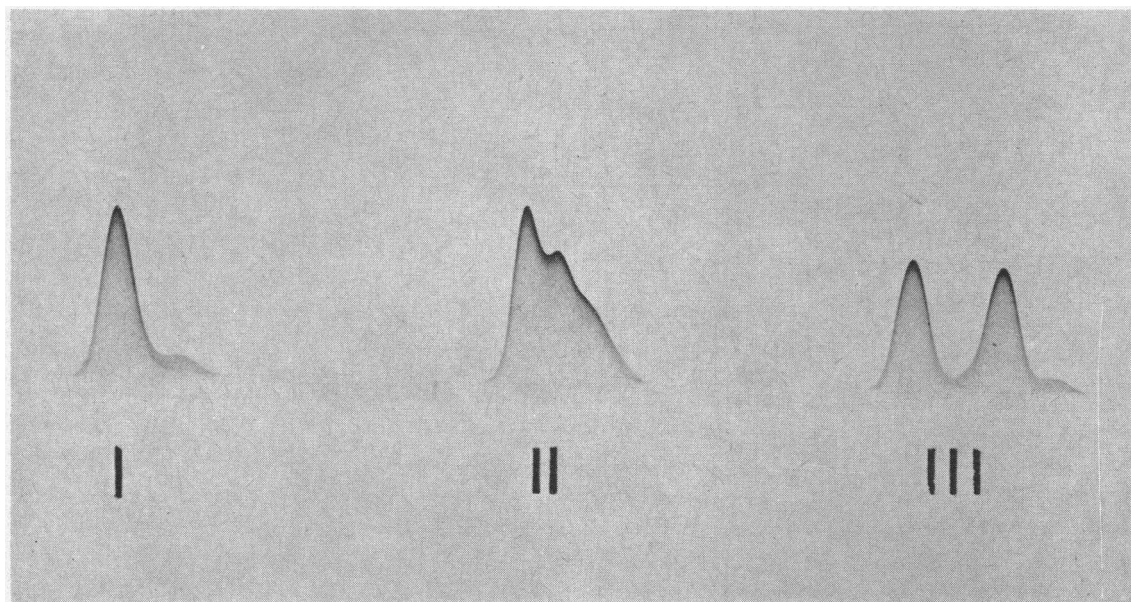* See Figure 2 for pedigree.
† Anomalous albumin.

FIG. 3. PRECIPITATION PATTERNS AFTER SERUM-ANTIALBUMIN CROSSED (TWO-DIMENSIONAL) ELECTROPHORESIS OF SERA WITH NORMAL ALBUMIN (I), WITH ABNORMAL BREADTH OF THE ALBUMIN (II), AND WITH BISALBUMINEMIA (III). During electrophoresis in the first dimension the anode was to the left, and in the second towards the top.

gether with the α_1-antitrypsin band (classical bis-albuminemia). On conventional paper electrophoresis of normal and anomalous albumin sera side by side, a slight difference in the breadths of the albumin zones may be observed. This contrasts with classical bisalbuminemia, where two distinct albumin bands appear. On gel electrophoresis the difference is less clear with buffers of lower ionic strength if electrophoresis is done with identical migration distance for the albumin front.

Sera from five unrelated patients chosen from 1,550 patients admitted to the Department of Orthopedic Surgery, Malmö General Hospital, regularly showed an anomalous broad albumin zone on fast agarose gel electrophoresis. Case histories are given in Methods. For comparison, screen electrophoresis was performed on sera from 793 healthy factory workers, 1,207 blood donors, and 1,200 sera from unselected hospital patients, Of the subjects from whom we took these 3,200 sera, one of the blood donors (S.-E.S.) showed an anomalous spreading of the albumin zone. His case history is given in Methods.

Investigations on sera of relatives of Proband 2. Sera from all available relatives of Proband 2 were studied (see Appendix). The pedigree is given in Figure 2, where the solid circles and squares de-

note subjects with anomalous albumin, which occurred in both sexes and in three consecutive generations. Quantitative paper electrophoresis of sera from the family members showed normal patterns and values except for a slight hypergammaglobulinemia in two subjects (I:1, II:2) and a slightly decreased α_1-globulin content in several. The albumin content was also estimated immunochemically and with methyl orange. The results obtained with the three methods showed a high correlation (Table I) whether the sera contained electrophoretically normal or anomalous albumin. The orosomucoid fraction was normal in all subjects except the hospitalized proband. It was normal after recovery. The immunological α_1-antitrypsin values were normal or decreased. The proband and his parents had half the normal concentration of α_1-antitrypsin, and one healthy brother (III: 3) had an α_1-antitrypsin concentration characteristic of α_1-antitrypsin deficiency.

The serum albumin in several normal subjects, in all members of the family presented, and in a patient with classical bisalbuminemia was studied by serum-antialbumin crossed electrophoresis. The three typical patterns obtained are given in Figure 3. The pattern of normal serum showed a main peak and a small percentage of albumin
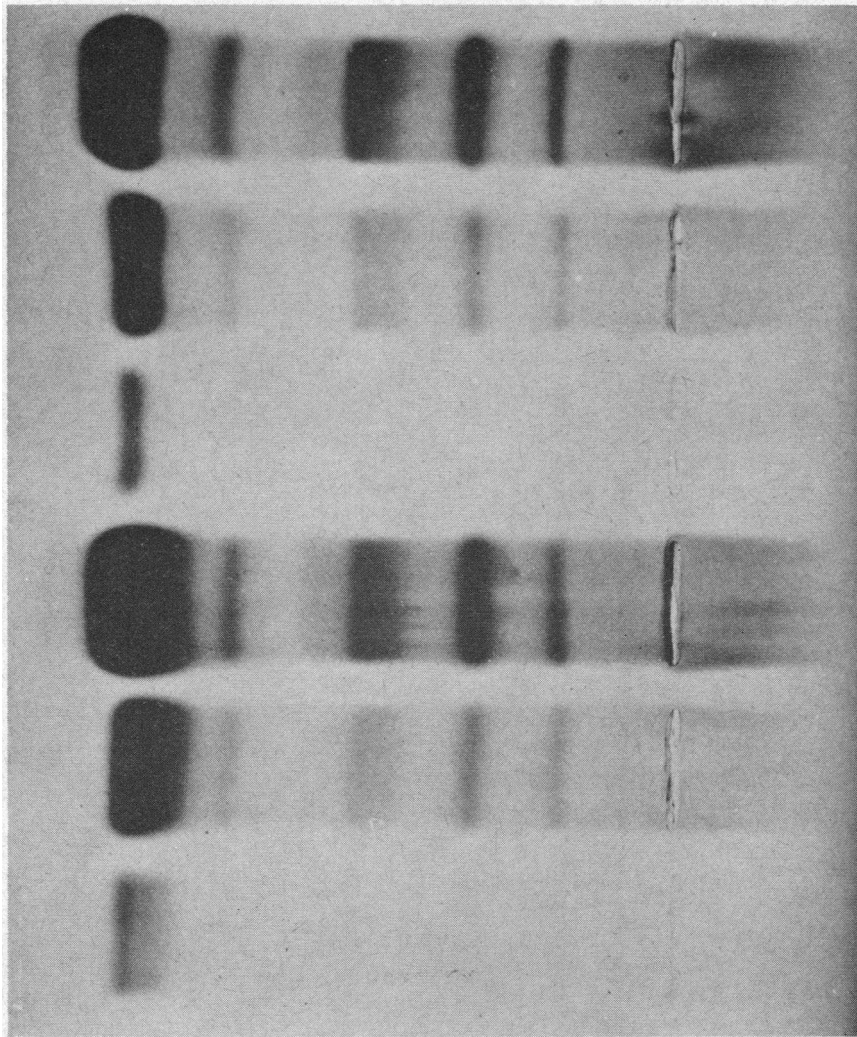
FIG. 4. AGAROSE GEL ELECTROPHORESIS OF SERUM WITH NORMAL (ABOVE) AND ANOMALOUS ALBUMIN
(BELOW) RUN AFTER APPLICATION OF DECREASING AMOUNTS.

molecules with slightly slower mobility. The sera with an electrophoretically broad albumin fraction contained two groups of albumin molecules differing slightly in rates of migration; the difference in charge was roughly half that seen in bisalbuminemia. When gel electrophoreses of sera from patients with an anomalous spread of albumin were run with a decreasing amount of serum, the molecules aggregated in two groups (Figure 4). Judging from the intensity of the color of the two groups of albumin molecules, we calculated the ratio between the normal and slow albumin species to be about 3 : 1.

Physicochemical studies on sera with anomalous albumin. To investigate whether the two types of

albumin molecules differed in shape or size, we filtered serum through a Sephadex G-200 column. The elution pattern of albumin coincided with that found for α_1-antitrypsin in sera with normal and anomalous albumin (Figure 5). Fractions from different elution zones were concentrated, and the proportion between the two albumin types in early and later appearing albumin fractions was estimated from the gel electrophoretic patterns. The albumin molecules with a low charge were slightly enriched in the albumin fractions that appeared first, and normal albumin mainly appeared in the latter part of the albumin elution zone (Figure 6).

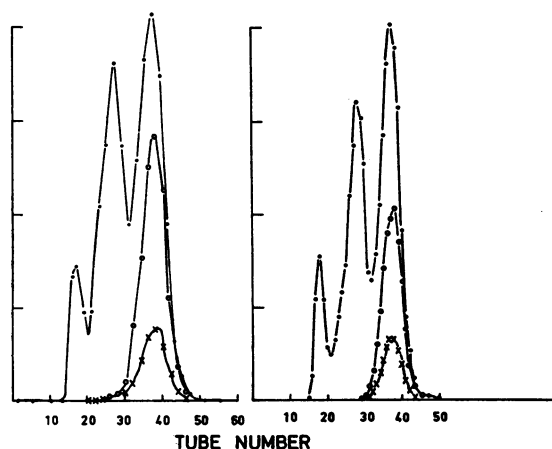On stepwise fractionation of serum with ammonium sulfate, the anomalous albumin was en-

FIG. 5. PATTERNS OBTAINED AFTER FILTRATION OF NOR-
MAL SERUM (RIGHT) AND OF SERUM FROM PATIENT 2 (LEFT)
THROUGH SEPHADEX G-200. Dotted curve denotes light
absorbancy (280 mμ), circle denotes albumin concentra-
tion, and crosses denote α_1-antitrypsin in arbitrary units.

riched in the early precipitating fractions. An al-
bumin fraction precipitated between 60 and 72%
saturation with ammonium sulfate was run on
DEAE-cellulose. The albumin molecules with a
lower charge were enriched in the first eluted frac-
tions on DEAE chromatography with a phosphate

buffer of increasing ionic strength and decreasing
pH. The albumin fractions eluted first consisted
of roughly equal parts of normal and slowly mi-
grating albumin. Pure albumin of normal charge
appeared later during the elution. These two
groups of fractions were concentrated and com-
pared in a Spinco ultracentrifuge. The sedimenta-
tion constants at infinite dilution in water at 20° C
were 4, 29 for the first eluted fraction and 4, 31 for
normal albumin. The patterns in the sedimenta-
tion velocity runs were identical, and the sedimen-
tation coefficient values increased with decreasing
concentrations.

On vertical starch gel electrophoresis no differ-
ence was seen in breadth of the albumin zone be-
tween sera with normal and anomalous albumin,
but an abnormal narrow band appeared between
the postalbumins and the "fast α_2-globulin zone"
(Figure 7). The ceruloplasmin content was nor-
mal. To determine whether the abnormal α_2 band
contained albumin, a slice of starch gel with sepa-
rated proteins was combined with an agarose gel
containing antialbumin. Electrophoresis of the
proteins into this agarose revealed that the ab-
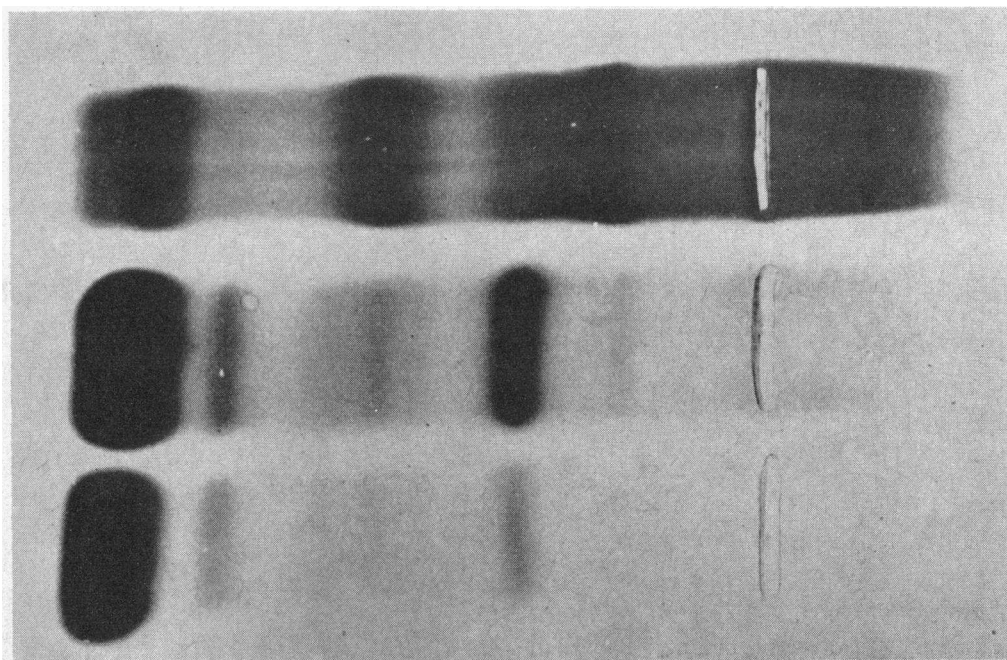normal band contained albumin (Figure 8), and

FIG. 6. AGAROSE GEL ELECTROPHORESIS OF CONCENTRATED FRACTIONS AFTER FILTRATION OF SERUM WITH
ANOMALOUS ALBUMIN THROUGH SEPHADEX G-200. Top pattern: tubes 30, 31; middle, tube 38; and bottom,
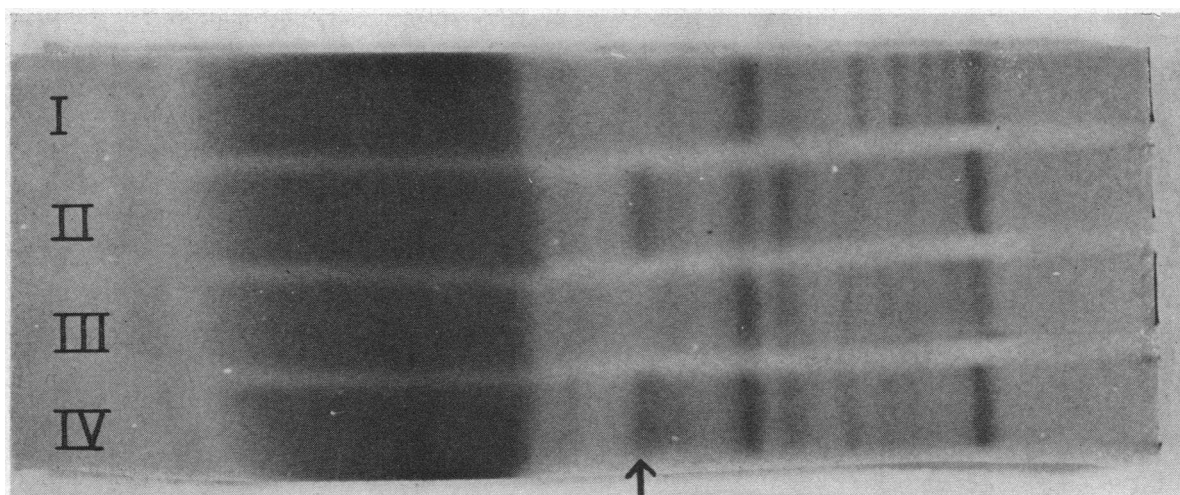tubes 46, 47.

FIG. 7. PATTERNS AFTER VERTICAL STARCH GEL ELECTROPHORESIS OF NORMAL SERA (I AND III) AND OF SERA WITH ANOMALOUS ALBUMIN (II AND IV). The arrow indicates the position of an abnormal band between the "postalbumins" and "fast α_2-globulins" in patterns II and IV.

that a minute albumin fraction normally occurs in the same electrophoretic zone. The sera were incubated for 8 hours with 0.1 M mercaptoethanol. The starch gel-antialbumin electrophoresis was repeated in the presence of 0.001 M mercaptoethanol in the starch gel. The albumin precipitate between the postalbumins and fast α_2-globulins disappeared from sera with normal and anomalous albumin.

Fractions enriched with anomalous albumin were treated with neuraminidase, and their agarose gel and starch gel electrophoretic patterns were compared with those of the untreated sample. No change was observed in the albumin zone when other components were electrophoretically retarded.

Normal serum was dialyzed against a large amount of urine from an individual with anomalous albumin. This caused no change of the mobility of the serum albumin.

The electrophoretic migration rate of a trace amount of albumin concentrated from the urine of a patient with anomalous serum albumin was normal.

Discussion

The zone spreading of albumin on electrophoresis of serum in stabilized media such as agar and starch gel cannot be explained entirely by the high albumin concentration and the buffer-ion interaction. If a gel strip is excised from the anode and cathode margins of the albumin zone after elec-

trophoresis and the proteins are re-run, albumin from the anode will show a higher mean mobility than that from the cathode (13). This indicates albumin heterogeneity and may be caused by structural differences or compounds firmly bound to albumin. There is recent evidence for chemical microheterogeneity of serum albumin with a number of albumin species differing in chemical properties (14–16), and it has been shown that only part of the albumin molecules (mercaptalbumin) in native serum contain reactive SH groups (17–19). The electrophoretic zone spreading of albumin in the series of patients presented here extends beyond the normal variation and may be due to the coexistence in the sera of two different albumin species, one with a normal and one with a lower rate of migration, but not so low as in classical bisalbuminemia. The electrophoretic difference among the three types of albumin is apparent from Figure 1.

Figure 2 gives a pedigree showing the occurrence of anomalous albumin (see Appendix). The results can best be explained by postulating that the affected individuals are heterozygous for an abnormal autosomal gene controlling albumin synthesis. The proband was heterozygous and one brother homozygous for α_1-antitrypsin deficiency. This can be ascribed entirely to chance, since the parents (II : 9, II : 10) of the proband were carriers of the rare gene for α_1-antitrypsin deficiency. Four other families under investigation show the
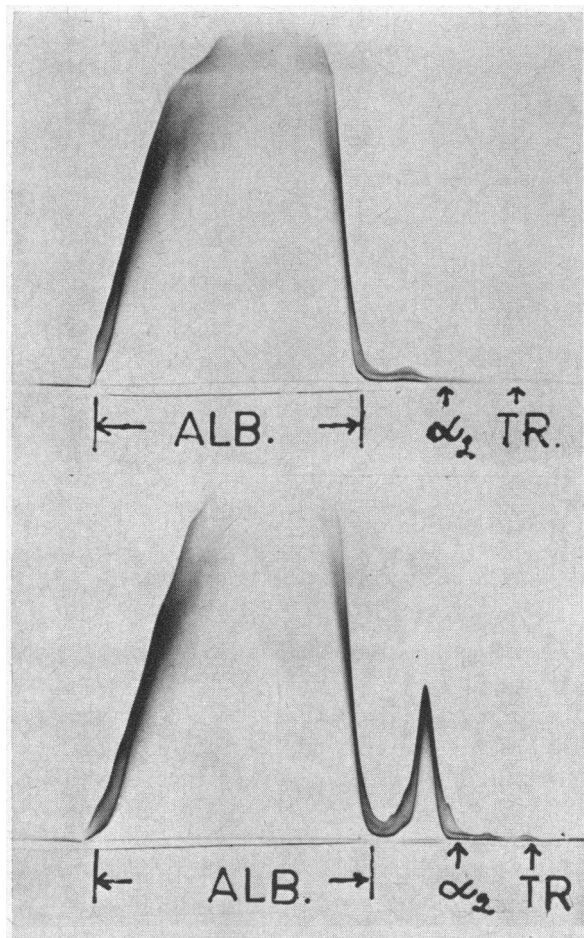
FIG. 8. THE PRECIPITATION PATTERNS OF NORMAL (ABOVE) AND ANOMALOUS ALBUMIN (BELOW) AFTER VERTICAL STARCH GEL ELECTROPHORESIS AND ELECTROPHORESIS IN AGAROSE GEL CONTAINING ALBUMIN ANTIBODIES. The second run was performed with the electric field turned 90°. TR indicates the position of transferrin, α_2 of "fast α_2-globulins," and ALB of the principal albumin zone.

albumin anomaly but not the gene for α_1-antitrypsin deficiency.

The protein partition found within the broad albumin zone is in conformity with the existence of two differently charged molecular species. The normal albumin type is of higher concentration, which is apparent from results of antigen-antibody (serum-antialbumin) crossed electrophoresis (Figure 3), salt fractionation, DEAE chromatography, and electrophoresis of diluted sera. The slightly earlier appearance of slow albumin in the gel filtration experiments suggests a somewhat higher molecular weight of anomalous albumin (Figure 6). However, ultracentrifugal study of a fraction

obtained after salt fractionation and DEAE chromatography containing roughly 50% anomalous albumin indicates that the albumin molecules with different charges were of identical molecular size. Albumin concentrated from the urine of a subject with anomalous serum albumin migrated at a normal rate. The leading antigenic determinants, the binding of bromophenol blue and methyl orange, seemed not to differ, since colorimetric and immunochemical albumin analysis gave conformable values and no spur formation was observed on immunoelectrophoresis or on double diffusion in agar gel.

On combined starch gel and immunoelectrophoresis, roughly 1% of the albumin molecules of normal sera appeared in the fast α_2 zone as a discrete fraction. This small fraction disappeared after reduction with mercaptoethanol before electrophoresis, which suggests that it represents a dimer of mercaptalbumin. On analysis of sera with anomalous albumin, the albumin in the fast α_2 zone on starch gel electrophoresis was always substantially larger than normal. It was, however, less than 10% of the total albumin and thus not entirely responsible for the increased electrophoretic width of the zone of anomalous albumin, which remained unchanged on agarose gel electrophoresis after reduction. The results indicate occurrence of a more easily oxidized mercaptalbumin in sera with anomalous breadth of the zone on agar gel electrophoresis. The early elution of some albumin on Sephadex chromatography may be explained by some dimer in the sample. The identical sedimentation constants and the symmetric peak for normal and anomalous albumin on ultracentrifugation are understandable, since the dimers were separated by DEAE chromatography before centrifugation.

In paralbuminemias the normal and the mutated genes cause synthesis of normal and anomalous albumin in roughly the same concentrations. In our patients albumin concentration was normal, and at least three-fourths of the albumin molecules had a normal charge. The electrophoretically anomalous part of the albumin may presumably be explained by the production of abnormal albumin or by an increased production of a positively charged substance firmly bound to albumin molecules with a normal degree of microheterogeneity. This link may sensitize the mercaptalbumin to

oxidation. The supposed substance must be firmly bound, since the electrophoretically retarded part of the albumin remained unchanged after salt fractionation, Sephadex filtration, and DEAE chromatography. The slight differences observed in precipitability and in chromatographic properties may be secondary to the change in charge.

No specific disease was associated with occurrence of anomalous albumin. Of the family of Patient 2, all eight relatives with anomalous albumin gave a history of intermittent back and joint pain, which was aggravated by heavy work (see Appendix). Of the 14 subjects with normal albumin, 2 had joint symptoms. No bone or joint deformities could be seen on examination of the family members, except for the proband's mother, whose left leg was shortened, probably because of a subluxation in the hip joint. Deafness and varicose ulcers of the leg were also seen in the family studied and in two other families under investigation. The five unrelated subjects with anomalous albumin were found on screen electrophoresis of 1,550 sera from the orthopedic department, but only one subject (blood donor) in 3,200 control subjects showed the same serum anomaly. This blood donor was in good condition, but he gave anamnestic data of back pain symptoms. Of the patients admitted to the orthopedic department, all five gave a long history of joint or bone symptoms.

Frazer, Harris, and Robson (20) have published data on a family with a "new genetically determined plasma protein" found during studies of families with deafness and goiter. On starch gel electrophoresis, the sign was a retardation of less than 10% of the paper electrophoretic albumin fraction. They observed what we believe is the dimer of anomalous albumin. The occurrence of deafness in their family is of interest, even though no link was found to the gene for the new plasma protein.

Although symptoms from supporting tissue in the unrelated patients and the family investigated are common in a general population, it is possible that anomalous albumin indicates a metabolic disturbance of the connective tissue.

Corroboration of this assumption requires extensive investigation of anomalous albumin per se and of special groups of patients.

Summary

The familial occurrence is described of an anomaly biochemically characterized by a slight decrease in charge and solubility of roughly one-fourth of the serum albumin molecules without deviation of the sedimentation constant from normal. The difference in charge is evident from electrophoretic and chromatographic properties. The anomaly is much more difficult to recognize by paper than by agarose gel electrophoresis. On starch gel electrophoresis sera with this anomaly show an abnormal band between the postalbumins and fast α_2-globulins, where a small albumin fraction is normally recognized. Both disappear after reduction with mercaptoethanol. Anomalous albumin seems to have a greater tendency to dimerize than normal albumin.

Five subjects with the anomaly were found among 1,550 patients admitted to a department for orthopedic surgery. One was found among 3,200 control cases screened with agarose gel electrophoresis. A family study indicates that the biochemical anomaly probably is the result of heterozygosity for an abnormal autosomal gene. High incidence of bone and joint complaints and bad hearing were common among the family members with anomalous albumin. The albumin anomaly may be secondary to an inborn metabolic error or caused by an abnormal albumin gene.

Appendix

Case histories of the relatives of Patient 2

Identification numbers refer to the pedigree (Figure 2). Twenty-one of the family members were interviewed, and 10 of them were examined clinically.

I:1. U.L., a 79-year-old man, had once wanted to be a blacksmith, but he had to give up his plans because the heavy work caused back pain and swelling of his wrists. He became a barber and later a bookshop assistant, and his arthralgia disappeared except for occasional spells. At the age of 48, he was operated on for umbilical hernia and at 72 for inguinal hernia. At our examination he had no back or joint pain, but he was nearly deaf.

I:2. K. J., a 77-year-old woman, had for many years painful swelling of the wrists and fingers as well as intermittent knee and back pain. At 74 she was admitted to her local hospital because of leg ulcers. She now has knee pain without exudation or capsular swelling. She cannot hear without a hearing aid.

I:3. A.J., a 68-year-old woman, had been tonsillectomized at 40 after recurrent peritonsillitis. At age 30 she had had venous thrombosis of the right leg, and since then

she intermittently has had ulcers of that leg. Since the age of 50 she has had pain in her fingers, which are deformed, especially in the proximal interphalangeal joints. Her hearing is impaired, her blood pressure is high, and she has angina pectoris.

I:4. A blacksmith died in 1938 at the age of 54. He had asthma during the last years of life and died of pneumonia. One of his daughters (II: 9) described him as a clever and alert man, who had only seldom complained of back or joint pain.

I:5. H.L., an 84-year-old woman, had pain in her left hip since age 50. During the last decades she has limped and used a stick.

II:2. U.M.L., a 43-year-old woman, has had no joint or back pain. Clinical examination revealed no skeletal abnormalities.

II:3. K.G., a 45-year-old factory worker, has had no joint or back symptoms and claims to be in good health.

II:4 K.G., a 43-year-old female clerk has had two children, born in 1947 and 1953. For many years she had had pain in her back and in most of her joints. At 38 she was admitted to the hospital because of back pain. X ray showed disk degeneration and reactive processes in the cervical and thoracic spine (spondylosis deformans).

II:5. G.J., a 39-year-old male clerk, felt well and had no bone or joint symptoms.

II:7. B.G., a 41-year-old woman, had an attack of lumbago at age 38. She also had symptoms of cholelithiasis and nephrolithiasis. She now feels well.

II:9. S.A., a 56-year-old woman, has had pain in her left hip since childhood. At about age 30 the pain increased, and at 40 she began to use a stick and to limp. At 40 she was operated on for cholecystitis. Her wrists and knee joints are swollen and painful. She has had leg ulcers.

II:10. J.A. is a 60-year-old symptom-free man.

II:11. H.A., a 53-year-old woman, has had for many years back and joint pain with no objective changes.

II:12. H.L., a 49-year-old male clerk, has had back pain since age 20. He had intended to be a blacksmith, but he could not because of severe back pain. He consulted an orthopedic surgeon, and after X-ray examination he was advised to discontinue heavy work. He now has pain in the back and legs.

III:1. S.P. is a 37-year-old healthy woman.

III:2. B.S., a 36-year-old woman, has had since age 10 episodes of severe back pain, especially in the lumbar region. X-ray examination at age 25 showed nothing unusual. She now has severe back pain when she has been working hard.

III:3. A.A. is a healthy 34-year-old policeman with no signs of respiratory insufficiency.

III:4. I.A. is a healthy 33-year-old woman.

III:5. K.E.A. is a healthy 30-year-old man. For many years he has played football regularly but now intends to stop because of pain in his left hip.

III:6. B.A. is the proband; see Patient 2 in Methods.

III:7. G.A. is a healthy 29-year-old woman.

III:8. L.A. is a healthy 28-year-old man.

III:9. T.A. is a healthy 26-year-old man.

III:10. R.A., a 29-year-old man, has had since the age of 1½ years diabetes mellitus treated with insulin. He has had no renal complications but has had slowly progressive retinopathy since 1957. He is now almost blind. Sometimes his wrist and finger joints are painful and swollen but he has no other bone or joint symptoms.

IV:1. K.S. is a healthy 17-year-old schoolboy with no bone or joint symptoms.

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