FAMILIAL PRIMARY SYSTEMIC AMYLOIDOSIS: AN EXPERIMENTAL, GENETIC AND CLINICAL STUDY*

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Familial primary systemic amyloidosis, a relatively unrecognized facet of the amyloid problem, represents an unusual disease process with protean manifestations. Genetic, clinical and pathological data with reference to this entity has been confirmed in four case reports in the world literature (1–4).

Maxwell and Kimball (1), on the basis of autopsy findings in three middle aged brothers, submitted the first report alluding to the familial aspect of systemic amyloidosis. The clinical data in these cases was not remarkable nor were the genetic implications discussed by these workers. Ostertag (2) in 1950 reported autopsy-proven amyloidosis on two brothers ages 35 and 39. There was suggestive clinical evidence that the mother of these patients and a daughter of them were afflicted with a similar process.

Andrade (3) in 1952 described a bizarre disease occurring endemically in the Oporto Region of Portugal. Locally it was known as "Mal dos peinhos" (foot disease). This insidious and relentless affliction was characterized by an essentially universal neurologic involvement. Paresis, particularly of the lower extremities, was a common finding. There was early impairment of thermal and pain sensitivities predominantly in the lower extremities. Gastroenteric disorders (flatulence, constipation, diarrhea) represented a distressing complication. Sexual disturbances in the male were the earliest and most constant complaints in the cases studied. The disease began in the second and third decades with an apparent predilection for males. In a number of cases gradual deterioration and death occurred in seven to ten years. Fifty-one of sixty-four cases reported were familial: ten families were genetically independent, two families were related, while thirteen cases were isolated and unrelated. While a large part of this material represents clinical and laboratory data, autopsies of two cases revealed definite histopathologic evidence of primary systemic amyloidosis.

Kantarjian and DeJong (4) added further genetic, clinical and pathological data to this entity. A father and two daughters were studied with positive necropsy findings in the father and one of the daughters. The syndrome described was not unlike that of Andrade (3) and consisted of neurologic signs and symptoms compatible with a severe progressive peripheral neuropathy, gastrointestinal symptomatology, and cardiovascular involvement. These workers further expanded the clinical spectrum in that they found endocrine abnormalities consisting of thyroid enlargement, low basal metabolic rate, amenorrhea, and impotence in the male. Remarkable ocular findings consisting of retinal exudates and exophthalmos were also described. On the basis of their genetic data they assumed the inheritance pattern to be a simple dominant.

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An unusual opportunity for the study of this inherited process presented itself when two cousins H. D. (case 16) and A. D. (case 11) were referred to our hospital in 1953 for the study of an unusual syndrome consisting essentially of neurologic and ophthalmologic symptomatology. Historical data secured from these patients suggested a reservoir of clinically similar cases in a genetically isolated community. Due to the remarkable degree of patient cooperation together with unstinted aid from physicians at the local level, sixty-six members of this pedigree agreed to submit to an intensive investigation of this inherited pathological process.

Case Report I. the propositus of this pedigree, H. D. (case 16), age 50 years, was admitted to the hospital April 16, 1953. His chief complaints were those of poor eyesight, generalized weakness and fatigue, mild recurrent chest pain on exertion, and numbness of the hands and legs.

The patient was uncertain as to the duration of his symptoms. Trauma to the left eye seemed to precipitate the onset of his visual difficulty even though recovery seemed to ensue after treatment. Two years prior to admission he noted the onset of "floating" spots in his left eye accentuated by prolonged eye strain. A similar process involved the right eye a year later. Apparently the onset of fatigue and weakness was insidious as he noted "tiredness" upon climbing stairs and while doing his farm work. Recurrent mild chest pain on effort coupled with slight breathlessness was of relatively recent origin. Continuous numbness and pain in the hands and feet represented the most troublesome complaint. According to the patient the hand discomfort was present for a "long time" while that of the lower extremities represented a new development of approximately two years duration. A concomitant incoordination further complicated the clinical picture. Nocturnal hand pain, unrelieved by any medication, resulted in the inability to sleep. The patient further volunteered the information that the "hand difficulty" had also troubled his father and his paternal uncle.

Physical examination: Temperature, pulse, and respiration were normal. Blood pressure was 162/110. No macroglossia was noted. The lungs were clear to auscultation and percussion. There was suggestive clinical evidence of cardiac enlargement. No murmurs or unusual heart sounds were heard. Examination of the abdomen gave essentially negative results. There were no remarkable skin changes.

The important findings on physical examination were those involving the ocular and neurologic systems. The external examination of the eye was normal as was ocular motility. The pupils were aniscoric, the left being larger than the right (6.5 mm.). Pupillary reflexes were prompt and equal. There was no abnormality of the anterior segments by slit lamp examination. Ophthalmoscopic examination revealed a massive degree of dense sheets of semi-opalescent hyaline-like opacities in the right eye. These opacities were attached in scattered fashion to the internal limiting membrane of the retina. Patchy bead-like deposits were noted on the surfaces of the retinal arterioles. The retinal veins were involved in a similar process. No other gross retinal pathology could be seen and the disc and macular area seemed to be uninvolved. The architecture of the left eye was obliterated by a similar and more severe process resulting in faint visualization of retinal details. A peripheral branch of the inferior temporal arteriole demonstrated a white bead-like deposit on its surface. Visual fields were done with only slight peripheral constriction being noted (5).

Neurological examination revealed a male with normal intelligence. There was decreased pin prick, light touch, and vibration sense bilaterally over the areas of median nerve distribution. There was peripheral blunting of superficial sensation over the upper and lower extremities. Deep sensibilities were essentially within normal limits excepting the second and third fingers on the right and the second finger of the left hand. There was bilateral thenar atrophy with paresis of the opponens and lateral lumbrical muscles (Fig. 1). The transverse carpal ligaments were thickened and appeared to be snugly attached to the

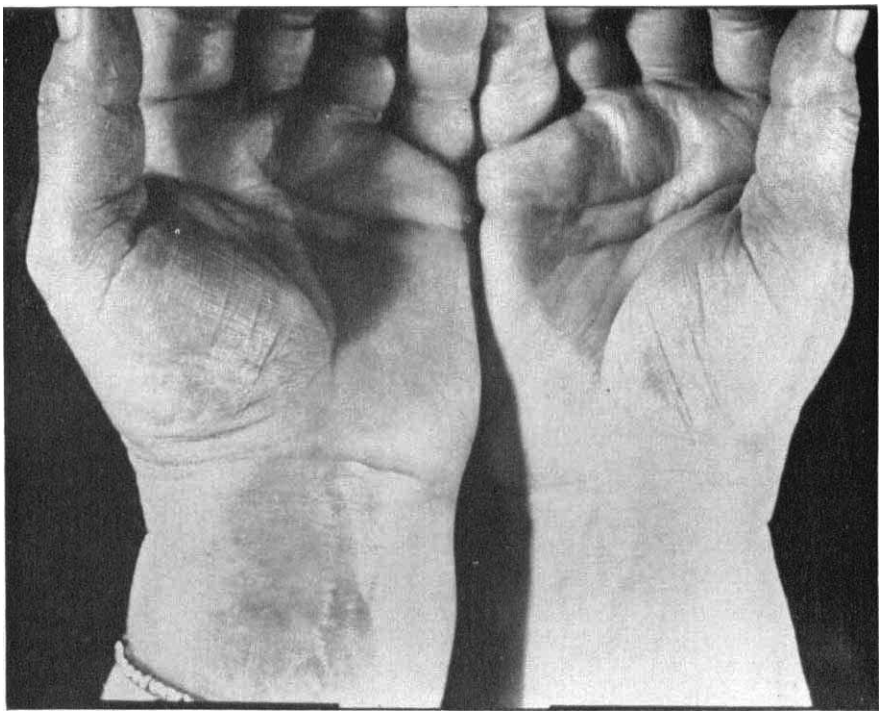

FIG. 1. Bilateral thenar atrophy in a 58 year old patient (case 1). The carpal tunnel syndrome was not present in this subject.

overlying structures (carpal tunnel syndrome). Deep tendon reflexes were hypoactive in the upper and completely absent in the lower extremities. Muscle strength seemed to be diminished universally.

Laboratory data: routine blood and urinalysis were normal. The urine tests for Bence-Jones protein were negative. The results of serologic tests were negative. A fasting blood sugar was normal. X-ray of the chest revealed cardiomegaly without parenchymal pulmonary disease. X-rays of the skull and colon were normal. Roentgen ray examination of the hands revealed evidence of hypertrophic arthritis. An electrocardiogram showed an incomplete left branch block, suggestive of myocardial changes. Spinal fluid examination was essentially negative. An intravenous congo red test was interpreted as being "positive" (with no record as to the amount absorbed). Microscopic examination of the gingival tissue revealed a thickening of the blood vessel walls which was suggestive of amyloid deposition. Free electrophoresis of the patient's serum revealed an atypical serum protein peak arbitrarily designated as α_2' (6).

The patient was discharged from the hospital April 18, 1953. His subsequent course was that of a slight but definite progression of his ocular, cardiologic and neurologic symptoms. He expired suddenly October 24, 1954 while resting at home.

An autopsy was done the same day. Significant gross findings were limited to the heart and lungs. The heart was markedly enlarged, weighing 840 grams. The epicardium was covered with small verrucous lesions in the region of the right ventricle measuring 5 mm. in diameter. The coronary vessels were patent and showed no appreciable evidence of atherosclerosis. Upon opening the chambers of the heart, the endocardial surfaces of the atria and the valves were somewhat roughened and pebbly having a tan-gray color. The chorda tendinae were grossly normal. The walls of the heart were markedly thickened; the left ventricle measuring 2.5 cm. in thickness and that of the right ventricle 1 cm. The cut

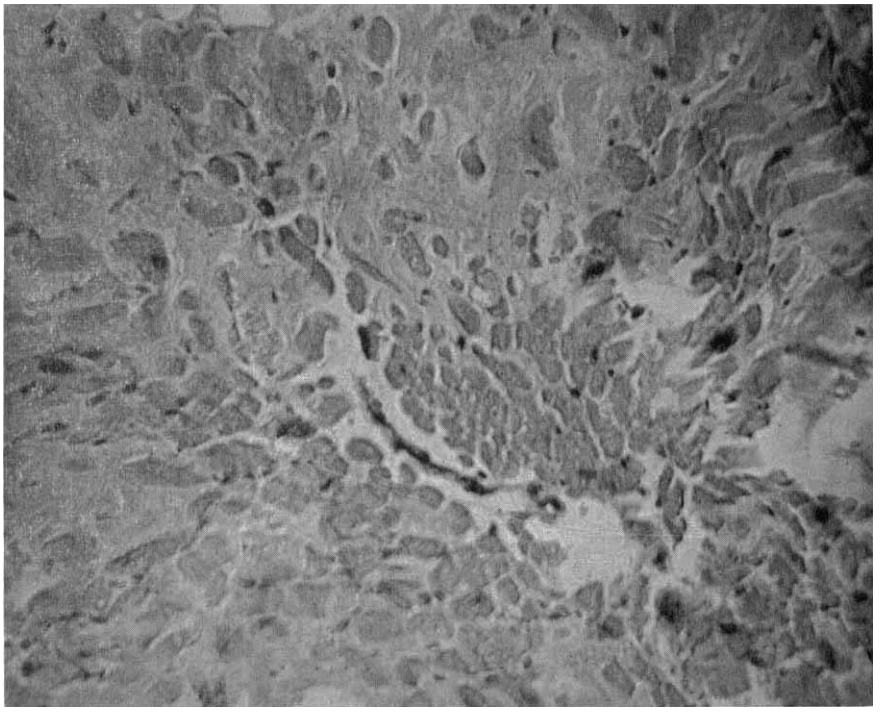

FIG. 2. Microscopic section of myocardium (case 16) (high power, H.E. stain) showing disruption of myofibril and large patchy areas of amyloid infiltration.

surface of this muscle was pale tan to pink in color. The intraventricular septum was similarly involved.

There were also found a bilateral pleural effusion with atelectasis of the lower lung segments. The liver, spleen, lymph nodes, pancreas, adrenal glands, the gastroenteric tract, the prostate, kidneys and skin were grossly normal. Unfortunately, no nerve tissue changes were described nor was any material secured for microscopic study. Eye examination was not permitted.

Histopathologic examination of the tissue (hematoxylin and eosin, congo red, and crystal violet stains) revealed extensive amyloid deposits in the myocardium and (Fig. 2, 3) in the vessels and substance of the tongue (Fig. 4). Marked vessel involvement of the larynx, liver, spleen, adrenal glands, pancreas, lungs, prostate, and kidneys was described. No amyloid was found in the bone marrow or skin. The final anatomic diagnosis was primary systemic amyloidosis with death being due to cardiac failure.

Case Report II. A. D. (case 11), age 52 years, a cousin of the propositus, (H. D., case 16) presented with symptoms consisting of numbness and pain in the hands and visual disturbances consisting of "floating specks" in the eyes. Symptoms referrable to the hands were first noted at 20 years of age and were markedly accentuated at rest and when exposed to cold. For an indeterminate period of time relief was attained by hard manual labor. However, currently the symptoms were accentuated both at labor and at rest. Treatment has been ineffective in alleviating the manual pain.

Symptoms referrable to the ocular system had their onset seven years prior to admission. There was a continuous progression of "floaters" and "specks" in the eye to the current disability of not being able to read a newspaper nor drive a car, nor to recognize familiar faces and figures.

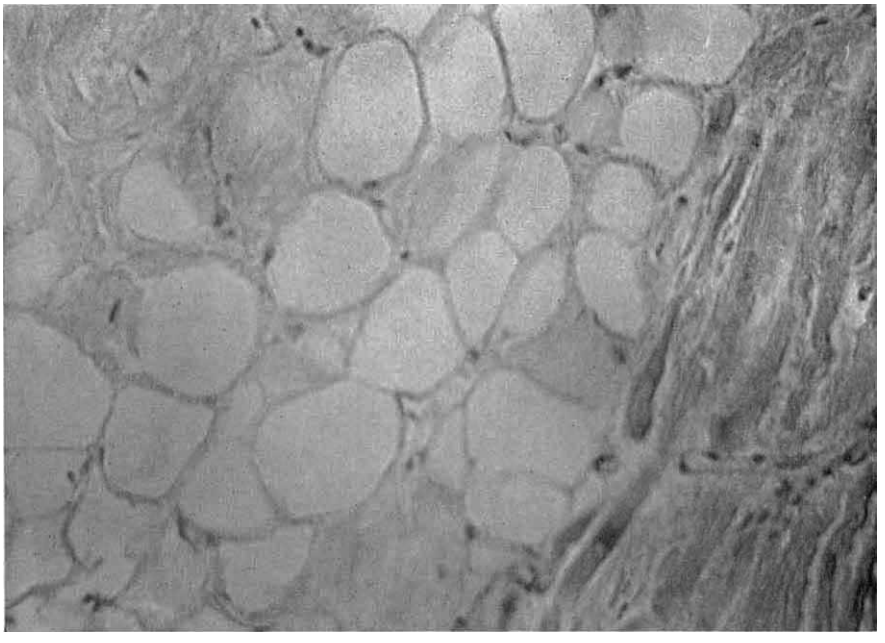

FIG. 3. Microscopic section of myocardium, case 16, (high power, H.E. stain) showing amyloid "rings" in the fat tissue of the heart.

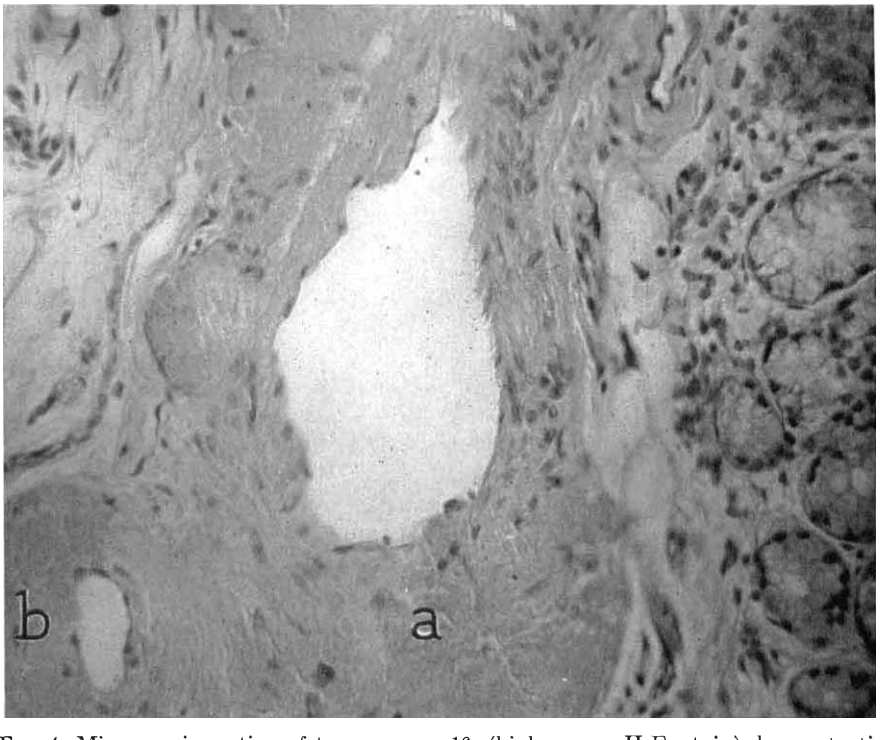

FIG. 4. Microscopic section of tongue, case 16, (high power, H.E. stain) demonstrating profuse amyloid involvement of a lingual artery (a). A similarly involved artery is located near the left lower section of the specimen (b).

Other less troublesome symptoms were those of epigastric distress after a fatty meal and a definite loss of potentia of long standing. The remainder of the history was essentially non-contributory.

Physical examination: temperature, pulse, and respiration were normal. The blood pressure was 130/90. Ocular examination revealed defective visual acuity with ability to count fingers limited to two feet. Pupil size and reflexes were normal. Slit lamp examination was essentially negative. Ophthalmoscopic examination revealed massive, obstructing semi-opalescent, sheath-like hyaline vitreous opacities in the right eye which obscured the retinal details except for glial proliferation from the retinal surface noted in the extreme periphery. The left retina was normal except for a localized white exudate on the surface of the inferior nasal arteriole. The visual fields, perimeter, and Bjerrum screen was normal to 3/300 and 1/1000 white test objects respectively on the left eye. A large central scotoma was found to 8.7/1000 white test object. Subsequent ophthalmoscopic examination two years later demonstrated an almost complete hyaline opacification of the vitreous which markedly limited the examination. However, bead-like deposits were noted on the superior nasal and temporal inferior nasal arterioles. In the extreme periphery of the retina the opacities assumed a coral-reef like appearance and seemed to be attached to the retinal surface. Examination of the nose and throat was negative. There was no evidence of macroglossia. The lymph nodes were not enlarged. Auscultation and percussion of the chest was negative. Examination of the abdomen revealed the presence of an enlarged, non-tender, non-nodular liver. The genitalia were those of a normal adult male. Rectal examination revealed no abnormalities. The skin of the hands was indurated, shiny and bound down resembling scleroderma in a remarkable fashion (Fig. 5). Neurologic examination revealed

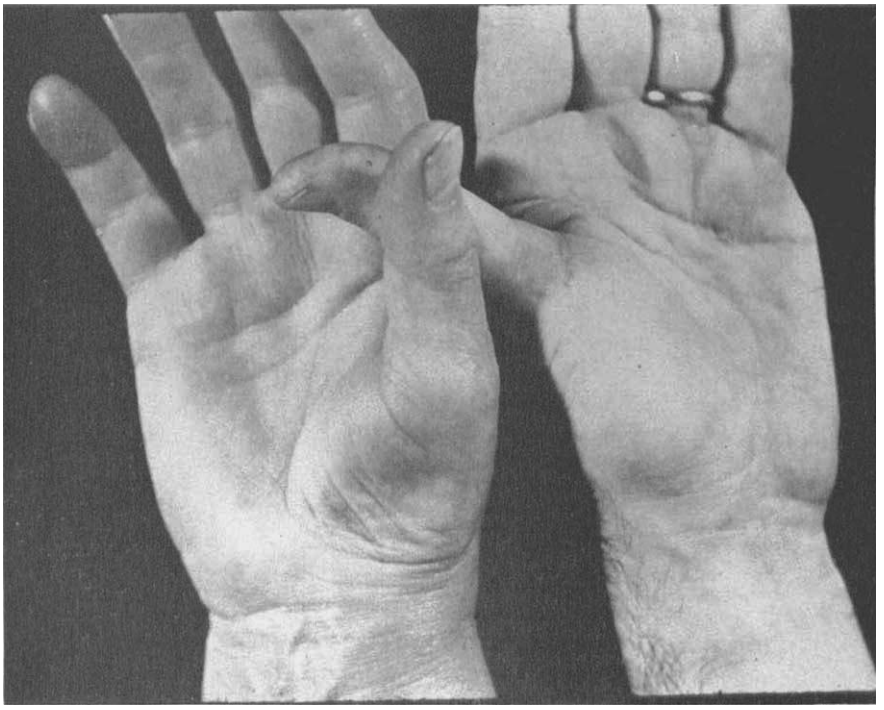

FIG. 5. Comparison of the parchment-like erythematous fingers and palm (case 1) of a 58 year old patient (left) with those of normal (right). Note also thenar atrophy.

a definite reduction in hearing in the left ear. A definite reduction in sensation to touch (cotton) and pain (pin prick) from the elbows to the finger tips was noted. This diminution of sensation increased distally. Similar findings were described below the knees in the lower extremities. Position sense was normal and there was no fasciculation or atrophy of the extremities. Hand strength was markedly reduced as was the ability to extend and flex the hands. Flexion, extension and abduction of the thumb was reduced. Abduction, extension, external and internal rotation of the shoulders was diminished. Flexion-extension weakness of the elbows and that of the wrists was prominent. Dorsal flexion of the feet was diminished otherwise the lower extremities were not remarkable. Peripheral pedal vessel pulsations were reduced bilaterally.

Laboratory data: routine blood and urinalysis were normal. The urine tests for Bence-Jones protein were negative. The results of serologic studies were normal. Blood urea nitrogen was 14.0 mgm. per cent. Bleeding time was 2 minutes 30 seconds and coagulation time 10 minutes 7 seconds. Cryoglobulin determinations were negative. Study of the liver function revealed a direct serum bilirubin of 0.12 mgm. and a total of 0.7 mgm., bromsulfalein retention 0 per cent, cephalin flocculation 1 plus at 48 hours, thymol turbidity 4.5 units, zinc turbidity 12.5 units, alkaline phosphatase 1.1 units, prothrombin time patient 15 seconds and control 14 seconds, and a serum cholesterol of 290 mgm. per cent. The electrocardiogram showed a moderate left axis deviation with occasional premature contractions. X-rays of the chest, skull and pelvis were normal. X-ray of the cervical spine revealed hypertrophic arthritic changes at C_5 and C_6. Pathological examinations of the gingiva and of the left dorsal index finger were negative with hematoxylin and eosin but positive for amyloid deposition with the congo red, crystal violet and periodic acid Schiff stains. Bone marrow examination was negative for evidence of multiple myeloma. Indirect laryngoscopy revealed no gross pathology of the vocal cords. Free electrophoresis of the patient's serum demonstrated an atypical serum protein peak ($\alpha_{2'}$).

PLAN OF INVESTIGATION

Extensive investigation of this pedigree, utilizing data secured by the family in this country and in Europe, reveals that the grandparents of H. D. (case 16) and A. D. (case 11) emigrated to this country from the Canton Berne, Switzerland in 1883. Unlike other emigres from Europe, the members of this family group came directly to a small community in northeastern Indiana without intermediate settlement thus maintaining relative genetic isolation.

The program of investigation of this pedigree encompassed four broad areas: 1. That of careful histories and physical examinations. 2. Comprehensive laboratory procedures consisting of routine blood, urine studies, and further analysis of the urine for Bence-Jones protein. Blood serology, screening procedures for hepatic, renal, and hemostatic function, cryoglobulin determination, electrocardiography, x-ray of the chest and bones, skin biopsies, and bone marrow examination completed the laboratory program of investigation. 3. Thorough study of the genetic aspects of the inheritance of this process utilizing familial, experimental, and legal data. 4. The application of experimental biochemical studies consisting of free electrophoresis of the serum and ultracentrifugal analysis of the serum lipoproteins (7, 8). The discovery of an atypical electrophoretic peak ($\alpha_{2'}$) in the two cousins suggested the possibility that perhaps this procedure (free electrophoresis) might represent a means of identification of those subjects stigmatized by the pathological process.

METHODS

Free electrophoresis. 2 ml. of fresh centrifuged serum were diluted with 6 ml. of the veronal buffer at pH 8.6, ionic strength of 0.1 M. The diluted serum was dialyzed for 18 hours against 500 ml. of buffer at 0° C. Electrophoresis was carried out on this dialyzed serum for one hour at 1.5° C. A Perkin-Elmer electrophoresis apparatus (Model 38) was used throughout this study.

The procedure for quantitation of the protein components involved the usual steps of the enlargement of the patterns, tracing by hand, and planimetry. The method is essentially that described by Tiselius and Kabat (10). The total protein content of the original serum was determined by the Biuret method (11, 12). Mobility calculations were carried out according to the method described by Longsworth (13).

Ultracentrifugal analysis. Blood specimens were drawn at times when the influence of dietary factors on lipoprotein levels was minimal. Lipoproteins were determined on fresh serum by ultracentrifugation at a density of 1.21 according to Lewis, Green and Page (14) modification of Gofman's technic (15). Cholesterol was determined by the method of Abell *et al.* (16).

RESULTS

Of the sixty-six subjects studied, twenty-nine showed an abnormal pattern in the α_2 globulin region. In fifteen cases, the presence of an atypical peak between α_2 and β globulin fractions, arbitrarily designated $\alpha_{2'}$ was demonstrated. In fourteen additional cases, the patterns were characterized by poor resolution in the α_2 area. Thirty-seven cases gave normal electrophoretic patterns. Representative electrophoretic patterns are presented in Figures 6, 7.

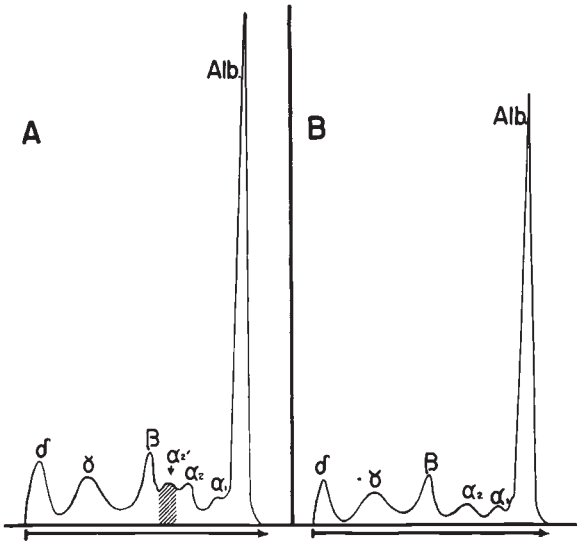

FIG. 6. Representative electrophoresis diagrams of serum in patients with primary systemic amyloidosis (A) and of normal serum (B). Alb., serum albumin; α_1, alpha-protein; α_2, alpha$_2$-globulin; $\alpha_{2'}$, abnormal component; β, beta-globulin; γ, gamma-globulin; δ, stationary anomalous boundary.

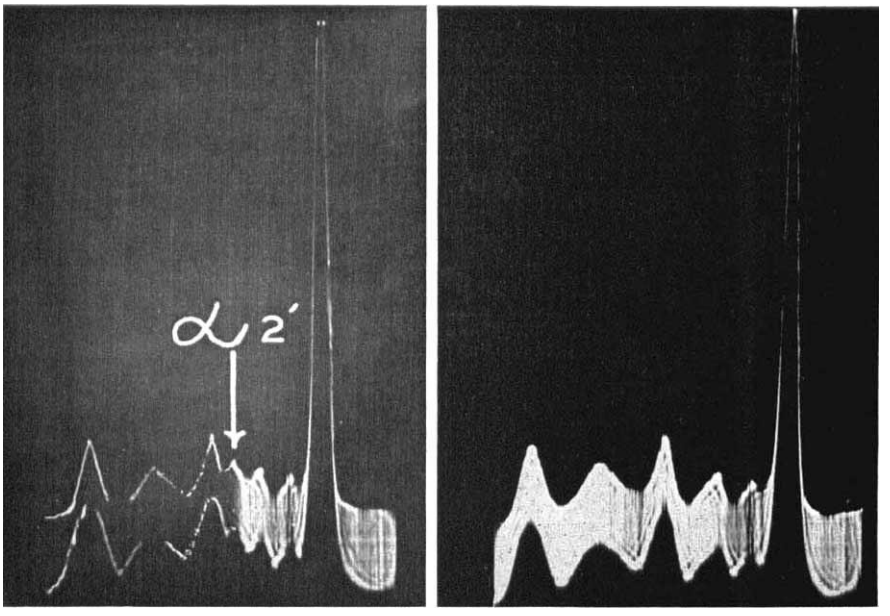

FIG. 7. Actual representative electrophoresis diagrams of serum in familial primary systemic amyloidosis (left) and of a normal serum (right). Note the atypical peak ($a_{2'}$) indicated in the left diagram.

No striking quantitative abnormalities could be demonstrated in these atypical electrophoretic patterns (Table I). The albumin content tends to be somewhat low and the total globulin content somewhat elevated. These differences, while suggestive are not significant. The percentages of α_1, α_2, and β globulins are normal in these subjects. Of the fifteen cases showing the atypical peak, eight had low values for α_2 globulin, seven had normal values. In fourteen cases with unresolved α_2 globulin areas normal values were present in twelve cases, two cases being slightly elevated. Here again the trend is suggestive but not significant.

Mobility calculations would seem to indicate that in the unresolved patterns the atypical peak is associated with the α_2 globulin fraction. In four cases the atypical peak appears to lie between the α_2 and β globulin, in three cases between the α_1 and α_2, and in eight cases the mobilities of both components fell within the normal α_2 range. On the basis of these data the position of the atypical peak remains in question. When the mobility of the peaks in question is related to the mobility of albumin, it would seem likely that the atypical peak does lie between α_2 and β globulins.

An attempt was made to determine whether a part of this atypical peak could be mucoprotein. Electrophoretic patterns were run on two atypical sera at pH 4.5 (17). No abnormality in mucoprotein could be demonstrated.

Lipoprotein determinations were carried out on thirty-three members of this pedigree. The lipoproteins of all members studied showed the normal comple-

TABLE I

Serum protein patterns in patients with familial primary systemic amyloidosis (6, 7)

No.	Case No.	Sex and Age	Total Protein	Albumin		α_1-Globulin		α_2-Globulin		$\alpha_{2'}$ Globulin		β-Globulin		γ-Globulin		Mobilities, u × 10^5 cm^2 sec^{-1} $volt^{-1}$					
				G./100 ml.	Per cent total protein	G./100 ml.	Per Cent total protein	G./100 ml.	Per cent total protein	G./100 ml.	Per cent total protein	G./100 ml.	Per cent total protein	G./100 ml.	Per cent total protein	Alb.	α_1	α_2	$\alpha_{2'}$	β	γ
Atypical patterns																					
1	1	F, 58	6.82	4.09	60.0	.29	4.2	.61	8.9	—	—	1.02	15.0	0.81	11.9	6.0	5.2	4.3	—	3.3	1.9
2	6	F, 33	6.75	4.17	61.8	.26	3.9	.41	6.1	.20	3.0	.66	9.8	1.02	15.1	5.8	4.9	4.0	3.4	2.8	1.3
3	9	F, 30	7.00	4.78	68.3	.24	3.4	.44	6.3	—	—	.74	10.6	.80	11.4	5.7	4.8	3.9	—	2.7	1.2
4	7	M, 5	6.81	4.16	61.1	.28	4.1	.37	5.4	.35	5.2	.96	14.1	.69	10.1	5.9	5.0	4.4	4.0	3.0	1.6
5	8	M, 3	6.83	4.24	62.1	.29	4.2	.43	6.3	.44	6.4	.89	13.1	.54	7.9	5.8	4.9	4.2	3.8	3.0	1.5
6	11	M, 3	7.17	3.97	55.3	.34	4.7	.34	4.7	.38	5.3	1.13	15.7	1.03	14.3	5.4	4.5	3.8	3.3	2.7	1.5
7	12	F, 22	7.62	4.34	57.0	.39	5.1	.50	6.6	.41	5.4	1.07	14.0	.91	11.9	5.4	4.6	3.9	3.4	2.7	1.5
8	13	M, 32	8.50	4.24	49.9	.36	4.2	.54	6.4	.65	7.7	1.21	14.2	1.50	17.6	5.9	5.1	4.2	3.7	3.0	1.5
9	14	M, 9	7.02	4.21	60.0	.32	4.5	.55	7.9	.34	4.8	.91	13.0	.69	9.8	5.7	4.8	4.1	3.7	2.8	1.5
10	15	M, 6	7.40	4.28	57.8	.31	4.2	.67	9.0	.30	4.0	.93	12.6	.92	12.4	5.4	4.7	4.1	3.7	3.0	1.7
11	16	M, 52	7.05	4.03	57.2	.30	4.3	.27	3.9	.54	7.7	.92	13.0	.98	13.9	5.7	4.9	3.9	3.6	2.9	1.6
12	17	M, 22	7.12	4.34	61.0	.28	4.0	.22	3.1	.39	5.5	.69	9.7	1.19	16.7	5.8	5.0	4.3	3.8	2.9	1.5
13	18	F, 17	7.39	4.27	57.8	.43	5.8	.20	2.7	.50	6.7	.95	12.9	1.04	14.1	5.7	4.8	4.1	3.7	2.7	1.3
14	19	F, 24	8.63	5.05	58.5	.41	4.8	.71	8.2	—	—	1.27	14.7	1.19	13.8	5.8	5.0	4.2	—	3.1	1.8
15	20	F, 47	7.17	3.79	52.8	.44	6.1	.51	7.1	.52	7.2	1.10	15.3	.82	11.4	5.9	5.0	4.3	3.7	3.3	1.9
16	21	M, 21	6.83	3.75	54.9	.31	4.6	.81	11.9	—	—	1.05	15.4	.90	13.2	5.9	5.1	4.3	—	3.2	1.9
17	23	F, 48	8.09	4.86	60.1	.23	2.9	.44	5.5	.44	5.5	.97	12.0	1.14	14.1	5.7	4.8	4.0	3.5	2.9	1.4
18	27	F, 18	6.50	3.85	59.3	.27	4.1	.73	11.2	—	—	.75	11.6	.90	13.9	5.9	5.0	4.0	—	3.0	1.5
19	26	M, 12	6.50	3.98	61.3	.26	4.0	.67	10.3	—	—	.95	14.6	.64	9.8	5.9	4.9	4.2	—	3.1	1.9
20	28	F, 73	7.12	3.76	53.5	.27	3.8	.37	5.2	.60	8.6	.91	13.0	1.12	15.9	5.7	4.8	4.1	3.6	2.9	1.5
21	29	M, 46	6.60	4.00	60.6	.24	3.6	.50	7.5	—	—	.86	13.1	1.01	15.3	6.0	5.1	4.1	—	3.1	1.6
22	32	M, 46	7.02	4.20	59.9	.32	4.5	.59	8.4	—	—	1.16	16.5	.76	10.8	5.7	4.9	4.0	—	3.0	1.5
23	33	F, 17	7.62	4.48	58.8	.42	5.5	.71	9.3	—	—	1.18	15.5	.84	11.0	5.6	4.7	3.9	—	2.9	1.6
24	34	M, 16	9.47	6.06	64.0	.61	6.4	.94	10.0	—	—	1.25	13.2	.61	6.4	5.5	4.6	3.7	—	2.7	1.2

25	35	F, 13	7.50	4.29	57.2	.37	4.9	.78	10.4	—	—	1.16	15.4	.91	12.1	5.7	4.8	4.0	—	2.8	1.4
26	36	F, 12	7.03	4.24	60.3	.32	4.5	.65	9.3	—	—	.96	13.7	.86	12.2	5.4	4.6	3.7	—	2.5	1.1
27	38	M, 69	7.62	4.70	61.7	.24	3.1	.20	2.7	.35	4.6	1.16	15.2	.97	12.7	5.4	4.7	3.9	3.6	2.8	1.5
28	39	M, 39	6.96	4.34	62.3	.27	3.9	.56	8.0	—	—	.84	12.1	.95	13.7	5.9	5.0	4.2	—	3.2	1.9
29	40	M, 73	7.07	3.84	54.3	.28	4.0	.67	9.6	—	—	1.05	14.8	1.22	17.3	5.5	4.7	3.9	—	2.7	1.2
									Normal patterns												
30		F, 33	6.84	4.31	63.0	.26	3.8	.47	6.9	—	—	.86	12.5	.94	13.8	5.7	4.7	3.9	—	2.8	1.5
31		M, 26	7.03	4.28	60.9	.30	4.2	.53	7.5	—	—	1.01	14.4	.91	13.0	5.3	4.4	4.0	—	2.9	1.3
32		F, 3	7.03	4.61	65.6	.32	4.5	.68	9.7	—	—	.84	12.0	.58	8.2	5.7	4.9	4.1	—	2.9	1.5
33		M, 42	6.84	4.13	60.4	.28	4.1	.70	8.7	—	—	1.03	15.1	.80	11.7	5.3	4.5	3.7	—	2.6	1.6
34		F, 53	6.88	4.03	58.6	.32	4.7	.67	9.8	—	—	1.21	17.6	.65	9.4	5.9	5.1	4.1	—	3.1	1.6
35		F, 34	7.61	4.65	61.1	.27	3.6	.75	9.8	—	—	1.27	16.7	.67	8.8	5.7	4.8	4.0	—	2.8	1.6

TABLE II

Serum lipoprotein values determined by ultracentrifugal analysis at density 1.21 in 33 members studied for familial primary systemic amyloidosis (8)

Case No.	Sex and Age	Atypical Serum Patterns	-S > 70	40–70	25–40	20–25	1–10	Serum Cholesterol	Remarks (Signs, Symptoms, Laboratory Data, etc.)
								mgm/100 ml.	
1	F, 58	Present	28	24	177	14	282	246	Neuropathy, skin biopsy
2	M, 32	Absent	<7	<7	141	19	199	215	Symptoms of peripheral neuropathy
3	M, 3	Absent	9	19	160	21	235	191	Negative
4	M, 20	Absent	<7	4	106	9	141	130	Negative
5	M, 7	Absent	6	24	152	7	141	129	Negative
6	F, 33	Present	7	7	152	14	177	179	Symptoms of neuropathy
7	M, 5	Present	*						Negative
8	M, 3	Present	†						Negative
9	F, 30	Present	7	9	129	14	152	147	Skin changes
10	M, 10	Absent	9	4	177	28	194	198	Negative
11	M, 52	Present	14	71	424	14	188	290	Neuropathy, skin biopsy, eye changes, hepatomegaly
12	F, 22	Present	14	14	139	24	199	210	Neuropathy, splenomegaly
13	M, 32	Present	118	122	354	28	258	326	Anemia, hepatomegaly
14	M, 9	Present	19	14	235	14	188	151	Negative
15	M, 6	Present	75	19	129	16	141	138	Negative
16	M, 52	Present	*						Neuropathy, eye changes cardiomegaly
17	M, 22	Present	14	19	141	7	164	126	Eye changes
18	F, 17	Present	—	7	106	16	247	162	Negative
19	F, 24	Present	14	16	152	7	118	168	Negative
20	F, 47	Present	24	24	97	21	—	—	Neuropathy, heart failure, hoarseness, gastro-intestinal
21	M, 21	Present	*						Negative
22	F, 64	Absent	14	28	398	<14	202	370	Neuropathy, hepatomegaly
23	F, 48	Present	24	55	290	12	133	266	Symptoms of peripheral neuropathy, tissue biopsy
24	F, 40	Absent	10	10	198	35	300	226	Neuropathy
25	F, 7	Absent	<7	9	157	12	188	159	Negative
26	M, 12	Present	7	9	223	14	129	168	Negative
27	F, 18	Present	108	24	164	14	118	158	Negative
28	F, 73	Present	*						Symptoms of peripheral neuropathy
29	M, 46	Present	12	35	280	19	235	226	Symptoms of peripheral neuropathy
30	F, 41	Absent	12	7	177	14	171	203	Negative
31	M, 32	Absent	7	16	188	9	106	178	Negative
32	M, 46	Present	9	52	280	12	100	233	Neuropathy, skin changes hepatomegaly
33	F, 17	Present	<7	7	152	19	141	181	Negative
34	M, 16	Present	4	7	118	7	118	165	Negative
35	F, 13	Present	26	14	152	7	101	184	Negative
36	F, 12	Present	*						Negative

TABLE II—*Continued*

Case No.	Sex and Age	Atypical Serum Patterns	-S > 70	40–70	25–40	20–25	1–10	Serum Cholesterol	Remarks (Signs, Symptoms, Laboratory data, Etc.)
								mgm/ 100 ml.	
37	M, 44	Absent	56	80	424	14	141	240	Hepatomegaly, gastro-intestinal
38	M, 69	Present	64	35	218	16	141	221	Neuropathy, cardiac insufficiency
39	M, 39	Present	*						Neuropathy
40	M, 73	Present	4	16	141	7	106	242	Neuropathy, cardiac insufficiency

* Lipoprotein determination not done.

† Material insufficient for analysis.

ments of components present in normal sera (-S > 70, 40–70, 25–40, 20–25, and 1–10) (Table II).

The average normal human serum lipoprotein values used as a standard were those of Lewis and Page (18). No normal determinations were available in the literature for the 1–17 year age range. Several cases reported in the present paper fell into this group. Those age groups for which normal lipoprotein values are available (18–34 and 35–60 years) indicate that these values increase with age. On the basis of this fact it might be speculated that normal values for the 1–17 age group would actually be somewhat lower than those for the 18–34 age group (19).

Lipoprotein concentrations were found to be within normal limits in six cases (4, 5, 19, 31, 34, 40). In three of these cases (4, 5, 31) all other data obtained were normal and the subjects were considered free of systemic amyloidosis. In the three remaining cases (19, 34, 40) with normal lipoprotein values, electrophoretic abnormalities were demonstrated.

Twenty-seven of the thirty-three subjects revealed abnormal lipoprotein values. Of this number fifteen were female, twelve were males. Ages ranged from 3–73 years; the females ranging from 3–73, the males from 6–73 years. The mean age for the females was 33 years, for the males 32 years. Fifteen of the twenty-seven cases were below 33 years of age.

Analysis of the data showed abnormalities in all components of the sera studied (Table II). The -S > 70 fraction had the least number of elevations (cases 13, 15, 27). The -S 40–70 component was elevated in four males (cases 11, 13, 32, 37). Only two females showed significant elevations in this fraction (cases 23, 27).

A rather consistent pattern of abnormality existed in the -S 25–40, 20–25, and 1–10 fractions. In the 25–40 component, abnormal values were found in eight males (cases 11, 13, 14, 26, 29, 32, 37, 38) and in seven females (cases 3, 22, 23, 25, 27, 33, 35). These elevations were very marked in two males, ages 6 and 12 years.

The -S 20–25 level was elevated in nine males (cases 2, 10, 11, 13, 14, 15, 26, 29, 38) and in ten females (cases 3, 6, 9, 12, 18, 20, 24, 27, 30, 33). Two of the

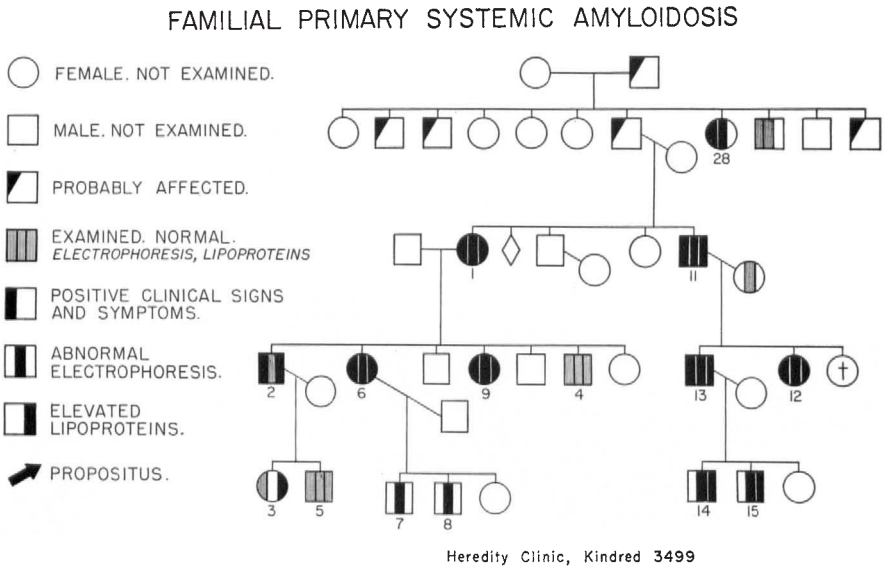

FIG. 8. Branch A, Kindred 3499

affected males were 6 and 12 years of age. Seven of the ten females were under 35 years of age, the youngest being a 3 year old child.

Appreciable increases in the measurable concentrations of the -S 1–10 fraction were noted in seven males (cases 2, 10, 11, 13, 14, 17, 29) and in five females (cases 1, 3, 12, 18, 24). The youngest male in this group was 9 years of age, the youngest female 3 years of age. The serum cholesterol levels were elevated in only five cases (1, 11, 13, 22, 23).

Correlation of Signs and Symptoms, Genetic Patterns and Experimental Data

In order to render the genetic profile more meaningful the pedigree* has been divided into cohesive branches labelled A (Fig. 8), B (Fig. 9), C (Fig. 10), D (Fig. 11). Branch A consisting of 14 cases (1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15) (Fig. 8), and branch D composed of 7 cases (10, 32, 33, 34, 35, 36, 37) (Fig. 11) respectively have been selected for discussion.

Correlation of clinical signs and symptoms of familial systemic amyloidosis with the electrophoretic findings were remarkable (Tables I and II). Of the fifteen subjects with the atypical electrophoretic peak, ten showed clinical signs and/or symptoms of the disease (cases 6, 11, 12, 13, 16, 17, 20, 23, 28, 40). Of the fourteen subjects whose electrophoretic patterns were unresolved in the α_2 globulin region, six had clinical signs or symptoms of the disease (cases 1, 9, 29, 32, 38, 39). There were thirteen subjects showing atypical electrophoretic patterns with no apparent manifestations of the disease. It should be noted that twelve of these thirteen cases were less than twenty-one years of age (cases 7, 8,

* The complete genetic profile of kindred 3499 is on file at the University of Michigan Heredity Clinic.

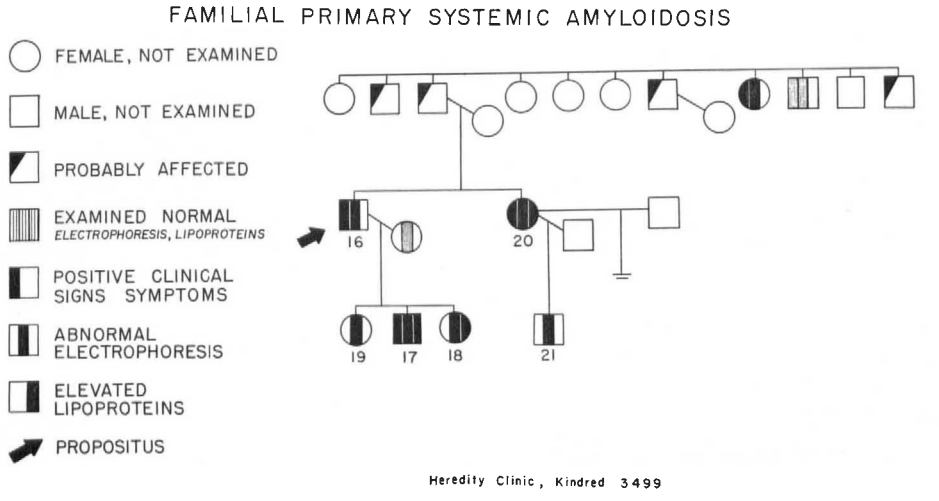

FIG. 9. Branch B, Kindred 3499

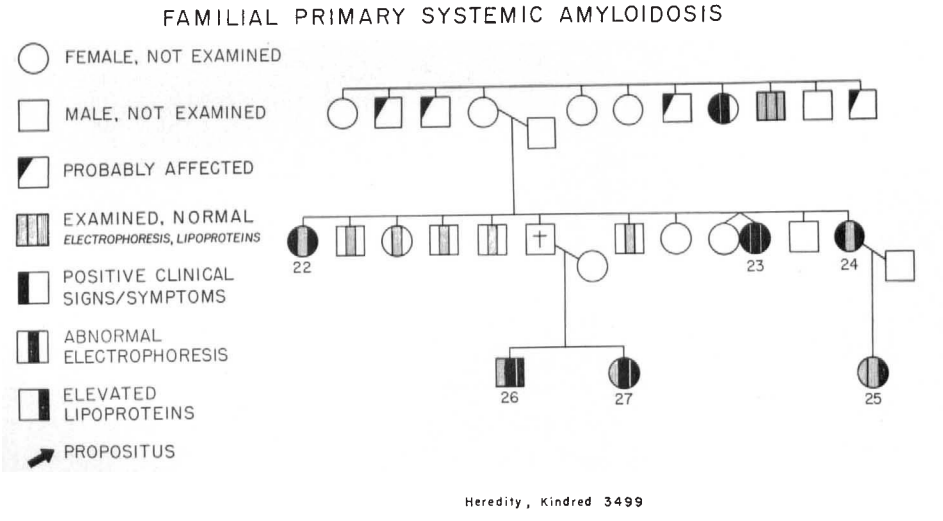

FIG. 10. Branch C, Kindred 3499

14, 15, 18, 21, 26, 27, 33, 34, 35, 36). Of this number seven were thirteen years of age or less.

The clinical data were next related to the results secured by ultracentrifugation. In sixteen cases an abnormal concentration of one or more -S fractions was accompanied by signs and/or symptoms of the disease. There was no sex preponderance noted in this correlation. Significantly, eleven apparently normal members, as determined by clinical study, demonstrated a definite increase in this class of molecules. Of this latter group nine were under 18 years of age.

The data secured by ultracentrifugation were then correlated with that ob-

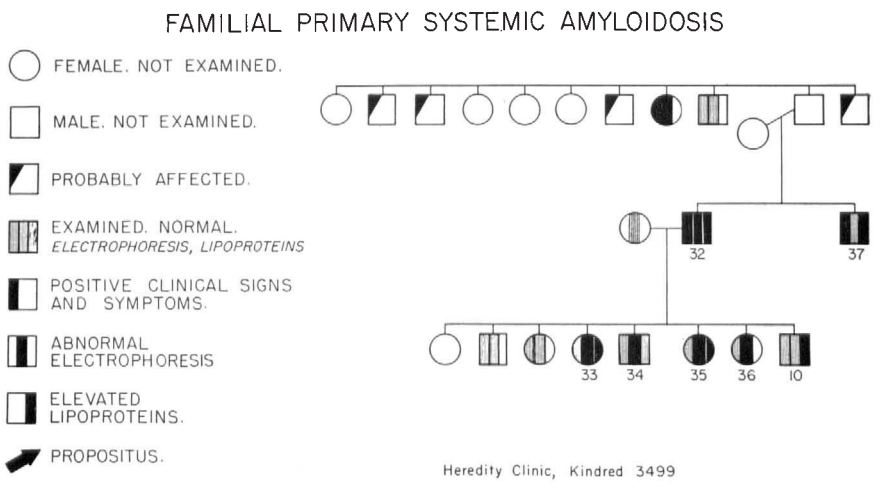

FIG. 11. Branch D, Kindred 3499

tained by free electrophoresis. In twelve cases wherein demonstrable quantitative evidence of an $\alpha_{2'}$ peak was found, ten cases (6, 11, 12, 13, 14, 15, 17, 18, 20, 23) (Table II) showed abnormal lipoprotein levels in the -S 25–40 and 20–25 fractions. In those cases wherein the α_2 electrophoretic peak was poorly resolved, an increase in the measurable concentrations of the -S 25–40 and 20–25 was found in nine cases (1, 9, 26, 27, 29, 32, 33, 35, 38) (Table II). In eight cases with normal electrophoretic patterns, lipoprotein components were found to be elevated.

Interesting correlations were found between lipoprotein abnormalities and the inheritance pattern. The data indicate varying degrees of genetic lipoprotein inheritance. The offspring of case 11 (Fig. 8), consisting of a daughter (case 12), son (case 13) and two grandsons (cases 14, 15) showed the most marked abnormalities in lipoprotein values. One member of this group (case 13), a male age 32 years, showed remarkable elevation in all of the lipoprotein fractions (Table II). His sons, ages 6 and 9 years respectively, likewise demonstrated unusual changes in the -S components.

The offspring of case 1 (Fig. 8), embracing a son (case 2), two daughters (cases 6, 9), four grandsons (cases 4, 5, 7, 8), and a granddaughter (case 3), revealed appreciable concentrations in the -S 20–25 and 1–10 fractions (Table II). Two members of this group (cases 4, 5) were entirely free of the disease by all methods of investigation. The most significantly affected member of this unit was a three year old child (case 3).

The offspring of case 32 (Fig. 11) which demonstrated abnormal lipoprotein levels in essentially the -S 25–40 fraction consisted of three daughters (cases 33, 35, 36) and a son (case 10) (Table II). A brother (case 37) showed similar changes.

There was no significant pattern of serum cholesterol elevation in any of the family segments studied.

DISCUSSION

Approximately one half of the sixty-six members of a large family pedigree have been shown by clinical, laboratory and experimental methods to be afflicted with clinical or sub-clinical primary systemic amyloidosis. In one case (number 16) of this membership, clinical suspicion was confirmed at autopsy. In three cases, (number 1, 11, 23), a positive tissue biopsy (skin and ovarian tissue) for amyloid deposition re-enforced the presumptive diagnosis of the disease process in question.

Evidence secured by this study points to a dominant form of inheritance. More significantly it emphasizes the importance of the electrophoretic serum and lipoprotein relationship to the pathological process. Data secured by these technics substantiate the clinical correctness of the dominant form of transmission (9).

The clinical signs and symptoms of familial amyloidosis described in this study confirms the observations of previous investigators. In this pedigree, at least, the broad clinical spectrum represents a predictable, concatenated series of events culminating in order of descending frequency in peripheral neuropathy, skin changes, hepatic enlargement and dysfunction, cardiovascular insufficiency, eye changes, gastrointestinal symptomatology, and splenomegaly. The occurrence of the carpal tunnel syndrome represents a previously unreported finding in the inherited form of the disease. In no case was there clinical evidence of macroglossia, bleeding tendency, or papulo-nodular dermatologic expression of the disease.

The extensive laboratory studies, which formed an important aspect of this investigation, served to effectively rule out co-existing or other disease processes (multiple myeloma). The experiences of previous workers as to the essentially noncontributory nature of common laboratory data has been confirmed. Nonspecific findings have consisted of x-ray proven cardiomegaly, various electrocardiographic abnormalities, and isolated hematologic abnormalities. The exhibition of the intravenous congo red test (case 16) presented only suggestive evidence of the amyloid process. This finding likewise substantiates the experiences of others. While it is still true that the pathological examination of tissue remains the single most important and generally accessible diagnostic aid, the caprice with which amyloid responds to the various staining procedures limits the usefulness of this approach.

The significance of the biochemical experimental approach to the study of inherited amyloidosis has been alluded to previously. This assumes even greater significance in that the investigative procedures available to the clinician today in the study of amyloid deposition do not permit clear-cut and final diagnostic clarification. The consistent demonstration in these patients with clinical or subclinical disease of an atypical $\alpha_{2'}$ peak by serum free electrophoresis may well represent a new technical aid for the clinician and the geneticist to study this disease. Evidence to date also suggests that its application in the diagnosis of the so-called isolated variety of primary systemic amyloidosis is justified. The

electrophoretic pattern in the latter form is consonant with those described in this study.

While the importance of electrophoretically and clinically positive individuals is not to be overlooked the detection of the subclinical case represents the greatest justification for the application of this technic. Just as in gout and familial hypercholesterolemia the clear and effective detection of the subclinical person vitalizes and validates the genetic statistical data. In this study thirteen cases were found in which clinical signs and symptoms were lacking, yet free electrophoresis revealed either a clearly delineated $\alpha_{2'}$ peak or poor resolution in the area between the α_2 and β globulins. When age in these individuals was correlated with the serum abnormalities an important finding was seen to emerge: twelve of these thirteen cases were under 21 years of age.

Several genetic and clinical possibilities suggest themselves as to this relationship. On the one hand, it might emphasize the importance of a time factor: that is, as these patients approach the second and especially third and fourth decades of life, the development of clinical amyloidosis represents a distinct possibility. Two cases to date (cases 12, 17), both in their early twenties, have progressed into the early phases of the "clinical crossover" (Table II). That this sequence of events may transpire seems most likely. On the other hand, due to the myriad inconstant relative capacities of inheritance in man, this serum abnormality may represent the only life long evidence of this process (carrier state). The carrier state might also indicate the individual with the appropriate genotype who does not develop the characteristic clinical findings until late adolescence, middle, or even old age. Or the carrier might be that person who has inherited a gene which produces in different persons different expressions of the disease which as yet, due to the limitations of physical findings and laboratory procedures, remains undetected. This observation assumes validity especially in primary systemic amyloidosis since an individual may show no pathological evidence of tissue involvement due to the vagary of amyloid staining, and yet at necropsy demonstrate ample evidence of the process. Further, the alleged carrier may be that individual who has inherited a gene which because of its extreme rarity presents and is known only in the heterozygous state.

The position of the atypical electrophoretic peak manifested by these patients suggests even further material for speculation. On the basis of mobility determinations this peak clearly seems to lie between the α_2 and β globulin fractions. When all atypical patterns are considered, the situation is by no means so well defined. Inspection of the unresolved patterns, however, and consideration of mobilities relative to albumin, support the original finding that peak resides in the area described.

Four possibilities concerning the nature of the atypical peak have been considered. The components might conceivably be a mucoprotein or a lipoprotein. It might arise from a split in the α_2 globulin fraction, or it might represent a protein of unknown, heterogeneous composition. These possibilities are, of course, not mutually exclusive. For example, the atypical component might be heterogeneous and might at the same time arise from the split in the α_2 component.

Part of the heterogeneous fraction could conceivably be mucoprotein, lipoprotein or both.

Attempts to demonstrate the existence of a mucoprotein abnormality in two patients who showed the atypical peak were unsuccessful. These data do not represent unequivocal evidence ruling out mucoprotein as a factor contributory to the atypical peak.

Ultracentrifugation of the serum lipoprotein in familial amyloidosis has not been previously reported. Review of the extensive literature as to the etiology of systemic amyloidosis revealed no previous suggestion that lipoprotein dysmetabolism might be involved in the process. Rather speculation has centered about the possible role of immunological reactions, plasma cell dysfunction, tissue ground substance changes, and macromolecular protein deposition. Despite the fact that the methodologies of these experimental technics are inherently different, it was believed that an investigation of lipoprotein function might clarify the nebulous area of amyloid genesis and subclinical case identification.

When correlated with the abnormal findings of free electrophoresis, ultracentrifugal analysis of serum lipoproteins was found to represent an additional experimental aid in diagnosis: twenty-seven of twenty-nine cases with the overt or subclinical process demonstrated an abnormal elevation in one or more fraction of these molecules. Furthermore, ultracentrifugal analysis was shown to be of value in those cases wherein poorly resolved electrophoretic patterns were obtained. In nine such cases serum lipoprotein elevations were found. Thus, not only was the identification of the elusive subclinical subject further augmented, but in eight cases was shown to have elevated lipoprotein components when electrophoretic findings were interpreted as being negative. Thus it would seem that the data obtained by these modalities is not only additive but is complementary as well.

A remarkable correlation was noted to exist between the results of these technics insofar as a more precise localization of the atypical area was concerned. Ultracentrifugal analysis in 18 cases showed that the -S 25–40 and 20–25 fractions, moving with a mobility similar to β and α_2 globulin fractions respectively were elevated. Thus, by means of these experimental methods, the residence of the abnormal biochemical changes in the area described is more clearly defined. While the subclinical individual has been identified by means of electrophoresis, the degree of biochemical inheritance has been only partly ascertained due to the limitations of the method. Ultracentrifugal analysis clearly demonstrated varying degrees of lipoprotein gene inheritance in different family segments of this pedigree. The most consistently transmissible elevated serum lipoprotein fractions were those of the -S 25–40 and 20–25 group. Correlation of these findings with sex or clinical disease appeared to be lacking.

Even though the data suggest that familial primary systemic amyloidosis may represent at least in part a disturbance in lipoprotein metabolism, numerous and as yet unknown factors may play a decisive role. The multitudinous properties and capacities of the blood vessel walls, the role of the ubiquitous connective tissue ground substance, the responsiveness of internal and other tissues to

amyloid deposition, and the little known intricacies of lipid metabolism and related enzyme systems may all in some manner, singly or together, influence the ultimate clinical course of the patient. Assuming these observations to be true, one is impressed by the fact that the gene appears to be crucial in the fundamental processes that are involved.

SUMMARY

Sixty-six members of a large family pedigree have been investigated for the presence or absence of clinical or subclinical inherited primary systemic amyloidosis by means of clinical, laboratory, and experimental biochemical methods. In twenty-nine of these cases evidence for the existence of this disease process has been obtained.

Based on these methods the inheritance of this disease has been shown to assume the dominant form. The exhibition of free electrophoresis of the serum and ultracentrifugation of serum lipoproteins has permitted effective identification of subclinical patients. The importance of this finding in terms of genetic data has been discussed.

The clinical findings in this pedigree, appearing with monotonous regularity in the third and fourth decades, have been shown to consist of a bizarre constellation: peripheral neuropathy, cardiovascular insufficiency, hepatic enlargement and dysfunction, unusual ocular manifestations, gastrointestinal symptomatology, and splenomegaly. The carpal tunnel syndrome has been described for the first time in familial amyloidosis.

The non-specificity and inadequacy of the usual laboratory procedures in the diagnosis of this process has been noted. Autopsy and the study of pathological tissue confirming the diagnosis has been presented.

The demonstration in these patients by means of free electrophoresis of an atypical peak migrating between the β and α_2 globulin areas, and the demonstration of elevated serum lipoprotein by means of ultracentrifugation have been emphasized. The use of these methods in the diagnosis of primary systemic amyloidosis has been recommended.

The correlation between these experimental methods and the clinical expression of the disease has been described. The additive and correlative features of these modalities has likewise been noted.

The demonstration by ultracentrifugation of abnormally elevated lipoproteins, particularly in the -S 25–40 and 20–25 fractions, suggest that at least in part familial primary systemic amyloidosis represents an inherited aberrancy in lipoprotein metabolism.

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