Serum Proteins in Ducks with Amyloidosis (34673)

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Amyloid frequently occurs spontaneously in the white Pekin duck (1, 2). The morphologic and staining characteristics of this amyloid have been described (2, 3). Electron microscopical observations show the same type of fibrils in the amyloid of the duck as in that of man (4, 5). The pathogenesis of amyloid in the duck is unknown; however it apparently is genetically influenced (2). Theories referable to experimental amyloidosis have been reviewed recently by Janigan and Druet (6).

Gray *et al.* (7) observed by electrophoresis on cellulose acetate strips increases in serum α_2- and β-globulins in mice with advanced spontaneously occurring amyloidosis. Milgrom *et al.* (8) found two antigenic components in extracts of amyloid containing human organs which were not detectable in extracts of organs without amyloid. Immunoelectrophoretic studies indicated the major component had the faster mobility and was stained with Sudan black. The minor component appeared in the α-globulin region of the electrophoretic field. Cathcart and Cohen (9) were unable to demonstrate any relationship between purified amyloid fibrils and whole human γ-globulin or components of γ-globulin. Janigan and Druet (6) found that repeated antigenic challenge in mice resulted in the cells of the immunologic apparatus becoming the source of an amyloid enhancing factor. These workers concluded that the reticuloendothelium and endothelial cells are involved in the deposition of the amyloid proteins.

The above studies suggest that amyloidosis in the duck might be correlated with possible changes in the plasma proteins. In order to explore the amyloidosis–plasma protein relationship, the present investigation was undertaken.

Materials and Methods. The ducks were white Pekins with and without amyloidosis. The blood was obtained from a severed carotid artery at the time of death. In obtaining plasma, the blood was prevented from coagulation by adding Versene, 1 mg/ml plasma, and the cells were removed by centrifugation. In the case of sera, the blood was allowed to coagulate. After syneresis, the clot and cells were removed by centrifugation. The plasmas and sera were dialyzed against 200 times the volume of the appropriate buffer for at least 24 hr, and then diluted to a concentration of approximately 1.0% as determined by the Goldberg refractometer. Frequently the sera or plasmas contained substantial amounts of lipid material which was best removed by centrifugation in the cold. The lipid material floated to the top and could be removed from the main portion of the liquid. Usually, 5 ml of plasma or serum were dialyzed against 1 liter of the buffer.

Duck sera and plasmas were examined for the distribution of the protein components by means of moving boundary electrophoresis. A total of 15 duck sera and 9 plasmas were analyzed; a majority in duplicate and in some cases in triplicate. The Spinco Model H electrophoresis and diffusion apparatus was used for most of the determinations. The methods for making the determinations and interpreting the data have been described elsewhere (10, 11). Several buffers were utilized including Veronal buffer, pH 8.6, ionic strength ($\Gamma/2$) 0.1; Tris–Veronal buffer, pH 8.6, 0.05 *M* Tris; Tris–Veronal–NaCl buffer, pH 8.6, 0.05 *M* Tris, 0.025 *M* NaCl; phosphate buffer, pH 7.7, ($\Gamma/2$) 0.2. Veronal buffer, pH 8.6, ($\Gamma/2$) 0.1 prepared according to Longsworth (12) was satisfactory and was used for most of the studies. Tris–Veronal–NaCl buffer, pH 8.6 was prepared by ad-

ding 9.51 g (0.05 mole) of Veronal to 6.055 g (0.05 mole) of tris-(hydroxymethyl)aminomethane (Tris) and 1.46 g (0.025 mole) of NaCl, and then diluting to 1 liter with distilled water. The Veronal buffer had a specific conductivity of 0.002995 to 0.003138 ohm^{-1} cm^{-1}. Electrolysis was allowed to proceed at 16 mA until the fast component had migrated across the field of view. The specific conductivity for the Tris–Veronal–NaCl buffer was 0.00235–0.00246 $ohm^{-1}cm^{-1}$. Electrolysis was allowed to proceed at 13.5 mA. An Aminco portable electrophoresis apparatus was used for the studies with this buffer. Considerable arbitrariness must be exercised in interpreting the patterns; however, every attempt was made to interpret the patterns consistently.

Results and Discussion. The electrophoretic patterns for the sera of ducks with and without amyloidosis are presented in Fig. 1. Duck 3330 was 494 days old when killed and the liver weighed 120 g. Large amounts of amyloid were present in the liver, spleen, and adrenals. Duck 3387 was 556 days old when killed and the liver weighed 54 g. There was no amyloid. The overall contour of the patterns suggest an increase in the region corresponding to the β-globulins (components 2 and 3). This region presents the hypersharp boundary known as the β-anomaly. Since lipid material causes turbidity in the protein solution, it was necessary to remove this material for the solution to transmit light.

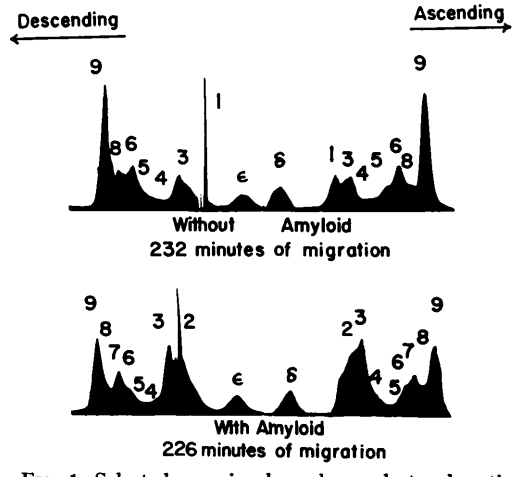

FIG. 1. Selected moving-boundary electrophoretic patterns of duck sera in Veronal buffer, pH 8.6, ($\gamma/2$) 0.1. The pattern for the duck without amyloid is that for D-3387. The pattern for the duck with amyloid is that for D-3330. The data for the analyses are in Table I.

The mobility and distribution data of the serum proteins for two ducks without and three ducks with amyloidosis are shown in Table I. Considerable variation exists in the plasma and serum proteins from different ducks. The data indicates no significant variation in the distribution of the components in frozen or fresh sera. There is a significant elevation in amounts of components 2 and 3 (mobilities 2.27–2.62 and 2.95–3.11, respectively) in ducks with amyloidosis. These mobilities suggest that these protein components are those designated β-globulins and possibly part of the γ-globulins in mammalians. The ducks with amyloidosis have a corresponding decrease in the fast component (mobility, 6.24–6.29) which corresponds to the albumin. Statistical analysis of the data in Table I, although limited in amount, indicates highly significant differences in the averages mentioned above. Critical ratios of 4.22 and 5.02, for these averages suggest that there is less than one possibility in 1000 that the differences are due to chance.

The patterns for the plasmas were much more difficult to interpret, and less difference was observed between the plasma from four ducks with and five without amyloidosis. There were two components faster than albumin in the plasmas of two of the ducks not having amyloidosis. No electrophoretic data on plasmas are included.

Samples of sera that had been collected from four ducks with and four without amyloidosis, and stored in the frozen state for periods of time ranging from several months to over a year were analyzed. The distributions of the components were similar to the data presented in Tables I and II. There was an increase in the β-globulins in the sera from ducks having amyloidosis. There were components faster than albumin in the sera of some of the ducks with and without

TABLE I. Mobilities and Percentage Distributions of the Serum Proteins of the Duck; Veronal Buffer, pH 8.6 (Γ/2) 0.1.

Component:	1		2		3		4		5		6		7		8		9	
Duck	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%
								Without amyloid										
D3494	1.85	8	2.36	21	2.99	17	3.52	4	4.31	6	4.89	13			5.42	7	6.03	24
D3494	1.94	10	2.48	20	3.04	16	3.67	3	4.50	5	5.15	13			5.75	6	6.36	27
D3494 (frozen)	2.13	11	2.72	22	3.15	12	3.66	5	4.50	4	5.09	14			5.68	6	6.21	24
D3494 (frozen)	2.28	11	2.93	20	3.39	11	4.07	3	4.92	7	5.73	19			6.36	6	6.97	23
D3387	2.19	12			3.10	16	3.85	5	4.43	7	4.85	15			5.41	7	6.11	38
D3387	1.69	15			2.97	14	3.85	4	4.40	6	4.92	14			5.40	9	6.09	38
Av	2.01	11	2.62	14	3.11	14	3.77	4	4.50	6	5.10	15			5.69	7	6.29	29
								With amyloid										
D3330			2.03	27	2.85	20	3.59	4	4.16	3	4.64	5	5.08	10	5.56	10	6.13	21
D3330			2.34	26	3.09	20	3.65	4	4.23	2	4.82	6	5.24	10	5.63	5	6.34	27
D3330 (frozen)			2.36	28	2.74	23	3.48	5	4.19	2	4.71	5	5.17	9	5.54	4	6.15	24
D3330 (frozen)			2.48	29	3.10	22	3.60	3	4.15	3	4.78	7	5.27	10	5.67	3	6.26	23
D3388	1.70	8	2.33	28	2.90	25	3.73	5	4.31	4	4.91	11			5.50	5	6.13	14
D3389			2.14	24	3.01	35			3.90	6	4.80	3	5.33	7	5.81	7	6.36	18
D3389			2.23	24	2.97	33			3.87	8	4.91	4	5.40	6	5.85	9	6.34	16
Av	1.70	1	2.27	27	2.95	25	3.61	3	4.12	4	4.79	5	5.24	7	5.65	6	6.24	22

TABLE II. Mobilities and Percentage Distributions of the Serum Proteins in Ducks; Tris–Veronal–NaCl Buffer.

	1		2		3		4		5		6		7		8		9	
	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%
3127[a]	0.00	10	1.52	3	2.70	7	3.62	4			4.46	8	5.46	6	6.50	34	7.68	28
3127-28[a]	0.00	12	1.59	5	2.33	3	3.10	8	3.84	5	4.74	8			6.25	40	7.26	19
3102[b]	0.00	11	1.04	10	2.06	23	3.09	12	4.19	3	4.79	5	5.76	13	6.60	23		
3102[b]	0.00	12	0.90	10	1.74	19	2.51	15	3.40	4	4.17	5	5.06	11	6.02	24		
3102[b]	0.00	10	1.22	14	2.14	18	2.83	13	3.80	4	4.56	6	5.48	10	6.29	25		

[a] Without amyloid.

[b] With amyloid and muscular dystrophy.

amyloidosis; however, the amounts of these faster components were higher in the sera from ducks without amyloidosis.

The study in which Tris–Veronal–NaCl buffer was used is shown in Table II. The sera from two ducks without amyloidosis, and the serum from one duck having amyloidosis with severe muscular dystrophy are included. The serum from the ducks without amyloidosis showed one component faster than albumin (mobility, 7.26–7.68). No components faster than albumin appeared in the serum from the duck with amyloidosis. The serum from the duck with amyloidosis has an increase in components 2, 3, 4, and 7 with a corresponding decrease in albumin. The increases were in the region of α-, β-, and part of the γ-globulins. These data are in general similar to those data resulting when Veronal buffer was used.

These data for the white Pekin duck with amyloidosis are consistent with those reported by Gray *et al.* (7) and Milgrom *et al.* (8) for elevation of the α- and β-globulins in mice and man with amyloidosis. These findings support the thesis that the amyloid in the duck is similar to that found in man and mouse.

Conclusions. The data indicate that in the sera from the white Pekin duck with spontaneously occurring amyloidosis there is an elevation in the β-globulins. Electrophoretic data using two buffers are reported. Variation exists in the electrophoretic patterns of plasmas from ducks with and without amyloidosis. Components faster than albumin occur in the sera and plasmas from some ducks—not all, and seem to be in larger concentrations in ducks without amyloidosis.

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