Acid Mucopolysaccharides in the Liver of Pekin Duck with Amyloidosis* (34112)

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Amyloidosis has been noted to occur spontaneously in the duck, especially the white Pekin duck (1–4). As the duck ages, the incidence of the disease increases and reaches a frequency in excess of 80% after 1½ years. Furthermore, amyloidosis in the Pekin duck is quite similar to that occurring spontaneously in humans; a genetic basis for each has been proposed (4–7 and others). This interesting model found in the duck offers another example to study biochemical properties of amyloid and pathogenesis of the disease.

Extensive chemical studies of amyloid tissue from humans, from a variety of sources (8–12), in an experimental model in mice (13), and in isolated amyloid fibrils (14) indicate an association of the acid mucopolysaccharides (MPS) with this fibrous protein. These studies concern the nature of MPS in the liver of Pekin ducks with gradations of amyloid deposits.

Materials and Methods. Pekin duck livers, involved with amyloidosis, and controls, were obtained from a colony of birds maintained at the University of Texas Medical Branch, Galveston. The ducks ranged in age from 11 to 14 months and were being fed on Purina Duck Startena and Growena. Food and water were available at all times. The tissues were received in the laboratory either frozen or fixed in formalin for the study of MPS composition. Sections were taken for histologic studies and were stained by hematoxylin and eosin and Congo red. The degree of involvement was approximated according to the histologic appearance for comparison to the MPS isolates.

The MPS were isolated, fractionated, and analyzed by methods that have been previously described in detail (12, 13). The total amount of MPS, a mixture isolated from the tissues, was estimated by analysis of uronic acid (15). Fractionation of the mixtures of MPS was achieved on Dowex 1-2 X(200–400 mesh) Cl^- columns and the fractions were analyzed for hexosamines and uronic acid by gas-liquid chromatography (16, 17). Differentiation of amounts of chondroitin sulfates A and C were performed by the method of Mathews and Inouye (18). Electrophoretic studies on cellulose acetate in a pyridine–formic acid buffer, 0.03 μ, pH 3.0, methylene blue, and alcian blue strain, were used for qualitative identification of MPS (19). These methods allow an estimation of the average content of individual MPS in the tissues. An example of multiple analyses and quantitation of MPS from one tissue sample is shown in Table I.

Results and Discussion. The results are summarized in Table II and Fig. 1. It can be seen from Table II that a good correlation exists between the degree of involvement as estimated by histologic survey and the total amount of MPS in the tissues. More than a tenfold increase occurs in the more advanced involvement of the liver. In these studies it was observed that increasing amounts of MPS correlated well with increasing amyloid infiltration within the liver. The increase, as indicated by the fractionation of MPS preparations, illustrated in Fig. 1, is due to changes in content of specific compounds. As has been found in other studies of amyloid, hep-

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TABLE I. Analyses of Acid Mucopolysaccharides from Liver of a Pekin Duck with Amyloid.[a]

Fraction[b]			Gas-liquid chromatography				
			Hexosamine		Hexuronic acid		
M NaCl	Uronic acid (mg)	Electrophoresis	Glucosamine (%)	Galactosamine (%)	Glucuronic (%)	Iduronic (%)	Mucopolysaccharide (%)
0.50	0.30	HA	100	—	100	—	2.7 HA
0.75	0.44	HS					
1.00	1.80	HS	100	Trace	83.6	16.4	47.8 HS
1.25	3.40	HS					
1.50	1.90	CS					13.5 CS-A
2.00	3.10	CS	9.0	91	51.0	49.0	23.2 CS-B
4.00	0.38	CS					6.6 CS-C[c]
							4.2 (Hep/KS?)

Abbreviations used: hyaluronic acid (HA); heparitin sulfate (HS); chondroitin sulfate (CS); heparin (Hep); keratosulfate (KS).

[a] Sample No. 2 of Table II and Fig. 1.

[b] Amount fractionated, 109 mg total (crude) MPS (uronic acid, 11%).

[c] Determined by the method of Mathew and Inouye (18).

aritin sulfate was uniformly increased in these samples; however, rather interesting in the duck liver is the striking increase of chondroitin sulfate B and, to a lesser content, chondroitin sulfates A and C. This observation is somewhat unusual, since increases of chondroitin sulfates to these proportions were not found in other amyloid tissues previously studied, although increases of chondroitin sulfate B have been detected before but not to this extent. An explanation of the marked increase of the total MPS with higher levels of both heparitin sulfate and the chondroitin sulfates is not apparent at this time, but the association of concomitant increases of two MPS, heparitin sulfate and chondroitin sulfate B, in several disease states involving vascular tissues have been reported earlier—

TABLE II. Correlation of Degree of Involvement of Amyloidosis of the Liver of Pekin Ducks with Content of Acid Mucopolysaccharides.

Duck sample	Age (months)	Sex	Degree of involvement[a]	Uronic acid (milligrams/gram defatted liver)[b]
Pooled (5)[c]	15–18	M & F	0	
Normal I				0.33
Normal II				0.36
No. 1	12	M	±	0.38
2	12	M	+	1.36
3	13.5	?	++	2.45
4	13	F	++	2.94
5	11	F	++	4.34
6	12	M	+++	5.75

[a] Estimates from histologic sections from minimal to severe.

[b] Total amount of acid mucopolysaccharides can be roughly estimated by multiplying values by 3.3.

[c] Number of ducks used for samples shown as duplicate determinations.

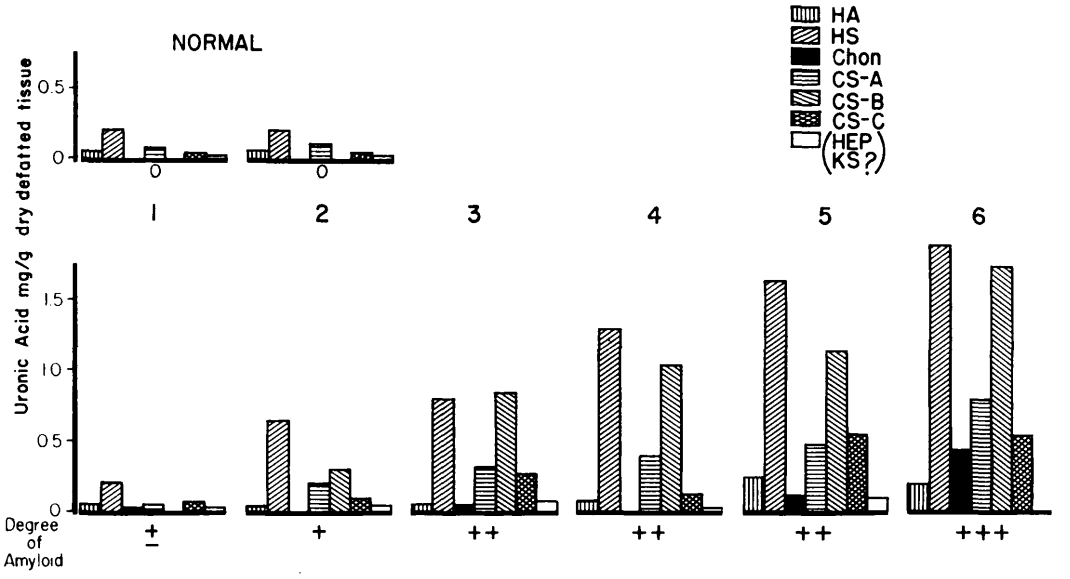

FIG. 1. Acid mucopolysaccharides (MPS) from normal and amyloid duck liver. The composition of MPS from six individual liver samples involved with different degrees of amyloidosis was compared with the MPS from two sets of pooled normal livers. Individual MPS are expressed as milligrams of uronic acid per gram dry, defatted tissue, and these values are approximately one third of the amount of MPS. For abbreviations, refer to Table II. A gradual increase of heparitin sulfate and chondroitin sulfates with increasing amloid involvement is apparent from the figure.

Hurler syndrome (20), atherosclerosis (21), diabetic nephropathy (22). In tissue culture, fibroblasts from individuals with Hurler syndrome have shown an increased synthesis of chondroitin sulfates A/C, in addition to heparitin sulfate and chondroitin sulfate B (23).

Comment. One of the most important aspects of the study of amyloid concerns the genetic origin and relationship to other causes for its development. In this respect, the Pekin duck is a good model, simulating certain forms of the disease as it occurs in humans. Information concerning a genetic basis will ultimately help to elucidate metabolic defects which lead to induction of the disease. Amyloidosis has been observed in man associated with a variety of conditions. The observations from study of MPS chemistry suggest that there is likely a metabolic link among the different syndromes of amyloidosis, whether of a genetic origin or not. This common defect is also suggested by study of amyloid from various sources by electron microscopy. Morphologically, there are no differences in the amyloid fibrils (24), and based on their appearance no distinctions within the syndromes of amyloidosis can be made. The Pekin duck model has advantages of being a readily available source of material, of presenting tissues with gradations of amyloid infiltration, and of yielding amyloid material from a related strain of individuals.

Summary. Studies on the liver involved with increasing amounts of amyloidosis and control tissues indicate that there is a marked increase in MPS content with severe amyloid infiltration. The increase of MPS was accounted for by increases in heparitin sulfate, chondroitin sulfate B, and somewhat less of chondroitin sulfates A and C. The consistent increase of heparitin sulfate in the liver of the ducks was similar to that observed in amyloidosis in humans and in experimentally induced disease. This remarkable increase of chondroitin sulfate B suggests a link to other vascular diseases and genetic connective tissue defects. The Pekin duck serves as an interesting model to study

spontaneously occurring amyloidosis.

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