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Grace P. Kerby

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Research Article

THE EFFECT OF INFLAMMATION ON THE HEXURONATE-CONTAINING POLYSACCHARIDES OF HUMAN PLASMA [1]

By GRACE P. KERBY

(From the Department of Medicine, Duke University School of Medicine, Durham, N. C.)

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The observation has been made (1) that polysaccharides containing uronic acid are increased in the plasma of patients with rheumatoid arthritis. In view of the probable identity of these polysaccharides with some components of ground substance (2–4), this is an observation of considerable interest. If it should apply only to patients with rheumatoid arthritis, it might be a reflection of basic changes in the metabolism of connective tissue in that disease. The present study was done to compare in this regard patients with rheumatoid arthritis and patients with inflammatory disease unrelated to rheumatoid arthritis. Increased levels of hexuronate-containing polysaccharides were observed in plasma from patients with rheumatoid arthritis. However similar increases were noted in the presence of nonrheumatoid inflammatory states.

EXPERIMENTS

Oxalated blood for plasma was obtained freshly from three groups of individuals: Group A, healthy ambulatory individuals and hospitalized patients with no evidence of inflammatory, hematologic, neoplastic, primary metabolic or connective tissue disease; Group B, hospitalized patients with no evidence of hematologic, neoplastic, primary metabolic or connective tissue disease, but with significant inflammatory reactions, for the most part either post-traumatic or secondary to pulmonary or urinary tract infection; and Group C, hospitalized or clinic patients with clinically unequivocal active rheumatoid arthritis, without regard to stage or grade. No patients were receiving adrenocorticosteroids. Most of the rheumatoid patients were on salicylates. C-reactive protein tests were negative in all individuals in Group A and positive in all patients in Groups B and C. With few exceptions, blood samples were obtained in fasting periods or at least three hours postprandially. Additional healthy laboratory personnel were studied before, during and after five day periods of salicylate ingestion in dosage sufficient to induce continuous mild to moderate symptoms of salicylism. C-reactive protein tests were negative throughout these studies except for a transient positive reaction in one individual, occurring during the period of salicylate ingestion and coincident with the occurrence of slight tenderness at site of a venipuncture.

Two series of observations were made, each series comprising three groups of patients as just defined. In Series I, euglobulins were precipitated by the mineral acid dilution method of Erickson, Volkin, Craig, Cooper, and Neurath (5) from duplicate 1 ml. and duplicate 5 ml. samples of plasma, to each of which a quantity of physiologic saline equal to one-half of the original plasma volume had been added. The 1 ml. samples were taken up to original plasma volume in alkaline saline, and protein content was determined by biuret reaction (6). Acid mucopolysaccharides were recovered from the 5 ml. samples by a previously described method (4) involving hydrolysis of proteins with crystalline pepsin and trypsin, dialysis of hydrolysates, and then precipitation of acid mucopolysaccharides with quarternary ammonium bromide. In Series II, acid mucopolysaccharides were recovered from duplicate 5.5 ml. samples of plasma by the method of Bollet, Seraydarian, and Simpson (3). Biuret determinations were done on 0.5 ml. aliquots removed just prior to precipitation with perchloric acid. In both series, hexuronates were measured by the carbazole method (7).

As noted previously (4), the method of Series I yields a polysaccharide fraction in which mucoprotein contamination is evidenced by disproportionately high yields of hexosamine. Consequently the data obtained by Series I are presented for group comparison with Series II and not for absolute levels of hexuronate. The method of Series II has already been noted by Bollet and associates (3) to show no evidence of mucoprotein contamination. The accurate recovery of added chondroitin sulfate (10 μg. added to 5.5 ml. of plasma) recorded by Bollet and coworkers was confirmed, as was the degree of accuracy possible with the method. Duplicate samples checked within 10 per cent in both series.

RESULTS

Samples of the material recovered were compared on paper chromatography with commercial chondroitin sulfate (General Biochemicals, Inc.) and with heparin (Liquaemin®, Organon, Inc.), using methods previously described (8). Metachromasia developed with toluidine blue. Mobility of the two components observed was identical with

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TABLE I

Hexuronate-containing polysaccharide levels in the euglobulin fraction of human plasma, recovered by Method I and measured by hexuronic acid content *

Subject groups (no. in group)	Euglobulins (mg.) per 100 ml. plasma	Hexuronic acid (μg.)	
		Per 100 ml. plasma	Per 100 mg. euglobulin
A—Noninflammatory 23	411 ± 94 (S. D.)	226 ± 75	56 ± 15
B—Inflammatory 26	748 ± 126	356 ± 118	48 ± 16
C—Rheumatoid 25	702 ± 189	372 ± 117	54 ± 13

* As noted in text, hexuronic acid levels determined by Method I are presented for group comparisons only. Disproportionately high hexosamine levels (4) indicate some degree of mucoprotein contamination of the fraction measured.

that already described (4) for the acid mucopolysaccharides recovered from Cohn fractions of human plasma.

Results of the survey of the three groups of individuals in Series I are summarized in Table I. As noted in the Experiments section, the data in this series are presented for group comparisons and not for conclusions regarding absolute levels of acid mucopolysaccharides.

Results of the survey of another three groups of individuals by the method of Bollet and associates (Series II) are presented in Table II. The protein levels were determined prior to perchloric acid precipitation.

In both series, the euglobulin fraction in Groups B and C was significantly greater than in Group A. This has been observed consistently in previous studies (9) and represents a nonspecific change found in any patient with an active inflammatory process. In both series the level of nondialyzable polysaccharides as measured by hexuronate content was higher in Groups B and C than in Group A. In Series I, the elevation was highly significant ($p < 0.01$) on comparison of Groups B and C with Group A. In Series II the elevation was highly significant ($p < 0.01$) on comparison of Group B with Group A and possibly significant ($p < 0.05$) on comparison of Group C with Group A.

The plasma levels of acid mucopolysaccharides

TABLE II

Plasma levels of hexuronate-containing polysaccharides, recovered by Method II (3) and measured by hexuronic acid content

Individual subjects	Group A Noninflammatory		Group B Inflammatory		Group C Rheumatoids	
	Euglobulins (mg.) per 100 ml. plasma*	Hexuronic acid (μg.) per 100 ml. plasma	Euglobulins (mg.) per 100 ml. plasma*	Hexuronic acid (μg.) per 100 ml. plasma	Euglobulins (mg.) per 100 ml. plasma*	Hexuronic acid (μg.) per 100 ml. plasma
1	643	152	802	207	1,090	181
2	544	157	923	216	1,020	148
3	615	163	1,061	178	932	148
4	632	171	878	231	915	159
5	421	157	1,361	165	783	224
6	307	135	895	229	829	212
7	643	121	1,061	173	999	253
8	623	120	1,072	177	736	166
9	706	118	969	252	669	151
10	857	127	1,021	122	1,094	235
11	596	159	1,227	177	906	145
12	570	168	1,118	129	1,062	123
13	809	118	1,320	122	1,411	137
Average of group (± S. D.)	613 ± 143	144 ± 21	1,054 ± 169	183 ± 43	957 ± 192	176 ± 42

* Before perchloric acid precipitation.

of subjects in Group A of Series II were considerably lower than the serum levels of normal individuals as reported by Bollet and co-workers (3).

By Method I, mild salicylism for five days in normal individuals produced no consistent change in amount of acid mucopolysaccharides recovered from plasma proteins.

DISCUSSION

The level of acid mucopolysaccharides recovered from plasma proteins of patients with rheumatoid arthritis was found to be increased as compared to a control group. This observation is in accord with an earlier report by Badin, Schubert, and Vouras (1). On evaluating a group of patients with a comparable degree of inflammation unrelated to rheumatoid disease, the level of acid mucopolysaccharides in plasma was found to be similarly increased on comparison with the control group. It is of interest that the increase in Series II (where mucoprotein contamination was not a factor) was more consistent in patients with nonrheumatoid inflammatory states than in the rheumatoid group. The increase in both instances paralleled fairly closely the increase in plasma euglobulins which was noted in the presence of any inflammatory process, including rheumatoid arthritis. Thus at present, the acid mucopolysaccharides must be classified with the numerous other substances known to occur in increased amounts in the plasma of patients with inflammatory processes in general. As a material whose study may elucidate some of the factors involved in the combination of acid mucopolysaccharides with protein, it offers promise, nonetheless. The striking difference between serum levels of acid mucopolysaccharides as reported by Bollet and associates (3) and plasma levels as noted in the present study is under further investigation now.

SUMMARY

The acid mucopolysaccharides recoverable from the euglobulin fraction of human plasma were found to be increased in patients with rheumatoid arthritis as compared to a normal group. The increase was nonspecific in that it occurred to an equal degree in patients with inflammatory processes unrelated to rheumatoid disease. The increase in acid mucopolysaccharides in inflammatory processes, both rheumatoid and nonrheumatoid, was accompanied by an increase in the euglobulin fraction of plasma from which the acid mucopolysaccharides were recovered.

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