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URINARY AND TISSUE GLYCOSAMINOGLYCAN PATTERNS IN HEPATIC ANGIOSARCOMA

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I. INTRODUCTION

The recent discovery of a relationship between vinyl chloride and angiosarcoma of the liver has received much attention (1-3). Although there are now systematic detection programs for vinyl chloride workers (3,4), there is as yet no specific chemical abnormality that serves as a good indicator of early, vinyl-chloride-induced liver injury and angiosarcoma. Alpha feto-protein has been a relatively valuable serological marker for hepatocellular carcinoma (5), but is has not as yet proven useful in the detection of angiosarcoma (6). New leads are needed if more specific tests are to be developed for angiosarcoma.

The literature suggests that the glycosaminoglycans in the urine and/or blood should be evaluated as a possible aid in early detection. The production of sulfated glycosaminoglycans is characteristic of malignant vascular tumors of the skin and some pathologists use this feature as a diagnostic aid (7). Barr and Bonin (8) observed a strong positive alcian-blue, glycosaminoglycan staining reaction in human angiosarcoma tissue and suggested than an attempt be made to qualitate and quantitate the production of glycosaminoglycans in the neoplasms, serum, and urine of those at risk. They pointed out that the urinary glycosaminoglycans may have diagnostic significance in angiosarcoma and, if so, a glycosaminoglycan spot test might easily be employed as a gross screening test of vinyl chloride production workers.

A number of other observations place the glycosaminoglycans in a relevant position with regard to angiosarcoma. Angiosarcoma is accompanied by connective tissue abnormalities (2,9) and changes in tissue, urinary, and blood glycosaminoglycans have been found to occur in many connective-tissue disorders -- including connective tissue disorders of the liver (10-14) -- as well as in hepatic cancer (15-17).

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The purpose of this study was to make a preliminary determination of the glycosaminoglycan patterns in tissue and urine associated with angiosarcoma of the liver and with vinyl-chloride-induced liver injury other than angiosarcoma and to compare these patterns with those in normal controls and those associated with other liver disease. Our goal was to evaluate the use of glycosaminoglycan patterns in the early detection of vinyl-chloride-induced liver injury and angiosarcoma and to explore the role of the glycosaminoglycans in the etiology of vinyl chloride injury.

II. PROCEDURES AND MATERIALS USED

Urine specimens were collected as occasional samples from: 9 normal controls; 9 individuals with histories of occupational exposure to vinyl chloride and having abnormal, liver, biochemical studies; 6 with "other" cancers prior to surgery; 3 with angiosarcoma; 8 with active viral hepatitis; 6 with cirrhosis; 2 with lung-liver metastases; and 4 with metabolic disorders of the liver (congenital and indirect hyperbilirubinemia).

In one case of angiosarcoma, 24-hr urines were collected on alternate days beginning 2 weeks prior to death.

Urine samples were collected without preservative and frozen at -76° until analysis. Specimens were divided into two 25 ml samples and one 5 ml sample. Urinary creatinine was measured on the 5 ml sample using a Technicon Autoanalyzer. The degree of urinary glycosaminoglycan polymerization was estimated by dialyzing one 25 ml sample for 24 hours in tap water; the sample was then treated identically to an undialyzed sample by the method of DiFerrante (18) using cetylpyridinium chloride as a precipitant. After resolubilization of the glycosaminoglycans in water, duplicate samples were assayed for total uronic acid by the modified carbozole reaction of Bitter and Muir (19). The remaining glycosaminoglycans were reprecipitated with cetylpyridinium chloride and separated into the wash, hyaluronic acid, chondroitin sulfate, and heparin fractions as described by Schiller et. al.(20). Each of the fractions was assayed for uronic acid (µg per mg of creatinine).

Autopsy tissue was obtained in 2 cases of hepatic angiosarcoma (tumor tissue and non-tumor tissue adjacent to tumor), 2 cases of cirrhosis, and in 3 control cases (gun-shot wound victims without liver pathology) and analyzed for glycosaminoglycans by a previously reported modification (21) of the method of Schiller et. al. (20). Uronic acid was determined in each of the hyaluronic acid, chondroitin sulfate, and heparin fractions.

Pieces of tissue were subjected to alcian-blue-periodic-acid-Schiff staining with and without hyaluronidase and diastase pretreatment. These procedures were carried out according to the methods described by Mowry (22).

Ascitic fluid was also obtained at autopsy in one case of angiosarcoma and analyzed for glycosaminoglycans. The fluid was centrifuged

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and the sediment analyzed as tissue above. The supernatant was treated by the method for urine described above.

III. RESULTS

A summary of the urinary glycosaminoglycan measurement is given in Table I. Normal controls had the least urinary glycosaminoglycans (measured as uronic acid) of all groups. All other groups showed some elevation. The levels in angiosarcoma, hepatitis, cirrhosis, and liver metastases were significantly elevated ($P < .05$) over normal controls. The cirrhotic group exhibited the greatest variance in urinary glycosaminoglycans. No significant differences were found in urinary creatinine levels between groups.

There were no significant differences between groups in either the percentage of the total glycosaminoglycans that was dialyzable (Table I) or in the percentage of the unfractionated total that appeared in the hyaluronic acid, chondroitin sulfate, and heparin fractions.

Seven of 9 vinyl-chloride-exposed individuals other than those with angiosarcoma had positive chondroitin sulfate fractions with negative hyaluronic acid and heparin fractions. This was true in only 3 of 32 other urines evaluated in this same manner.

The pattern of daily glycosaminoglycan excretion prior to death due to angiosarcoma in one individual is given in Figure 1.

Total tissue glycosaminoglycan levels for angiosarcoma tumors, fibrotic tissue adjacent to tumors, cirrhotic liver tissue and normal liver tissue are shown in Figure 2. Fractional hyaluronic acid, chondroitin sulfate, and heparin levels are given in Figure 3.

Histochemically, angiosarcomatous tissue exhibited a strong alcian-blue positive staining reaction. Alcian-blue staining was only slightly less in "non-tumor" tissue adjacent to tumor masses. The staining reaction in tissue from normal liver was very weak and only slightly stronger in cirrhotic liver tissue. The strong alcian-blue reaction in angiosarcomatous tissue did not occur if sections were pretreated with hyaluronidase.

Ascitic fluid sediment was uronic-acid-positive in only the hyaluronic acid fraction -- 112 µg uronic acid per gram of dry, defatted sediment; ascitic fluid supernatant contained 1.7, 1.2, and 0.2 µg uronic acid per ml in the hyaluronic acid, chondroitin sulfate, and heparin fractions, respectively.

IV. DISCUSSION

The literature indicates that normal male creatinine excretion is 1.5 g per 24 hrs (23). Thus, our normal mean (Table I) of 3.2 ± .4 µg cetylpyridinium chloride-precipitable uronic acid per mg creatinine falls in the middle of the normal ranges reported by Varma et. al. (24),

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2.6 - 4.7 µg/mg; DiFerrante and Rich (25), 2.9 - 4.8 µg/mg; and Kao and Leslie (26), 1.8 - 4.9 µg/mg.

Although our study was not controlled for age, Goldberg and Cotlier (27) have shown that urinary glycosaminoglycan excretion is constant from ages 20-70. Manley et. al. (28) have shown that the proportion of urinary glycosaminoglycans in the chondroitin sulfate fraction is constant from ages 20-70. Manley et. al. also reported that the chondroitin sulfate fraction is highest at birth and that it gradually drops until age 20, suggesting that urinary chondroitin sulfate reflects tissue growth.

The fact that we found no differences between groups in the creatinine concentration is significant in that it indicates that occasional samples do reflect 24-hour excretion when normalized to creatinine. Precedent for expressing glycosaminoglycan measurements as a function of creatinine content in occasional urine samples has been established by DiFerrante and Rich (25) and Pennock (29). Manley et. al. (28) have shown that the creatinine/uronic acid ratio is steady from ages 20-70.

Our results indicate that the liver diseases evaluated are accompanied by elevated urinary glycosaminoglycan excretion. Our tissue data suggests that this reflects liver-tissue glycosaminoglycan elevation and conforms to the reports by others that both hepatic connective tissue disorders (10-14) and hepatic cancer (15) result in increased hepatic glycosaminoglycan levels. It may be significant that the angiosarcoma patients had half the urinary glycosaminoglycan excretion of patients with liver metastases and that our analysis of angiosarcomatous tumor tissue exhibited half the glycosaminoglycan content reported by Kojima et. al. (15) for hepatocellular carcinoma.

While our data suggest that liver disease results in a decrease in the proportion of highly polymerized glycosaminoglycans, variance was large within each group and none of the differences between groups were statistically significant.

Although we have not completed the characterization of isolated glycosaminoglycan fractions, our data indicate: 1) that the chondroitin sulfates are the primary urinary glycosaminoglycans in both normal controls and in disease states; 2) that the chondroitin sulfates and heparin are the dominant glycosaminoglycans in normal and cirrhotic livers (Figure 3). Chondroitin sulfate is elevated in the fibrotic, non-tumor, portions of angiosarcomatous livers while heparin is the predominant glycosaminoglycan in tumor tissue. Hyaluronic acid is also apparently elevated relative to chondroitin sulfate in angiosarcomatous tumors (Figure 3); and 3) that hyaluronic acid is the sole glycosaminoglycan in ascites fluid sediment.

These qualitative data are in general agreement with those reported by others. Goldberg and Cotlier (27), Douglas et. al. (30), and Varma et. al. (24) have reported that the chondroitin sulfates are the predominant urinary glycosaminoglycans. Varma et. al. reported that 2/3 of urinary glycosaminoglycans are chondroitin-4-and chondroitin-6-sulfate and this agrees with our data on normal controls and on those with liver disease.

Kojima et. al. (15) reported that in hepatocellular carcinoma

tissue, chondroitin sulfates and hyaluronic acid were increased 33 and 10 times, respectively, over amounts found in healthy livers; the heparin and heparan sulfate proportions dropped. This contrasts with our data on angiosarcoma tissue, i.e. heparin and hyaluronic acid increased 5 and 10 times, respectively, over normal tissue; chondroitin sulfate levels rose but fell in proportion to other glycosaminoglycans. Galambos and Shapira (10) reported that the chondroitin sulfates are dominant in normal livers and in hepatic fibrosis, but Kojima et. al. (15) report that chondroitinase-resistant and hyaluronidase-resistant glycosaminoglycans are dominant. Kuroda et. al. (31) also reported that heparan sulfate is the dominant glycosaminoglycan in the normal liver. Our histochemical observation that nearly all of the increased alcian-blue positive material in angiosarcomatous livers was susceptible to hyaluronidase digestion suggests that the observed chondroitin sulfate elevation is due to chondroitin-4- and/or chondroitin-6-sulfate.

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The increases in liver and urinary glycosaminoglycans may well reflect an important role of these substances in the process of fibrogenesis and in tumor growth. Galambos and Shapira (10) reported that hyaluronic acid was elevated during hepatic fibrogenesis. If a similar fibrotic process is operative in angiosarcoma, it may be that the observed tumor-tissue heparin increase is reflective of tumor growth. We did observe a four- to six-fold greater heparin level in tumor tissue than in adjacent, non-tumor tissue.

The observation that the chondroitin sulfates tend to be the exclusive uronic-acid-positive constituents in the urine of individuals is paradoxical in that those glycosaminoglycan fractions that are most elevated in angiosarcomatous tissue are those that are absent from the urine of individuals who may well have early, vinyl-chloride-induced liver injury. This pattern may be due to the selective action of lysosomal, glycolytic enzymes in the liver and/or may reflect the role of the chondroitin sulfates in early fibrotic changes in the liver. Certainly the potential usefulness of this pattern in early detection warrants the more complete evaluation now ongoing in our laboratory.

V. SUMMARY

Glycosaminoglycans were measured in urine and tissue of patients with hepatic fibrosis and hepatic cancer including vinyl-chloride-exposure-associated liver injury and angiosarcoma. Angiosarcoma, hepatitis, cirrhosis, and liver metastatic patients exhibited significantly elevated glycosaminoglycan excretion. Angiosarcoma tissue exhibited elevated glycosaminoglycan levels with the greatest increases in the heparin fraction. Histochemically, angiosarcomatous tissue gave a strong alcian-blue staining reaction which could be prevented by pretreatment with hyaluronidase. Although vinyl-chloride-exposure-associated liver injury other than angiosarcoma was not accompanied by a significantly elevated glycosaminoglycan excretion, this condition tended to be associated with a urinary glycosaminoglycan excretion pattern in which the chondroitin sulfate fraction was the only uronic-acid-positive fraction.

TABLE I. Urinary Glycosaminoglycan Levels in µg Uronic Acid per mg Creatinine by Liver Diseases Category

Patient group	Cases	µg uronic acid per mg creatinine (± 1 S.E.)	% uronic acid not dialyzable (± 1 S.E.)
normal control	9	3.2 ± .4	65 ± 5
vinyl chloride exposed	9	4.1 ± .4	39 ± 6
other cancer	6	4.5 ± 1.5	39 ± 6
other liver disease	4	5.1 ± 0.8	37 ± 13
angiosarcoma	3	7.6 ± 1.6	41 ± 22
hepatitis	8	8.5 ± 1.8	52 ± 14
cirrhosis	6	12.7 ± 3	53 ± 9
liver metastasis	2	13.8 ± .9	42

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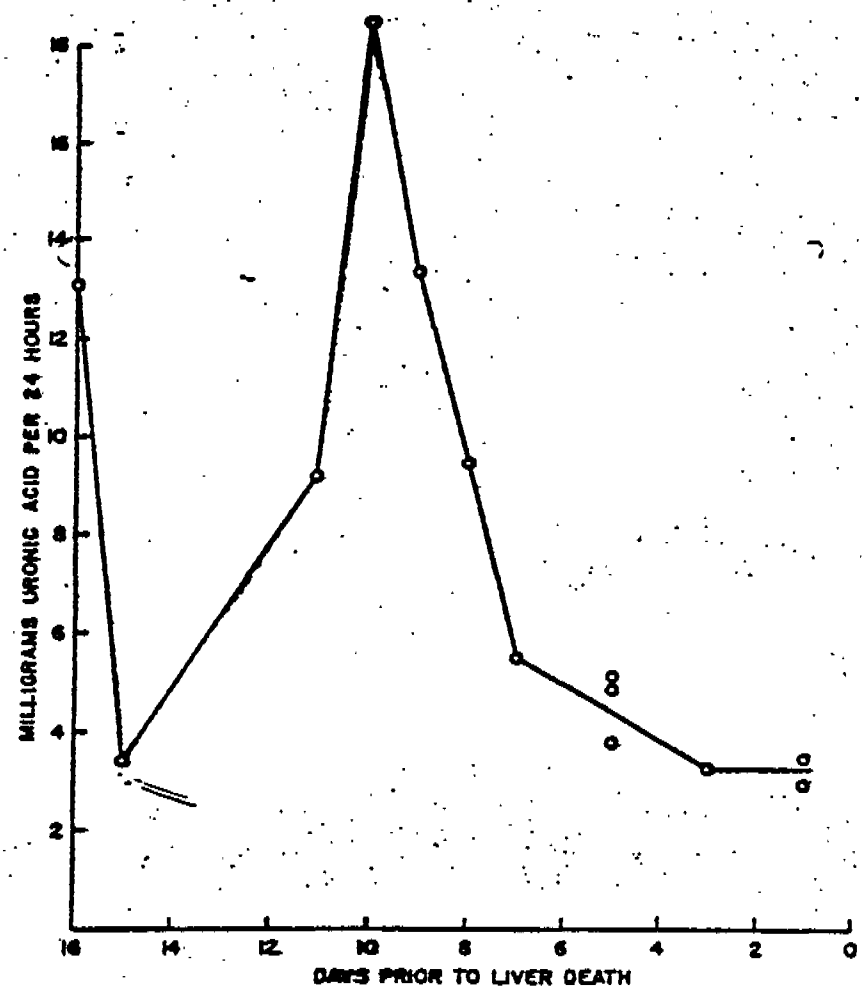

FIG. 1. Urinary glycosaminoglycan output in one angiosarcoma patient during the 16-day period prior to death.

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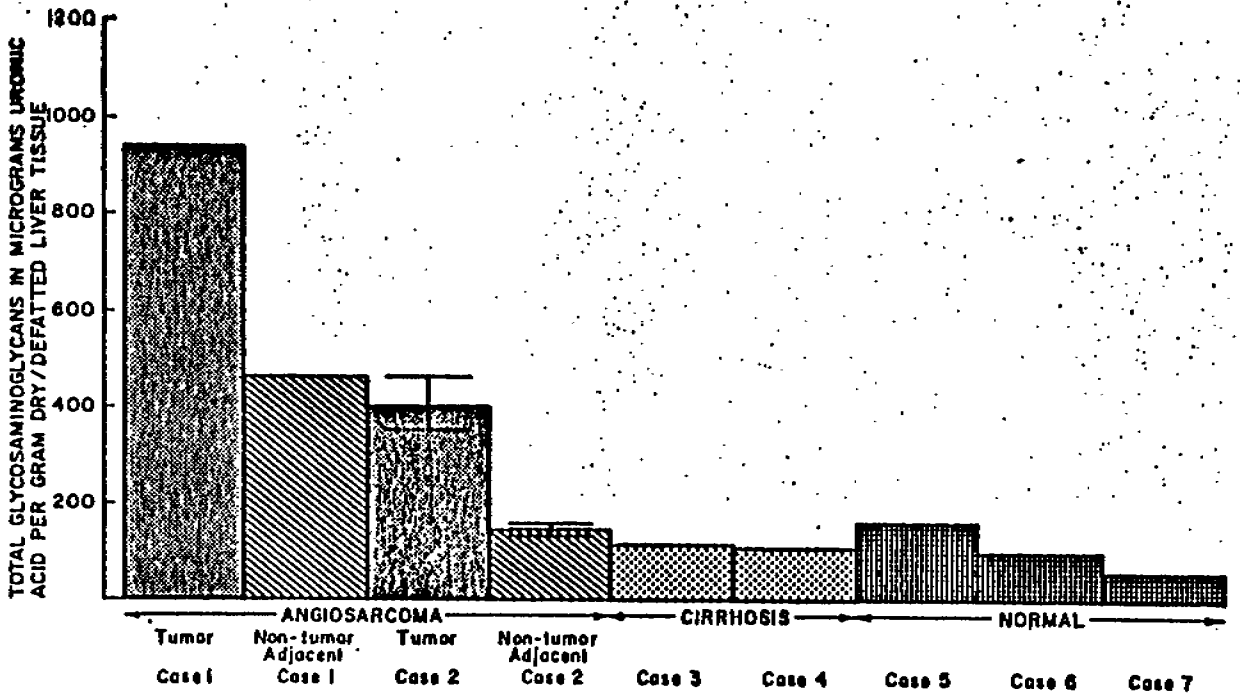

FIG. 2. Glycosaminoglycan concentration in angiosarcomatous, cirrhotic and normal human liver tissue.

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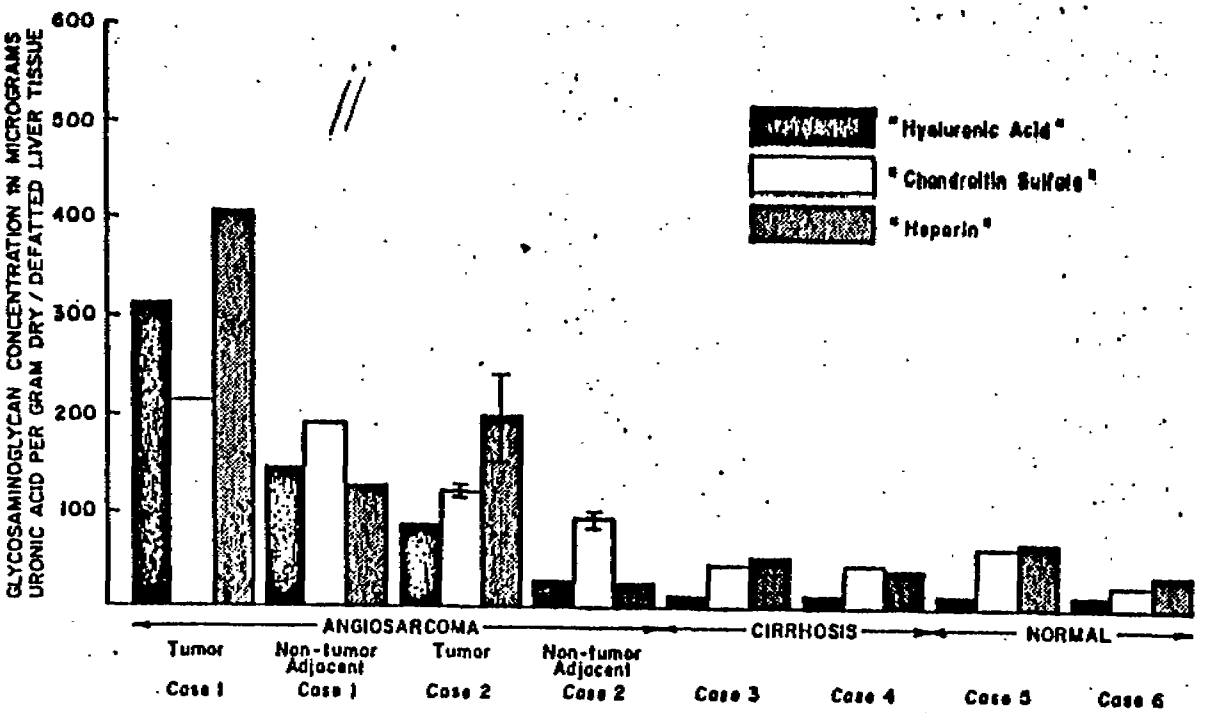

FIG. 3. Fractional concentrations of glycosaminoglycans in angiosarcomatous, cirrhotic and normal human liver tissue.

VI. REFERENCES

1. Creech, J. L. and Johnson, M. N. Angiosarcoma of the Liver in the Manufacture of Polyvinyl Chloride. J. Occup. Med. 16: 150-151, 1974.

2. Falk, H., Creech, J. L., Heath, D. W., Johnson, M. N., and Key, M. M. Hepatic Disease Among Workers at a Vinyl Chloride Polymerization Plant. JAMA 230: 59-63, 1974.

3. Makk, L., Creech, J. L., Whelan, J. G., and Johnson, M. N. Liver Damage and Angiosarcoma in Vinyl Chloride Workers: A Systematic Detection Program. JAMA 230: 64-68, 1974.

4. Creech, J. L., Makk, L., Whelan, J. and Tamburro, C. H. Hepatotoxicity Among Polyvinyl Chloride Production Workers During First Year of Surveillance Program. Gastroenterology 67: 786, 1974.

5. Kohn, J. and Weaver, P. C. Serum Alpha Fetoprotein in Hepatocellular Carcinoma. Lancet 2: 334-336, 1974.

6. Tamburro, C. H., Makk, L. and Creech, J. L. Unpublished observation.

7. Girard, D., Johnston, W. C., and Grahm, J. H. Cutaneous Angiosarcoma. Cancer 25: 868-883, 1970.

8. Barr, R. and Bonin, M. "Letters." JAMA 231(9): 914, 1975.

9. Popper, H., and Thomas, L. B. Alterations of Liver and Spleen Among Workers Exposed to Vinyl Chloride. Ann. NY Acad. Sci. 246: 172-194, 1975.

10. Galambos, J. T., and Shapira, R. Natural History of Hepatitis: IV Glycosaminoglycuronans and Collagen in the Hepatic Connective Tissue. J. Clin. Invest. 52(11): 2952-2962, 1973.

11. Koizumi, T., Nakamura, N., and Abe, H. Changes in Acid Mucopolysaccharide in the Liver in Hepatic Fibrosis. Biochim. Biophys. Acta. 148: 749-756, 1967.

12. Kojima, J. Studies on the Metabolism of Hepatic Connective Tissue in Fibrosis of the Liver. Med. J. Osaka Univ. 16: 419-429, 1964.

13. Rubin, E. Autoradiographic Characterization of Sulfated Acid Mucopolysaccharides in Experimental Cirrhosis. J. Histochem. Cytochem. 14: 688-689, 1966.

14. Patrick. R. S. and Kennedy, J. S. The Synthesis of Sulfated Mucopolysaccharide at Sites of Hepatic Fibrosis Induced by Carbon Tetrachloride, Amyloidosis, and the Implantation of Catgut. J. Pathol. Bacteriol. 88: 549-555, 1964.

15. Kojima, J., Nakamura, N., Kanatani, M. and Ohmori, K. The Glycosaminoglycans in Human Hepatic Cancer. Cancer Res. 35(3): 542-547, 1975.

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16. Anghileri, L. J. Metabolism of Acid Mucopolysaccharides in Hepatoma and in Normal Liver. Oncology 30: 304-317, 1974.

17. Yamamoto, K., and Teryama, H. Comparison of Cell Coat Acid Mucopolysaccharides of Normal Liver and Various Ascites Hepatoma Cells. Cancer Res. 33: 2257-2264, 1973.

18. DiFerrante, N. M. The Measurement of Urinary Mucopolysaccharides. Anal. Biochem. 21: 98-106, 1967.

19. Bitter, T., and Muir, H. A Modified Uronic Acid Carbazole Reaction. Anal. Biochem. 4: 330-334, 1962.

20. Schiller, S., Slover, G. A., and Dorfman, A. A Method for the Separation of Acid Mucopolysaccharides: Its Application to the Isolation of Heparin from the Skin of Rats. J. Biol. Chem. 236(4): 983-987, 1961.

21. Kupchella, C., and Steggerda, F. The Distribution of Acid Mucopolysaccharides in the Canine Gastrointestinal Mucosa. Trans. NY Acad. Sci. 34: 351-360, 1971.

22. Mowry, R. W. Alcian Blue Techniques for the Histochemical Study of Acidic Carbohydrates. J. Histochem. and Cytochem. 4: 407, 1956.

23. Sunderman, F. W. and Boerner, F. Normal Values in Clinical Medicine. W. B. Saunders. Philadelphia, p. 353, 1949.

24. Varma, R. S., Varma, R., Allen, W. S., and Wardi, A. H. Urinary Excretion of Acid Mucopolysaccharides in Schizophrenia. Biochem. Med. 11(4): 358-369, 1974.

25. DiFerrante, N. and Rich, C. The Determination of Acid Aminopolysaccharide in Urine. J. Lab. Clin. Med. 48: 491-494, 1956.

26. Kao, K. and Leslie J. Micro Fractionation and Determination of Urinary Glycosaminoglycans. Biochem. Med. 9(4): 317-326, 1974.

27. Goldberg, J. and Cotlier, E. Specific Isolation and Analysis of Mucopolysaccharides (Glycosaminoglycans) from Human Urine. Clin. Chim. Acta. 41: 19-27, 1972.

28. Manley, G., Severn, M. and Hawksworth, J. Excretion Patterns of Glycosaminoglycans and Glycoproteins in Normal Human Urine. J. Clin. Pathol. 21: 339-345, 1968.

29. Pennock, C. A. A Modified Screening Test for Glycosaminoglycan Excretion. J. Clin. Path. 22: 310, 1969.

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30. Douglas, C., Nowak, J. and Danes, B. Mucopolysaccharides in Urine During Normal Human Development. Pediatr. Res. 7: 724-727, 1973.

31. Kuroda, J., Saito, S., Seno, N., Nagase, S., and Anno, K. Isolation and Chemical Characterization of Mucopolysaccharides from Rat Tumors. Cancer Res. 34(2): 308-312, 1974.