STUDIES ON THE BIOCHEMISTRY OF CONNECTIVE TISSUE

E. Mead Johnson Award Address

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University of Chicago

Introductory Remarks: "Dr. Dorfman, who is just barely past the 40-year mark, did his undergraduate work at the University of Chicago, and in 1939 received his Ph.D. in biochemistry. For the next 3 years Dr. Dorfman was a Research Associate and an Instructor in Biochemistry at his Alma Mater. It was during this period that he worked with Dr. Felix Saunders of the Department of Biochemistry and Dr. Stewart A. Koser of the Department of Bacteriology on bacterial metabolism and growth factors. In 1943, Dr. Dorfman became a student again and in 1944 received the M.D. degree. Following his graduation in medicine, he served an internship at Beth Israel Hospital in Boston and a pediatric residency at the University of Chicago.

"From 1946 to 1948 there was a tour of duty in the Army; practically the entire time was spent as Chief of Biochemistry at the Army Medical Department Research and Graduate School in Washington, D.C.

"Since March 1948, Dr. Dorfman has been connected with the Pediatric Department at the University of Chicago as Assistant Professor, Associate Professor and Professor. He has also served as Director of Research at La Rabida Jackson Park Sanitarium and more recently as Director of the La Rabida-University of Chicago Institute.

"Albert Dorfman has become well known for his studies on the physiology of connective tissue, and it is for his work in this field that he has been selected to receive the E. Mead Johnson Award of the American Academy of Pediatrics."

IT IS with profound thanks both to the Academy of Pediatrics and to Mead Johnson and Company that I accept this generous award. Although persistence in research can only be sustained by inner pleasure and conviction, all investigators are sufficiently insecure in this conviction as to receive immeasurable satisfaction and stimulus from a recognition such as this award represents.

My own interest in the biochemistry of connective tissues spans a period of 11 years. During this time it has become increasingly obvious that a satisfying approach to the many ramifications of this biologic and medical problem could only be made by a joint effort. The complexity of methods and background no longer permits a professional approach to problems of biology and medicine by a single individual. The investigator working alone must either severely limit his approach or risk a wasteful amateurish performance. If one wishes the pleasure and satisfaction of a broader view of a biologic problem, cooperative effort is mandatory. I have permitted myself this brief detour in order to emphasize the contributions of my colleagues. Although many individuals have been associated with this work, and I will refer to their contributions in the appropriate portions of this discussion, I would like to pay particular tribute to J. Anthony Cifonelli, Martin B. Mathews, Saul Roseman, and Sara Schiller, who have at various times been senior members of our research group. Their intelligence, imagination and devotion to investigation have been responsible for much of the progress that has been made.

The role of connective tissues as a sup-
The ground substance has been long recognized but it is becoming increasingly clear that these tissues have other physiologic functions. Diversity of function is paralleled by physical and chemical differentiation. Despite heterogeneity, however, all connective tissues have similarities of structure and origin. The extracellular portions are composed of fibers imbedded in an amorphous ground substance. Both the fibers and certain other components appear to originate from metabolic activities of fibroblasts.

The fibers of connective tissue are probably of two chemical types, collagenous and elastic. While considerable progress has been made toward understanding both the structure and metabolism of collagenous fibers, only limited information is available concerning the origin and nature of elastic fibers. Specific characteristics of individual connective tissues are determined by the relative proportions of fibers and ground substance as well as the chemical nature and physical properties of both the fibers and ground substance components. The amorphous ground substance of connective tissues is a complex mixture, the composition of which varies. In certain locations special characteristics are obvious. The matrix of cartilage appears to be composed primarily of a high concentration of a complex of chondroitinsulfuric acid-A and a protein, while synovial fluid contains a hyaluronic acid-protein complex. In addition to such specialized connective tissues, ground substance contains important protein components, among which are soluble precursors of fibers. Many reports have appeared on the character of collagen precursors, but few studies pertinent to the precursors of elastin are available. Specific proteins found in firm linkage with acid mucopolysaccharides are also present. The work of Shatton and Schubert and Matthews has demonstrated that cartilage contains macromolecular complexes composed of chondroitinsulfuric acid and a noncollagenous protein.

In recent years an increasing investigative effort has been applied to the study of the acid mucopolysaccharides. These compounds are linear polyelectrolytes of high molecular weight. They are composed of repeating disaccharide units which contain an acetylated hexosamine and a uronic acid. In some cases a sulfate group is present, probably on the hexosamine moiety. One known compound, keratosulfate, has a hexose substituted for the uronic acid; another compound, heparin, has no N-acetyl group, but instead a sulfamic acid linkage.

Figure 1 illustrates the repeating disaccharide unit of chondroitinsulfuric acid-A as established by Davidson and Meyer. The compound consists of N-acetylglactosamine, glucuronic acid and sulfate combined in equimolar proportions. The sulfate is arbitrarily placed. The glycoside linkages
are alternately 1,3 and 1,4. This compound is the principal component of cartilage ground substance and has been termed chondroitinsulfuric acid-A. The work of Mathews in our laboratory, suggests that it is a linear compound, each chain having a molecular weight of approximately 50,000. In cartilage these chains appear bound to a protein core by covalent linkages resulting in a macromolecular complex with a minimum molecular weight of 4,000,000. Even a cursory inspection of the structure of this compound immediately suggests physicochemical properties of considerable interest. The presence of both carboxyl and sulfate groups on each repeating unit confers a high negative charge. Such a highly charged polyelectrolyte, linear in configuration, interacts with water and cations such as Na⁺.

In considering the ionic composition of extracellular fluid, due consideration must be given to the electrostatic effects of the negative charges of the acid mucopolysaccharides as well as proteins. The relative importance of this phenomenon in controlling hydration and electrolyte concentration will depend on the concentration of these substances exerting such effects. Unfortunately, adequate data are not yet available to assess quantitatively this question. It is clear that in a tissue such as cartilage the amount of chondroitinsulfuric acid is sufficient to "bind" substantial amounts of cations. An important possibility is that in certain locations such as in basement membranes the mucopolysaccharide concentration may be sufficient to result in concentration of cations.

While it is beyond the scope of this paper to pursue further the details of these concepts, it should be emphasized that these properties of acid mucopolysaccharides should be considered in problems concerned with exchange of electrolytes between circulation and extracellular fluid and between extracellular fluid and cells. More specific interactions such as their role in binding calcium for the initiation of calcification have been already suggested by various investigators.

The acid mucopolysaccharides have other physiologic activities. Some of these are detailed in Table I. This list is probably not complete, but is included only to indicate the diversity of physiologic and pathologic processes which may be influenced or mediated by this group of substances.

It seems likely that specific functions result from both the quantitative localization of polysaccharides as well as the specific chemical and physicochemical properties of the individual substances. There is increasing evidence of a multiplicity of acid mucopolysaccharides in mammalian tissues. Figure 2 lists the chemical composition of the known acid mucopolysaccharides. It is probable that this list is still incomplete. Although specific physiologic functions of these substances have been inadequately studied, it is clear that even closely related substances may exhibit markedly different properties. Studies with Grossman have shown that chondroitinsulfuric acid-B exhibits striking antithrombic properties in contrast to chondroitinsulfuric acid-A which has no effect on the coagulation system.

These preliminary considerations have suggested that a detailed study of the chem-

| TABLE I |

**Possible Role of Acid Mucopolysaccharides in Physiologic and Pathologic Processes**

- Control of electrolytes and water in extracellular fluid.
- Calcification.
- Wound healing.
- Resistance to infection.
- Lubrication.
- Blood coagulation.
- Clearing activity.
- Maintenance of stable transparent medium (eye).
istry and metabolism of acid mucopolysaccharides might be a fruitful approach to many biologic and medical problems. It would be neither possible nor appropriate to review all of the investigations carried out along these lines. I have chosen to present briefly the results of two lines of investigations which pertain more specifically to problems of pediatric interest.

The first of these is concerned with the influence of diabetes mellitus on acid mucopolysaccharide metabolism. This has been carried out as part of a program concerned with both the enzymatic mechanisms involved in the biosynthesis of acid mucopolysaccharides and the factors influencing their metabolism in mammals.

In earlier work carried out with Roseman, Cifonelli, Ludowieg, Moses and Mayeda, methods were devised for the study of the biosynthesis of hyaluronic acid using isotopically-labeled precursors. It was shown that the glucosamine and glucuronic acid portions of hyaluronic acid formed by a strain of Group A streptococcus were derived from glucose and that acetate serves as a precursor of the acetyl group.9 This knowledge facilitated a study of the rates of metabolism of acid mucopolysaccharides in mammalian skin. Experiments carried out with Schiller established for the first time normal rates of metabolism of both hyaluronic acid and the sulfated mucopolysaccharide of skin of normal rats and rabbits.10-12 The results disclosed that, contrary to older conceptions regarding the stability of connective tissues, the acid mucopolysaccharides turn over rapidly. The half-life time for hyaluronic acid was found to vary between 2.4 and 4.5 days while that of the chondroitin sulfonic acid fraction was found to vary between 7.6 and 10.7 days. The more rapid turnover of the hyaluronic acid was of considerable interest and emphasized the specificity of properties of different components of ground substance.

These experiments, which established the parameters of normal metabolism, have permitted the study of possible deviations from normal under specific experimental conditions.

The importance of investigating the effect of insulin on mucopolysaccharide metabolism was suggested by observations on infants of diabetic mothers. The presence of a nonpitting edema in such infants has been long recognized. That this edema might be related to the polysaccharides of the dermis was suggested by the observation that the intercutaneous injection of hyaluronidase resulted in collapse of the turgid skin. It seemed possible that the infant of the

<table>
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<th>AMINO SUGAR</th>
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<th>SULFATE</th>
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<td>CHONDROITIN SULFURIC ACID B (#-HEPARIN)</td>
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<td>GLUCURONIC ACID</td>
</tr>
<tr>
<td>HEPARIN</td>
<td>GLUCOSAMINE (N-SULFATED)</td>
<td>GLUCURONIC ACID</td>
</tr>
<tr>
<td>HEPARIN MONOSULFURIC ACID</td>
<td>GLUCOSAMINE</td>
<td>(?) +</td>
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</table>

Fig. 2. The composition of acid mucopolysaccharides of connective tissue.
The involvement of insulin in the metabolism of acid mucopolysaccharides was further indicated by the finding that both the glucosamine and uronic acid portions of the molecule derive from glucose. Since insulin is concerned with the utilization of glucose and since the early steps of mucopolysaccharide biosynthesis and other pathways for the utilization of glucose are probably identical, it seemed reasonable to postulate a role for insulin in the biosynthesis of these compounds. Experimental study of this phenomenon in the human was not feasible, but those ideas could be readily tested in diabetic animals.

Diabetes was induced in rats by a single subcutaneous injection of alloxan monohydrate 150 mg/kg of body weight. Animals that did not lose weight were eliminated. Three weeks after the administration of the alloxan, concentrations of glucose in the blood of rats, selected at random, ranged from 410 to 592 mg/100 ml. In treated animals, insulin was injected subcutaneously in daily doses of 20 or 40 units/kg of body weight. This dose was varied because continued administration of the higher dose resulted in evidence of hypoglycemia in some animals. During insulin treatment, concentrations of glucose ranged between 25 and 45 mg/100 ml of blood.

Sixty rats were divided into three experimental groups of equal size. One group of animals was used 3 weeks after the administration of alloxan. A second group served as untreated controls, while a third group of normal animals was maintained with half the average daily food intake for 3 weeks prior to, and during, the experiment. The weight loss of the latter group was similar to that of the diabetic animals. Each of the 60 rats was injected once subcutaneously with 80 μc of acetate-1-C\textsuperscript{14} and 2.7 μc of Na\textsubscript{2}S\textsuperscript{35}O\textsubscript{4}, as an isotonic mixture. Ten rats in each group were killed 1 and 5 days after the injection.

Figure 3 presents the isotope concentration of the mucopolysaccharides isolated from the skin of these groups of animals. The diabetic animals show a striking decrease in the uptake of C\textsuperscript{14} by the hyaluronic acid and of C\textsuperscript{14} and S\textsuperscript{35} by the chondroitinsulfuric acid fraction. No such decrease was evident in the fasted animals. Since the weight loss by the animals on restricted food intake duplicated that lost by the diabetic rats, weight loss per se appears to have no influence on the uptake of C\textsuperscript{14} and S\textsuperscript{35} by the mucopolysaccharides of skin. The rate of disappearance of isotopes from both polysaccharides is also illustrated in Figure 3. The C\textsuperscript{14} concentration of the polysaccharides of the diabetic rats on the fifth day has been omitted since the radioactivity of this sample was too low for accurate counting. While half-life times calculated from two points are not accurate, the values serve to indicate gross changes in turnover. The apparent half-life times of 2.6 days for hyaluronic acid and 11.0 and 10.8 days for chondroitinsulfuric acid (based on the concentrations of C\textsuperscript{14} and S\textsuperscript{35}, respectively) found in the skin of normal animals, agree with those obtained previously from more detailed decay curves.\textsuperscript{11,17} In the diabetic animal, however, the turnover is considerably slower as evidenced by an apparent half-life of 4.5 days for hyaluronic acid and 20.9 days for chondroitinsulfuric acid (based on concentration of S\textsuperscript{35}).

The use of acetate-1-C\textsuperscript{14} effects specific labeling of the N-acetyl component of the mucopolysaccharide molecule.\textsuperscript{11} Since acetate utilization is decreased in the diabetic animal,\textsuperscript{15,14} it may be argued that the observed results mirror alterations in acetate metabolism. Although the similarity of the C\textsuperscript{14} and S\textsuperscript{35} data would appear to invalidate this objection, another experiment was undertaken utilizing glucose-U-C\textsuperscript{14} as well as Na\textsubscript{2}S\textsuperscript{35}O\textsubscript{4}. In the same experiment the effect of insulin on both normal and diabetic rats was studied. Four experimental groups were used. At appropriate times, as designated below, each animal received a single subcutaneous injection of an isotonic mixture containing 6.7 μc of glucose U-C\textsuperscript{14}
and 13.3 μc of Na₂S³⁵O₇. Two groups were made diabetic as described above. Three weeks later one-half of the diabetic animals were injected with the radioactive mixture. The remainder were treated daily with 20 or 40 units of insulin per kilogram of body weight for 1 week before the administration of the radioactive material and daily thereafter until killed. The two other groups of animals consisted of nondiabetic rats. One group served as a normal control while the other was injected daily with 20 or 40 units of insulin per kilogram of body weight, before and after receiving the isotopes.

Eight to ten rats in each group were sacrificed at intervals of 1, 5 and 17 days after the administration of radioactive material and the hyaluronic acid and chondroitinsulfuric acid fractions were isolated from the respective pools of skin.

Figure 4 indicates a marked decrease in incorporation of isotope in both the hyaluronic acid and chondroitinsulfuric acid in diabetic as compared with normal rats. This finding is entirely in accord with the results obtained when acetate-1-C¹⁴ was employed as a precursor. Calculation of half-life times for hyaluronic acid again showed some prolongation in diabetic animals (5.0 days compared with 3.8 days for normal) although these differences were not as striking as those observed for chondroitinsulfuric acid or those obtained in the previous experiment.

The data concerning chondroitinsulfuric acid demonstrate, as in the previous experiment, a marked inhibition of isotope uptake in diabetic animals, although the differences in decay (half-life times) are not as evident (Fig. 5).

The administration of insulin to diabetic animals restores the defect of uptake toward normal as illustrated by the data for C¹⁴ in hyaluronic acid (Fig. 4) and for both C¹⁴ and S³⁵ in chondroitinsulfuric acid (Figs. 4 and 5). The half-life times were actually shorter than normal in this group (HA-C¹⁴, 1.9 days; CSA-C¹⁴, 3.9 days, and CSA-S³⁵, 6.3 days). This is not surprising since the animals were hypoglycemic. The effect of insulin in normal rats was somewhat variable and not as striking as in the diabetic animals, probably because the dia-

![Figure 3](image-url)
abetic animals are more sensitive to insulin.

The results of these experiments indicated a decreased capacity to metabolize acid mucopolysaccharides in diabetic animals. A fall in concentration of these substances might therefore be anticipated. Since methods for isolating the mucopolysaccharides are not quantitative, attempts were made to estimate possible changes in mucopolysaccharide concentrations by utilizing the method of isotope dilution. The results, indicated in Table II, demonstrate a marked decrease in concentration of hyaluronic acid and a less striking decrease in concentration of chondroitinsulfuric acid. These determinations of pool size permitted the calculation of the turnover rates presented in Table II. The difference between diabetic and normal animals is evident.

The sizes of the hyaluronic acid and chondroitinsulfuric acid pools in the skin of the fasted animals were not measured so that turnover rates comparable to those calculated for the normal and diabetic rats could not be obtained.

These experiments clearly demonstrated that the metabolism of acid mucopolysaccharides is impaired in the diabetic animals and is restored toward normal by the action of insulin. These findings may find importance in the explanation of the delayed wound healing, susceptibility to infection and accelerated vascular degeneration in diabetes mellitus. Their relationship to the pathologic findings in infants born to diabetic mothers requires further elucidation.

I should like to turn now to a different

### TABLE II

<table>
<thead>
<tr>
<th>Pool Size*</th>
<th>Turnover Rate†</th>
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<tr>
<td>(mg/100 gm)</td>
<td>(mg/100 gm/day)</td>
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<tr>
<td><strong>Hyaluronic acid</strong></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>215</td>
</tr>
<tr>
<td>Diabetic</td>
<td>88</td>
</tr>
<tr>
<td><strong>Chondroitinsulfuric acid</strong></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>187</td>
</tr>
<tr>
<td>Diabetic</td>
<td>145</td>
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</table>

* Pool size as determined by isotope dilution method and expressed as mg/100 gm of acetone-defatted skin.

† Turnover rate = $\frac{100 \times 1.14}{t_1}$. 

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Fig. 4. A comparison of the $^{14}C$ concentration of hyaluronic acid and of chondroitinsulfuric acid of the various groups 1 (left bar) and 5 (right bar) days after the administration of glucose-$^1C^4$ and Na$_2$S$_2$O$_4$. 

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kind of study of acid mucopolysaccharides. The Hurler syndrome or gargoylism (lipochondrodystrophy, dysostosis multiplex) was first definitively described by Hunter in 1917. His report was concerned with two brothers, aged 8 and 10 years, who were dwarfed. They were deaf and had widely spaced teeth, short necks and protuberant abdomens with hepatosplenomegaly. In addition, they had inguinal hernias, short, broad and thick hands and flexion deformities. One of the patients had a cardiac murmur. These patients had no mental retardation or corneal opacity. A subsequent report by Hurler in 1919 presented two male patients who, in addition to the characteristics described, demonstrated mental retardation and corneal opacities. A considerable number of cases of this syndrome have been described; this literature has been recently admirably reviewed by McKusick. The presence of large vacuolated cells in many tissues suggested that the Hurler syndrome should be classified as a storage disease. Whereas earlier investigators considered the material to be of a lipid nature, Lindsay and co-workers thought the material to be a glycoprotein while Dawson suggested it was a complex polysaccharide. The first real progress in identifying the storage material came from the work of Brante who isolated, from tissues (liver and meninges) obtained at necropsy, a substance which he considered to be an acid mucopolysaccharide. The analyses for sulfate, hexosamine and uronic acid indicated that the material was similar to or identical with that of chondroitinsulfuric acid. He suggested that the Hurler syndrome might represent an inborn error of acid mucopolysaccharide metabolism. Somewhat later, Uzman also isolated from tissues material thought to be polysaccharide in nature although poorly characterized.

On the basis of our own interest in the metabolism of acid mucopolysaccharides, this syndrome seemed profitable for investi-

![Fig. 5. A comparison of the S concentration of chondroitinsulfuric acid of the various groups after administration of glucose-U-C and NaS-OSO. The bars in each group, reading from left to right, represent values 1, 5, and 17 days after the injection of the isotopes.](image)
Migration. The studies have been carried out in collaboration with Lorincz. Current knowledge regarding the origin of the acid mucopolysaccharides suggests formation in connective tissues, probably primarily by fibroblasts. Since the abnormal materials are present in a wide variety of cell types in the Hurler syndrome, it seemed possible that they were formed in connective tissue cells and then carried to other organs. If this were true, then an excess of mucopolysaccharides might be expected in the blood and urine of patients afflicted with the disorder. Since no necropsy material was available, an investigation was undertaken of mucopolysaccharides in the urine of a patient with the Hurler syndrome. This patient is a 6-year-old Negro girl who was referred to Bobs Roberts Hospital by Dr. Eugene Diamond. She has the grotesque skeletal and facial deformities, hepatosplenomegaly and corneal clouding characteristic of this syndrome.

After dialysis against cold running tap water, 48-hour urine samples containing thymol were concentrated in vacuo at 50°C to one-tenth the original volume. Sodium hydroxide was added to make a 2% solution and, after 24 hours at room temperature, dialysis against cold running tap water was repeated. Ten milligrams of once crystallized trypsin was added and the mixture dialyzed against phosphate buffer (0.1 M, pH 8.0) at 38°C, with external stirring for 4 to 5 days. Protein was precipitated by the addition of one-half the volume of 40% trichloroacetic acid and was removed by centrifugation after standing at 4°C for 1 hour. After dialysis against distilled water, the solution was concentrated to approximately one-tenth the previous volume, and the “crude mucopolysaccharide” fraction was precipitated by the addition of 4 volumes of 95% ethanol in the presence of 2% sodium acetate. The precipitate which formed after 48 hours at 4°C was collected by centrifugation, washed with absolute ethanol and ether, and dried in vacuo in a

![Graph](Fig. 6. Pattern of distribution of mucopolysaccharides after separation on a Celite slab in phosphate buffer, pH 7.0; 250 volts D.C.; 100 milliamperes.)
These "crude mucopolysaccharide" fractions were purified by slab electrophoresis as described by Schiller et al.10 One hundred fifty to two hundred milligrams of "crude mucopolysaccharide" per 48-hour sample were isolated from the urine of this patient, as compared with 20 to 50 mg/48-hour sample obtained from the urine of normal individuals of comparable age and sex. The "crude mucopolysaccharide" obtained from normals, when subjected to slab electrophoresis, yielded insufficient material for further identification. Recently, DiFerrante and Rich27 isolated from 23 liters of pooled normal human urine 140 mg of an acid mucopolysaccharide which was indistinguishable from chondroitin-sulfuric acid-A.

Purification of this crude material by slab electrophoresis resulted in the demonstration of two different mucopolysaccharides. The electrophoretic pattern is illustrated in Figure 6. It will be noted that the ratio of hexosamine to uronic acid (as obtained by the carbazole method) is higher in the more rapidly migrating fractions than in the more slowly migrating peak. This low carbazole value has been previously found to be characteristic of chondroitinsulfuric acid-B.

Figure 7 presents the analytic data for this fraction and similar fractions obtained from the urines of two other patients. They are compared with theoretic analyses for chondroitinsulfuric acid-B. Other properties which serve to identify this polysaccharide are shown in Figure 8.

The finding of this polysaccharide was

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**Table 1: Analyses of the mucopolysaccharides isolated from the urines of three patients with Hurler syndrome.**

<table>
<thead>
<tr>
<th></th>
<th>HEXOSAMINE</th>
<th>N</th>
<th>URONIC ACID</th>
<th>N-ACETYL</th>
<th>SO4</th>
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<td>CSA-B (THEORETICAL)</td>
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<td>0.40</td>
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</tbody>
</table>

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**Figure 7.** Analyses of the mucopolysaccharides isolated from the urines of three patients with Hurler syndrome. The fraction referred to here is the one that migrates most rapidly on slab electrophoresis.

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**Figure 8.** Properties of the polysaccharide isolated from the urine of patients with the Hurler syndrome. These are the properties of the more rapidly migrating peak.
fortuitous since it came at a time when this particular polysaccharide was being investigated in our laboratory for other reasons. Chondroitinsulfuric acid-B had previously been shown to be present in skin, gastric mucosa, and beef lung. Recent investigations by Hoffman, Linder, and Meyer²⁸ and by Cifonelli, Ludowieg and Dorfman,²⁹ have shown that chondroitinsulfuric acid-B contains L-iduronic acid in place of the D-glucuronic acid present in chondroitinsulfuric acid-A and hyaluronic acid. L-iduronic acid is an isomer of D-glucuronic acid, differing only in the position of the hydroxyl group on carbon 5. Figure 9 illustrates the relationship between the two substances.

Recently, Grossman and Dorfman⁵ have shown that in contrast to chondroitinsulfuric acid-A, chondroitinsulfuric acid-B shows considerable antithrombic activity in the presence of plasma. Its potency, relative to heparin, varies with thrombin concentration. At low thrombin concentrations, it is more active than heparin, while at high thrombin concentrations it loses activity and actually antagonizes the antithrombic activity of plasma. With whole blood its anticoagulant activity is 5% that of heparin.

The chemical nature of the "slow peak" has not been clearly delineated. Analyses of this substance together with those of a fraction isolated from the liver of a patient succumbing to this disease, are presented in Figure 10. The finding of acid mucopolysaccharides in the urine made imperative the establishment of the relationship between the stored material and the excreted substances. Through the heroic efforts of Dr. Ira Rosenthal of the Department of Pediatrics, University of Illinois, we have recently obtained necropsy material from an 11-year-old girl severely affected by the disease. Somewhat surprising was the finding that material isolated from the liver appears similar in properties to the minor component found in urine of the patients we had studied previously. Other properties of this substance are shown in Figure 11. This substance appears similar in properties to a compound isolated from liver by Jorpes and Gardell³⁰ and named by them heparin monosulfuric acid. The identity of their material with ours has so far not been established.

The relationship between the two polysaccharides is not clearly evident. Preliminary study of the mucopolysaccharides isolated from the urine of an affected sibling of the patient from whom necropsy material was available, indicates that it is similar to that isolated from the liver. More recently, we have obtained urine as well as necropsy material from another typical patient. In contrast to the results described above, this patient appears to have chondroitinsulfuric acid-B in both liver and urine.

The isolation and identification of the mucopolysaccharides from the urine of a number of patients represents a tedious and time-consuming endeavor. For some purposes a simplified screening test seems desirable. Fortunately, a method previously developed¹¹ for the assay of hyaluronidase was found applicable to urine after only minor modification. This is based on the fact that acid mucopolysaccharides react with albumin (at a pH of 3.70) to produce turbidity. Under appropriately controlled conditions of pH, ionic strength, temperature and time, this method can be used for quantitative estimation of the concentration of acid mucopolysaccharides. Whereas sufficient studies have not yet been completed to validate this as a test for the Hurler syndrome, it has already been found to be of considerable value for screening purposes. The test is performed as follows:

The urine is centrifuged and dialyzed with external stirring against distilled water.
for at least 3 hours. One and one-half milliliters of urine (usually diluted to twice the original volume) is mixed with 0.5 ml of 0.3 M phosphate-citrate buffer, pH 5.6, containing 0.45 M NaCl. To this mixture is added 10 ml of the acid albumin reagent prepared as previously described. The resultant turbidity can be quantitatively estimated after 10 minutes by reading in a photoelectric colorimeter. This method can be used for the quantitative estimation of excreted acid mucopolysaccharides. Insufficient data are yet available to recommend this as a diagnostic test. Preliminary studies indicate that the two polysaccharides so far discovered in the urine of patients with the Hurler syndrome behave differently in this test.

The urines of 15 additional patients with Hurler syndrome have been examined and in every case except one have been found positive. The single exception is a mildly affected individual belonging in the famous family described by Beebe and Formel. No positive tests have so far been obtained in normal urine or urines of a limited number of patients with other diseases.

A discussion of the nature of the metabolic anomaly in the Hurler syndrome would seem premature. Clinical and family studies have suggested that at least two types of the disease may exist. In some families, such as that described by Beebe and Formel, the disorder is limited to males. Such patients appeared to be less severely affected, frequently surviving to adulthood and lacking corneal opacities. In other families the disease appears in both sexes and results in corneal opacities, more severe neurologic involvement, with death in late childhood. Our experience to date indicates the excretion of mucopolysaccharides in both forms. A correlation between the clinical manifestations and the type and quantity of mucopolysaccharides is not yet possible.

The fundamental biochemical defect is not yet obvious. Whether the excretion or

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Fig. 10. Analyses of material isolated from the urine of a patient with the Hurler syndrome compared with polysaccharide isolated from liver. The urine fraction is the one which migrates more slowly on slab electrophoresis.

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<th>&quot;SLOW PEAK OF URINE&quot;</th>
<th>LIVER FRACTION</th>
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<tbody>
<tr>
<td>HEXOSAMINE</td>
<td>GLUCOSAMINE</td>
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<tr>
<td>ANTITHROMBIC ACTIVITY</td>
<td></td>
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<tr>
<td>EFFECT OF TESTICULAR HYALURONIDASE</td>
<td></td>
</tr>
<tr>
<td>$[\alpha]_D^{20}$</td>
<td>$+39^\circ$</td>
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Fig. 11. Properties of the more slowly migrating peak.
deposition of polysaccharides results from an increased formation, a decreased breakdown, or a faulty deposition of polysaccharides is not yet clear. Brante first suggested that Hurler syndrome should be considered a heritable disorder of mucopolysaccharide metabolism. Whereas this serves as a working hypothesis, the true nature of the defect awaits further elucidation.

In this presentation I have attempted to present progress reports regarding two aspects of mucopolysaccharide metabolism. Our studies were originally stimulated by a desire to understand better the fundamental mechanisms operative in rheumatic diseases. In the course of these investigations we have been led into unexpected pathways which we hope have cast at least a glimmer of light on other obscure problems.

SUMMARY

The nature and diversity of acid mucopolysaccharides of connective tissue have been discussed. It is probable that these substances play a variety of physiologic roles.

Insulin affects the rate of synthesis of acid mucopolysaccharides in skin. There is a striking decrease in concentration in the skin of diabetic animals. This is restored toward normal by the administration of insulin.

Acid mucopolysaccharides are excreted in the urine of patients with Hurler syndrome. At least two different polysaccharides are involved. The amount of each in the tissues and urine vary in different patients.

REFERENCES


RESPONSE OF THE ADRENAL CORTEX TO CORTICOTROPHIN FROM HUMAN INFANTILE PITUITARY GLANDS, J. H. Solem et al. (Lancet, 1:414, February 22, 1958.)

This is the preliminary report of investigations aimed at determining the cause for the low urinary excretion of 17-ketosteroids during the first 6 to 8 years of life. This could be due either to a lack of the appropriate trophic hormone in the pituitary in infancy or to an immaturity in the adrenal glands of infants. The authors prepared an extract from the pituitary glands of infants less than 2½ years of age obtained at necropsy. The preparation was tested in an adult. This subject had been shown to excrete up to 82 mg/day of 17-ketosteroids following the injection of ACTH gel. The extract from the pituitary glands of infants given in the same way caused an excretion of 17-ketosteroids up to 91.9 mg/day. This indicated that the extract from the pituitary glands of infants contained a corticotropic substance capable of causing normal stimulation of the adrenal glands of an adult. This suggests that the low urinary excretion of 17-ketosteroids in infancy and early childhood is due to a limitation in the capacity of the adrenal glands to elaborate 17-ketosteroids rather than to a lack of corticotropic hormone in the pituitaries of infants.
in the economic setting of the "shot and formula" epoch? The realization of the best in child care is governed by economics as surely as by education, even though pediatricians may be trying to give the best possible care without regard for compensation.

Unless some representative agency troubles to acquaint the public with the meaning of the new pediatrics, how can the pediatrician expect to practice it? One should not have to look far to discover an organization that could help the practicing pediatrician by preparing the public to appreciate the difference between comprehensive care and pill peddling.

The conscientious practicing pediatricians better consider their plight and insist upon help before some other enterprising group persuades the public that a little knowledge of child care can be applied by any general practitioner to meet their needs at the attractive price possible with mass production and quick turnover.

Should the problem be avoided because it has to do with "public relations," as though declaration of one's position was the exclusive privilege of hucksters and politicians? Even a statesman or a leader cannot exert due influence without public understanding. If the ideals being proclaimed as characterizing the goals of pediatricians are to shape the future care of children, they must become incorporated into the public consciousness.

The organization which seizes the opportunity to play the role of responsible statesmanship, through appropriate cultivation of public appreciation of the potentialities of the new pediatrics, will be sure to earn a loyal following and, more important, foster the welfare of the children of the future. Should this subject be relegated to a committee pondering on long-term policies or made the object of prompt effective action?

The Letters to the Editor section of PEDIATRICS is available to even the most humble who may be stimulated to offer thoughtful comment on some aspect of these provocative questions.

CHARLES D. MAY, M.D.

ERRATUM

PEDIATRICS, Volume 22, page 576, 1958: In Figures 3, 4, and 5 of the paper by Dorfman, "counts per million" should read "counts per minute."
STUDIES ON THE BIOCHEMISTRY OF CONNECTIVE TISSUE: E Mead Johnson
Award Address
Albert Dorfman
_Pediatrics_ 1958;22;576

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### Award Address

Albert Dorfman

*Pediatrics* 1958;22;576

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