The Hunter Corrective Factor

PURIFICATION AND PRELIMINARY CHARACTERIZATION

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SUMMARY

Fibroblasts from patients with the Hunter syndrome are deficient in a specific protein, designated "Hunter corrective factor," which is required for the degradation of sulfated mucopolysaccharide. This factor has now been purified 120-fold from normal human urine by (NH₄)₂SO₄ fractionation, gel chromatography on Sephadex G-200, passage through anti-albumin Sepharose to remove albumin, a major contaminant, and finally, preparative polyacrylamide gel electrophoresis. The last procedure separates two "iso-factors," which are probably charge isomers; both differ in charge from the Hunter factor derived from fibroblast secretions.

The molecular weight of urinary Hunter factor is estimated at 65,000 by polyacrylamide gel electrophoresis and 114,000 by gel filtration.

The most highly purified preparation of urinary Hunter factor shows a single protein component in polyacrylamide gel electrophoresis at pH 8, but can be resolved into several bands by isoelectric focusing in polyacrylamide gel. It is free of the common lysosomal glycosidases and sulfatases, as well as of factors effective in other mucopolysaccharidoses (Hurler, Scheie, Sanfilippo A and B, and Maroteaux-Lamy).

The Hunter corrective factor accelerates the degradation, by Hunter fibroblasts, of dermatan sulfate labeled in the sulfate or galactosamine moieties, as well as of exogenously added proteodermatan [³⁵S]sulfate. The effect of the factor persists in the recipient cells with a half-life of 2 days.

The Hunter syndrome (mucopolysaccharidosis II) is a genetic disorder of mucopolysaccharide metabolism, the only one of the mucopolysaccharidoses to be transmitted as an X-linked recessive (1). Its clinical manifestations (skeletal dysmorphism, hepatosplenomegaly, cardiovascular problems, and frequently though not always, mental retardation) result primarily from massive intralysosomal deposition of dermatan sulfate and heparan sulfate (2, 3). Fragments of these mucopolysaccharides are also excreted in urine in unusually large amounts.

Fibroblasts derived from the skin of Hunter patients perpetuate the metabolic error in tissue culture and accumulate excessive dermatan sulfate (4, 5). The accumulation and increased turnover time of dermatan sulfate can be ascribed to inadequate degradation (6).

The abnormal metabolism of sulfated mucopolysaccharide by Hunter fibroblasts can be corrected, i.e. changed toward normal, by adding to the culture medium a particular protein found in fibroblast secretions or in urine derived from individuals who do not have the Hunter syndrome (7, 8). Thus the defect in the Hunter syndrome can be equated with a deficiency of this protein, designated the Hunter corrective factor, which is required for mucopolysaccharide degradation.

This paper describes a procedure for the purification of the Hunter corrective factor from normal human urine, some investigations of its properties and function, and comparison with the Hunter corrective factor from fibroblast secretions (9). Similar studies have been reported for the analogous but distinct Hurler corrective factor (10) and Sanfilippo A corrective factor (11).

MATERIALS AND METHODS

Reagents—H₂[³⁵SO₄]₄ and D-[6-³H]glucosamine were obtained from New England Nuclear Corp.; Sephadex G-200 and Sepharose 4-B from Pharmacia; Agarose from Marine Colloids, Inc.; human serum albumin from Sigma Chemical Co.; DEAE-cellulose, Whatman microgranular DE-52 from H. Reeve Angel, Inc.; Bio-Gel P-2, from Bio-Rad; cyanogen bromide from Eastman; Ampholine carrier ampholytes, pH 3 to 6 (batch no. 29) from LKB Instruments; substrates for lysosomal enzymes from Sigma and Calbiochem, except for p-nitrophenyl-β-N-acetylglucosaminide, purchased from Cyclo Chemical Company. Goat antiserum against human serum albumin was a gift of Dr. J. Robbins, National Institute of Child Health and Human Development.

Cell Culture—Skin fibroblasts from normal individuals and from mucopolysaccharidosis patients were maintained in culture as previously described (6, 7).

Assay of Corrective Factor Activity—Addition of Hunter factor to the culture medium reduces intracellular accumulation of radioactive mucopolysaccharide in Hunter fibroblasts exposed to [³⁵SO₄]. Correction in Hunter fibroblasts follows the same kinetics as in Hurler (10) or Sanfilippo A (11) fibroblasts; the definition of a unit is analogous, one unit being the factor activity that gives
half-maximal correction in Hunter fibroblasts and is calculated as previously described (10).

Conditions for assay were less stringent than those specified for the Hunter factor (10). Several fibroblast lines from well-diagnosed Hunter patients were used, from 1 to 5 days after subculturing, at cell densities ranging from 0.5 to 1.0 mg of cell protein per 100-mm Falcon Petri plate (denser or older cultures were less sensitive to correction). However, for any one experiment one single subculture was used; where the experiment required activity determinations at different times, the samples were stored frozen and assayed in one batch.

Preparation of Anti-albumin Sepharose—The immunoglobulin G fraction of 124 ml of goat antiserum against human serum albumin was prepared by precipitation with 50% saturated ammonium sulfate and DEAE-cellulose chromatography (12), yielding 3.1 g of purified IgG. The amount of specific antibody was found to be 795 mg by quantitative immune precipitation (13). The IgG fraction, 140 ml, containing 35 mg of protein and 5.4 mg of specific antibody per ml, was dialyzed against 0.2 M sodium citrate buffer, pH 6.0, and coupled to 140 ml of packed Sepharose 4-B by the method of Cuatrecasas (14). The Sepharose had been activated with 300 mg of cyanogen bromide per ml of packed gel; coupling was performed in 0.2 M sodium citrate buffer, pH 6.0. The product was washed with 20 liters of 0.1 M NaCl over a period of 10 hours; only 10% of the protein was found in the washes, indicating that about 90% had been coupled.

To test its antigen-binding capacity, the anti-human serum albumin Sepharose was equilibrated with 0.1 M sodium phosphate buffer, pH 7.0, and poured into a Pasteur pipette to give a bed volume of 2 ml. A solution of human serum albumin in the same buffer (2.28 mg, 0.5 ml) was passed through the small column at a rate of 0.05 ml per min; 2-ml fractions were collected with a Buchler refrigerated fraction collector at 0°C.

Results and Discussion

Purification of Hunter Factor

Step 1. Ammonium Sulfate Fractionation—Freshly collected morning urine from healthy men in the military (110 liters) was saturated to 70% by addition of 450 g of ammonium sulfate per liter, and the precipitate was collected as previously described (10). This and all subsequent steps were performed at 0°C.

The sticky precipitate was suspended in 500 ml of water, stirred on a magnetic stirrer for 1 hour, and the suspension centrifuged. One ml of the supernatant fluid was removed for assay, while the remainder, 630 ml, was further fractionated by the addition of 155 g of ammonium sulfate (giving about 50% saturation), the pH being held at 6.0 by periodic additions of 0.5 M NaH2PO4. After 1 hour, the precipitate was removed by centrifugation and 147 g of ammonium sulfate were added to 700 ml of the supernatant fluid (ca. 80% saturation), the pH again being maintained at 6.0. The precipitate was collected by centrifugation after 1 hour and redissolved in a minimal amount (112 ml) of 0.01 M sodium phosphate buffer, pH 6.0, in 0.15 M NaCl ("90 to 80% fraction"). Recovery in this and all subsequent steps is listed in Table I.

Step 2. Chromatography on Sephadex G-200—The 50 to 80% ammonium sulfate fraction was dialyzed overnight against two changes, 6 liters each, of 0.5 M NaCl in 0.01 M sodium phosphate, pH 6.0, and subjected to filtration on Sephadex G-200. As seen in Fig. 1, top, the Hunter factor is eluted in a peak located at the ascending limb of a large protein peak. The latter contains

1 J. Derge and E. F. Neufeld, unpublished experiments.
TABLE I  
Summary of purification

| Purification step | Total $A_{280}$ units | Total activity | Specific activity | Purification | Yield |
|-------------------|-----------------------|---------------|------------------|--------------|-------|
| 1. Ammonium sulfate |                        |               |                  |              |       |
| 0-70%             | 12,300                | 540,000       | 44               | 1            | 100   |
| 50-80%            | 3,510                 | 512,000       | 146              | 3            | 95    |
| 2. Sephadex G-200 chromatography |            |               |                  |              |       |
| Column 1          | 274                   | 293,000       | 1,440            | 33           | 73    |
| Column 2          | 166                   | 322,000       | 1,950            | 41           | 60    |
| 3. Anti-albumin Sepharose |            |               |                  |              |       |
| Pool 1            | 102                   | 290,000       | 2,550            | 58           | 48    |
| Pool 2            |                       |               |                  |              |       |
| Pool 4            |                       |               |                  |              |       |
| Pool 5            |                       |               |                  |              |       |

Fig. 1. Chromatography on Sephadex G-200. The column dimensions were 10 X 89 cm; void volume, 2160 ml; flow rate, 100 ml per hour; fraction volume, 20 ml. Column and sample had been previously equilibrated with the elution buffer, 0.01 M phosphate buffer, pH 6.0, in 0.5 M NaCl. Top, 50 to 80% ammonium sulfate fraction, 110 ml, $A_{280} = 32$; bottom, Fractions 180 to 210 of above pooled, concentrated to 62 ml, and re-applied to the same column.

predominantly albumin, as determined by electrophoresis and immunodiffusion.

The fractions with the highest specific activity, 180 to 210, were pooled and concentrated to a volume of 62 ml by ultrafiltration on a Diaflo PM-30 membrane in an Amicon 400 ultrafiltration cell (50 p.s.i. pressure from a nitrogen tank over a period of about 4 hours at 4°C). The concentrate was rechromatographed on Sephadex G-200 (Fig. 1, bottom); fractions with the highest specific activity, 170 to 210, were pooled and concentrated as above to 26 ml. The second gel filtration removed much but not all of the albumin.

**Step 3. Removal of Albumin on Anti-albumin Sepharose—** Attempts to remove residual albumin by conventional techniques failed because albumin accompanied Hunter factor in all procedures performed at pH 6.0 or higher (chromatography on DEAE-cellulose or hydroxylapatite, or electrophoresis in polyacrylamide gel), whereas Hunter factor activity could not be preserved in procedures requiring more acidic pH. Removal of albumin by affinity chromatography on anti-albumin Sepharose proved an adequate solution to this problem.

The concentrate from the second gel filtration column was dialyzed overnight against two changes, 500 ml each, of 0.1 M sodium phosphate, pH 7.0. It contained 54 mg of albumin, as estimated by passage of an aliquot over a small antibody-Sepharose column.

A preparative scale affinity column was made by packing 105 ml of settled anti-albumin Sepharose, with a binding capacity for 72 mg of albumin, into a column (2 X 33.5 cm) which was then equilibrated with 0.1 M sodium phosphate buffer, pH 7.0. The G-200 concentrate was passed through this column at room temperature, at 30 ml per hour, and fractions of 5.4 ml were collected. Fractions with an absorbance at 280 nm greater than 0.1 were pooled and concentrated by ultrafiltration, as above, to a volume of 6.5 ml. From the absorbance of the eluate, it could be calculated that 55 mg of albumin had been adsorbed. Complete removal of albumin was demonstrated by immunoelectrophoresis by means of the procedure of Scheidegger (21). The material applied to the anti-albumin Sepharose column exhibited a strong precipitin arc, while the eluate showed none, even at twice the protein concentration.

**Step 4. Preparative Polyacrylamide Gel Electrophoresis—** The gel filtration step had provided fractionation based on molecular size. Gel electrophoresis was therefore applied at a relatively nonrestrictive pore size, which provided separation based predominantly on net charge. The selected pH of 8.0 was as low as compatible with recovery of activity. At this pH and at a gel concentration of 5% T the factor mobility was high ($R_F = 0.8$ to 0.9), separation from neighboring bands was adequate, and short fractionation times could be expected to yield increased resolution due to reduced diffusion.

Fig. 2 shows that under these conditions Hunter factor activity is separated into two “isofactors,” characterized by $R_F$ values of 0.91 and 0.76. These isofactors are probably charge isomers,
since molecular sieving was relatively ineffective under the electrophoretic conditions used; moreover, upon gel filtration on Sephadex, Hunter factor activity appeared homogeneous. 

The eluate was reanalyzed in the same electrophoretic system (at 7.5% T) with the results shown in Fig. 3. The activity profile of the faster migrating iso-factor is coincident with two broad protein bands (R_F, 0.64 to 0.70 and 0.49 to 0.56). Comparison of the relative stain intensities of these bands with the corrective factor activity of the fractions from which they are derived (Fig. 2) shows that the faster of these two bands cannot be the one responsible for the activity.

The peak of the more slowly migrating iso-factor activity is coincident with a single band of protein (R_F = 0.44). The coincidence suggested that the protein of R_F = 0.44 could be the Hunter factor. The proposition was tested by analytical polyacrylamide gel electrophoresis of Pool 4 at various gel concentrations. The results were described in Table II. Although both parameters differ slightly, statistical analysis of the joint 95% confidence limits of K_R and Y_0 revealed that the two curves were not significantly different, i.e. that the Hunter factor activity is indistinguishable from the protein band in the buffer system employed.

However, gel electrophoretic analysis at a single pH does not appear sufficient evidence for a claim to isolation of electrophoretically homogeneous Hunter factor, particularly since analytical gel electrophoresis was carried out in the same buffer system as preparative fractionation. In view of the fact that the factor is poorly soluble and readily inactivated below pH 5, a meaningful variation of fractionation pH values was not possible. Therefore, isoelectric focusing in polyacrylamide gel was carried out on the most purified material as an additional test for homogeneity. It was found that Pool 4 could be fractionated into 5 species (with apparent pI values of 3.00, 4.10 (major), 4.50 (minor), 5.00, and 5.50).

Fig. 3 shows that R_F values, with the exception of those for Fractions 30 to 40, are not characteristic constants for each component, but appear to vary in regular fashion as a function of elution volume. Such variation has been previously observed in preparative electrophoresis (R. A. Yadley and A. Chrambach, manuscript in preparation) and is ascribed to the fusion of two adjacent band distributions in different proportions.

Table II includes KR and Y_0 values for fibroblast factor in line F, which differ from those in line E, because deoxygenation of the gel solution was achieved by evacuation in the first instance and argon gassing in the second (see “Materials and Methods”). Since the KR values of urinary and fibroblast factor are indistinguishable when measured under the same conditions (lines C and D, respectively) the two factors must be of the same molecular size.
puter programs for testing the identity of proteins on the basis of joint confidence envelopes of \( K_R \) and \( Y_0 \), for calculating confidence limits of molecular weight from gel filtration data, and for optimization of conditions in preparative gel electrophoresis.

Ten \( \mu g \) of protein, or 20 to 30 units of Hunter factor activity were subjected to electrophoresis at various polyacrylamide gel concentrations. Gels were stained for protein, or sliced and assayed for activity (9); \( R_F \) values were determined and \( K_R \) and \( Y_0 \) values calculated as described (15).

### Table II
Parameters descriptive of molecular size (\( K_R \), \( K_V \)) and of net charge (\( Y_0 \)) of Hunter factor preparations

| Hunter factor                          | No. of points | \( K_R \) Value | \( Y_0 \) Value | Molecular weight |
|----------------------------------------|---------------|-----------------|-----------------|-----------------|
| A. Activity, Pool 4                    | 5             | 0.1008\(^a\)    | 0.0115          | 2.60 0.71       |
| B. Protein of Pool 4                   | 4             | 0.0791\(^a\)    | 0.0042          | 1.73 0.17       |
| C. Activity, Urine, Step 2             | 6             | 0.1007\(^a\)    | 0.0095          | 2.89 0.64       |
| D. Activity, Urine, Step 2             | 4             | 0.0073\(^a\)    | 0.0320          | 3.21 0.14       |
| E. Activity, Fibroblast secretions     | 5             | 0.0754\(^a\)    | 0.0083          | 2.14 0.50       |
| F. Activity, Fibroblast secretions     | 4             | 0.1007\(^a\)    | 0.0095          | 2.89 0.64       |

* Denaturation by argon.
\(^a\) Concentrate from the first Sephadex G-200 column.
\(^b\) \( K_R \) was determined on the preparative Sephadex G-200 column, calibrated with seven standard proteins (10).
\(^d\) Ref. 9; denaturation by evacuation.

Molecular Size and Net Charge of Hunter Corrective Factor

The physical parameters determined by polyacrylamide gel electrophoresis and by gel filtration for the urinary Hunter factor activity are listed in Table II and compared with the corresponding values for the preparation derived from fibroblasts. The activity of Pool 4 and that of crude urinary concentrate from Step 1 are indistinguishable with regard to molecular size (\( K_R \)) and of net charge (\( Y_0 \)) from the factor derived from fibroblast cultures (lines A, C, and E, respectively). The molecular weight of fibroblast Hunter factor had been estimated in earlier experiments (9) at 65,000, with 95\% confidence limits between 32,000 and 115,000 (line F in Table II). 2

The free mobility (\( Y_0 \)) appears similar for crude urinary factor (line C) and the purified activity of Pool 4 (line A), whereas Hunter factor in fibroblast secretions appears to be a more negatively charged species (line E).

These conclusions, based on \( K_R \) and \( Y_0 \) values and their standard deviations, are supported by a more rigorous treatment, the consideration of joint 95\% confidence limits for \( K_R \) and \( Y_0 \).

The observed difference in free mobility, \( Y_0 \), between Hunter factor from fibroblast secretions and from urine may be due to tissue-specific differences. In addition, the urinary factor itself is heterogeneous, as shown by electrophoretic resolution of isofactors. Since the factor preparation from fibroblasts was derived from cells of one individual, whereas that of the urinary factor was derived from a pooled population, genetic polymorphism is a possible cause for the heterogeneity. However, secondary alterations of the factor after release from the cell, or during purification, are at present equally plausible explanations.

\( d \) Dr. David Rodbard provided unpublished methods and computer programs for testing the identity of proteins on the basis of joint 95\% confidence envelopes of \( K_R \) and \( Y_0 \), for calculating confidence limits of molecular weight from gel filtration data, and for optimization of conditions in preparative gel electrophoresis.

Gel filtration of the urinary Hunter factor at either 0.01 or 0.5 ionic strength, pH 6.0, gives a higher molecular size estimate than polyacrylamide gel electrophoresis at pH 8.0 and 0.015 ionic strength (14,000, with 95\% confidence limits of 77,000 and 170,000; line E in Table II). The reasons for this discrepancy are unknown. In contrast to the Hunter corrective factor (10), the Hunter corrective factor shows no aggregation at low ionic strength.

### Specificity

Purified Hunter factor has no effect on fibroblasts of individuals with other mucopolysaccharidoses. Fifty units of Hunter factor applied to cells of the Hurler, Scheie, Sanfilippo A and B, and Maroteaux-Lamy genotypes revealed less than 0.5 unit toward any of these. The Hunter factor has no effect on sulfated mucopolysaccharide accumulation of normal cells.

The factor was equally effective on fibroblasts of patients with the mild (adult) or severe (juvenile) form of the Hunter syndrome (22).

### Duration of Corrective Effect

When Hunter factor is removed from the culture medium, its effect persists with a half-life of 2 days (Fig. 4), a figure comparable to that found for Sanfilippo A factor, 2 days (11), but shorter than that for Hurler factor, 9 days (10). Fig. 4 shows no significant difference in the persistence of corrective activity of the two urinary isofactors.

### Function of Hunter Corrective Factor

Acceleration of sulfated mucopolysaccharide degradation by Hunter factor has been documented previously (7). It was shown that addition of the factor to Hunter fibroblasts not only reduces the accumulation of intracellular \( ^{35}S \)mucopolysaccharide, but also reduces its turnover time. The increment metabolized in the presence of factor can be quantitatively recovered in the medium as dialyzable fragments.

Further evidence has now been obtained with proteoglycan. \( ^{35}S \)Sulfate supplied exogenously. As seen in Table III, the substrate is degraded to a much greater extent in normal cells than in Hunter cells. The addition of factor to Hunter cells.
increases the breakdown to a normal level; control experiments showed that it has no effect on normal cells. The ethanol-soluble breakdown product behaves as inorganic sulfate on Bio-Gel P-2.

The degradation requires entry of the proteodermatan sulfate into the cells, since it is not produced by medium previously incubated with normal or Hunter cells. An effect of Hunter factor on the uptake (pinocytosis) of the substrate is excluded by the data shown in Table III. Incubation of the substrate with Hunter factor (followed by boiling to inactivate the factor) did not render the substrate more degradable by Hunter fibroblasts.

Full restoration of the degradative process by the factor is confirmed by examining the effect of the factor on mucopolysaccharide labeled in the hexosamine moiety. Cells were incubated with [3H]glucosamine for 3 days, after which radioactive medium was replaced by unlabeled. The presence of factor in the chase medium caused the disappearance of two peaks of radioactive material, as demonstrated by chromatography on Sephadex G-200 (Fig. 5). These correspond in elution position to two peaks of [35S]mucopolysaccharide, the chase of which is likewise accelerated by Hunter factor. The major peak (Fractions 35 to 48) has been identified as containing primarily dermatan sulfate by the criteria of chromatography on DEAE-Sephadex, electrophoresis in 0.1 M ZnSO4, and hydrolysis followed by co-chromatography of the radioactive hexosamine with b-galactosamine on Dowex 50 (23).

The Hunter corrective factor, therefore, assists Hunter cells in degrading dermatan sulfate of exogenous or endogenous origin. This suggests that the factor may be a catabolic enzyme. If so, it must be distinct from the common lysosomal enzymes, since there was no detectable activity toward the p-nitrophenyl

![Graph](image)

**Fig. 4.** Duration of corrective effect. Measurements were carried out essentially as described previously (10), except that confluent plates (1.5 mg of cell protein) were used to minimize cell growth during the experiment. ○, Pool 2 (fast-moving iso-factor); •, Pool 5 (slow-moving iso-factor).

| TABLE III |
| Degradation of proteodermatan [35S]sulfate in presence and absence of Hunter factor |

Each culture plate (ca. 3 mg of cell protein) received 5 ml of medium containing 35,000 cpm of proteodermatan [35S]sulfate, with or without 22 units of Hunter factor (Pool 5), as indicated. After incubation for 24 hours, the medium was removed and cells collected by trypsinization. Intracellular [35S]mucopolysaccharide was determined as the radioactivity insoluble in boiling 80% ethanol (6). Radioactive material in the medium was separated into ethanol insoluble (= residual mucopolysaccharide) and ethanol soluble (= fragments) by addition of 3 volumes of absolute ethanol, boiling, cooling on ice for 1 hour, and centrifugation. There was no significant radioactivity in any other fraction.

| Cells | Factor | Intracellular mucopolysaccharide | Radioactivity in medium | Degradation* |
|-------|--------|---------------------------------|-------------------------|--------------|
| Hunter | -      | 13,300                          | 2,200                   | 13           |
| Hunter | +      | 3,200                           | 10,400                  | 76           |
| Normal | -      | 4,200                           | 12,300                  | 75           |

* Degradation (%) = fragments in medium (cpm) / fragments in medium (cpm) + intracellular mucopolysaccharide (cpm) × 100.

![Graph](image)

**Fig. 5.** Accelerated disappearance, in the presence of factor, of mucopolysaccharide labeled in the hexosamine moiety. Ten milliliters of medium, containing 35,000 cpm of proteodermatan [35S]sulfate, with or without 22 units of Hunter factor (Pool 5), as indicated. After incubation for 24 hours, the medium was removed and cells collected by trypsinization. Mucopolysaccharide was determined as the radioactivity insoluble in boiling 80% ethanol (6). Radioactive material in the medium was separated into ethanol insoluble (= residual mucopolysaccharide) and ethanol soluble (= fragments) by addition of 3 volumes of absolute ethanol, boiling, cooling on ice for 1 hour, and centrifugation. There was no significant radioactivity in any other fraction.

| Cells | Factor | Intracellular mucopolysaccharide | Radioactivity in medium | Degradation* |
|-------|--------|---------------------------------|-------------------------|--------------|
| Hunter | -      | 13,300                          | 2,200                   | 13           |
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| Normal | -      | 4,200                           | 12,300                  | 75           |

* Degradation (%) = fragments in medium (cpm) / fragments in medium (cpm) + intracellular mucopolysaccharide (cpm) × 100.
derivatives of α- and β-D-galactose, α- and β-D-glucose, α-D-mannose, α-L-fucose, N-acetyl-β-D-glucosamine and N-acetyl-β-D-galactosamine. Nor was there detectable aryl sulfatase A or B activity. For these experiments, 9 units of the most purified preparation, Pool 4, were used. The absence of detectable p-nitrophenyl-β-D-galactosidase activity in purified Hunter corrective factor, as well as in purified Hurler and Sanfilippo A corrective factors (10, 11) should lay to rest the once popular hypothesis (e.g. 24–27) that a deficiency of that enzyme is the primary defect of the three mucopolysaccharidoses.

Enzymatic activity related to mucopolysaccharide degradation has been attributed to corrective factors in three instances. The corrective factor for Sanfilippo A fibroblasts removes sulfate from the mucopolysaccharide stored by these cells, probably heparan sulfate with a high N-sulfate content (11). The corrective factor for cells from a patient with an atypical mucopolysaccharidosis (28) appears to be β-glucuronidase.6 The factor corrective for Hurler and Scheie fibroblasts has been identified as α-L-iduronidase (29). Prolonged incubation of Hunter factor with dermatan sulfate isolated from Hunter cells (labeled in the sulfate, uranic acid, or galactosamine moieties) has so far failed to reveal enzymatic activity of the factor. Such negative results must be interpreted with caution, since incubation conditions may have been inappropriate. One must not, however, ignore the possibility that Hunter factor affects mucopolysaccharide degradation by some indirect mechanism.

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Characterization

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