CHICK EMBRYO FIBROBLASTS PRODUCE TWO FORMS OF HYALURONIDASE

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ABSTRACT

Cultured chick embryo fibroblasts derived from skin and skeletal muscle exhibit hyaluronidase activity both associated with the cell layer and secreted into the medium. Although both forms of the enzyme have a number of similar characteristics (R. W. Orkin and B. P. Toole, 1980, J. Biol. Chem. 255), they differ in thermal stability at neutral pH and in behavior on ion-exchange chromatography. Both forms of the enzyme are equally stable at acidic pH for long intervals, but the cell-associated hyaluronidase is significantly less stable than the secreted form at neutral pH and at temperatures ≥30°C. Neither the presence of proteases nor inhibitors of hyaluronidase appear to be involved in this thermal lability of the cell-associated enzyme. Chromatography of the two forms of hyaluronidase on carboxymethyl cellulose reveals that most (60-90%) of the secreted form of the enzyme elutes at a lower ionic strength than the cell-associated enzyme. Treatment of the secreted form of hyaluronidase with neuraminidase shifts its elution profile on carboxymethyl cellulose toward that of the cell-associated form, and also decreases its thermal stability at neutral pH. In contrast, treatment of the secreted form of hyaluronidase with alkaline phosphatase has no detectable effect. These data suggest that the secreted hyaluronidase differs from the cellular form in possessing additional sialic acid residues which endow the former with increased stability in the extracellular milieu.

Regulation of hyaluronate levels by hyaluronidase may be critical for specific aspects of morphogenesis and tissue remodelling. Numerous studies have correlated high levels of hyaluronate with cell proliferation and migration and reduced levels of this glycosaminoglycan (apparently resulting from the action of hyaluronidase) with the onset of overt differentiation (see reference 30, for review). However, neither the manner in which hyaluronate exerts its effects on cell behavior nor the mode by which hyaluronidase regulates hyaluronate levels has been well defined.

Until recently, the mechanism of hyaluronidase action at the cellular level could not be studied directly because the enzyme had not been detectable in cultured cells (3, 15). In preceding papers, we have recently detailed the synthesis and secretion of hyaluronidase by primary and passaged cultures of chick embryo muscle- and skin-derived fibroblasts, as well as its production by a number of mammalian cell lines, and have partially characterized the enzyme (21-23). High levels of enzyme were found to be present both associated with the cells and in the media of these cultures. Both enzyme fractions had the pH optimum of 3.7 and displayed no activity at neutral pH (23), typ-
rical of lysosomal hyaluronidase (4). The secreted forms, but not the intracellular forms, of several lysosomal exoglycosidases have been shown to contain recognition ligands, in most cases a 6-phosphomannosyl moiety, which mediate specific uptake of these enzymes (see references 19 and 26 for review). Thus we compared the cell-associated and secreted forms of hyaluronidase for chemical differences which might relate to this receptor-mediated uptake. We have not found evidence for phosphomannosyl groups on hyaluronidase, but have observed that the secreted form of the enzyme differs from the cellular enzyme both in thermal stability at neutral pH and in behavior on ion exchange chromatography. These differences in the two forms of the enzyme appear to be caused by the presence of additional sialic acid residues in the secreted form of hyaluronidase.

MATERIALS AND METHODS

Preparation and Assay of Hyaluronidase from Cell Cultures

Fibroblast cultures prepared from 10-d chick embryo skin and from 12-d chick embryo thigh muscle (see reference 23) were grown to confluence in complete serum-containing medium (nutrient medium F-12 with 10% fetal calf serum and 1% antibiotic-antimycotic solution, Grand Island Biological Co. [GIBCO], Grand Island, N. Y.) and then incubated for variable periods (at least 48 h) in serum-free culture medium. Some cultures were prepared by the Massachusetts Institute of Technology Cell Culture Center. Hyaluronidase was isolated separately from the cell layer and from the serum-free medium, as previously described (23). Unless otherwise indicated, all manipulations were done at 4°C. Briefly, the medium was collected, concentrated by ammonium sulfate precipitation at 65% saturation, and the precipitates of serum-free culture medium were adjusted to 0.02 M ammonium sulfate precipitation, as described above, and the hyaluronidase activity remaining in the two samples (37°C vs. 4°C preincubation) was compared.

In some experiments, protease inhibitors were added to the samples before preincubation at neutral pH. These included (a) 1 mM phenylmethylsulfonyl fluoride (PMSF) dissolved in 95% ethanol, (b) 1 mM para-chloromercuribenzoic acid (PCMB) dissolved in 50 mM NaOH, (c) a mixture containing 100 mM N-acetyl-L-cysteine, 5 mM benzamidine-HCl, and 10 mM EDTA (20), (d) 0.1% soybean trypsin inhibitor (SBTI), 0.1% N-ethylmaleimide (NEM), alone and in combination.

For mixing experiments, known aliquots of hyaluronidase-containing samples from cell extracts and from medium samples were preincubated at neutral pH, either alone or after mixing together. After the preincubation at neutral pH and 37°C for 3 h, samples were corrected to the appropriate ionic strength and pH with formate buffer and incubated with hyaluronate substrate as indicated above. Control samples were preincubated at neutral pH at 4°C.

Ion-Exchange Chromatography on Carboxymethyl Cellulose

Samples of cell extracts and resolubilized ammonium sulfate precipitates of serum-free culture medium were adjusted to 0.02 M sodium formate, 0.03 M NaCl, pH 3.7 (0.2X formate buffer) by either dilution or dialysis and applied to columns of carboxymethyl cellulose (CMC) (17 X 80 mm) (CM-52, Whatman), previously equilibrated with 0.2X formate buffer, pH 3.7. Routinely, two successive step elutions of 50-75 ml each were applied, one at 0.1 M NaCl, 0.02 M sodium formate, pH 3.7, and the second at 0.2 M NaCl, 0.02 M sodium formate, pH 3.7. 5-ml fractions were collected and assayed individually for hyaluronidase activity as indicated above. Hyaluronidase-containing frac-

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200 ml of 0.02 M sodium formate, pH 3.7, was applied. 5-ml aliquots of the latter were removed, incubated with sialyllactose (Sigma Chemical Co.) as substrate. Released, free \( p \)-nitrophenol was assayed colorimetrically (9). Under these conditions, complete digestion of the substrate was obtained.

RESULTS

Thermal Stability at Neutral pH of the Cell- and Medium-derived Forms of Hyaluronidase

Comparison of the activities of the two forms of the enzyme after incubation over a varying temperature range from 4° to 37°C (Table I) or at fixed temperatures for increasing incubation times (Fig. 1) demonstrated that, at neutral pH, the secreted, medium-associated form of hyaluronidase is significantly more thermally stable at 30°-37°C than the cell layer-associated form of the enzyme. Similar results were obtained with hyaluronidase prepared from confluent cultures of chick embryo skin or muscle fibroblasts. Table I shows that, although both cell- and medium-derived samples retained complete activity when neutralized and maintained at 4°C, incubation for 3 h at 30°C and neutral pH led to an ~30% loss in cell-associated hyaluronidase activity, whereas the secreted medium form of the enzyme lost only ~5% of its activity under these same conditions. Likewise, a similar incubation at 33°C resulted in

| Temperature (°C) | Initial enzyme activity | Initial enzyme activity |
|------------------|--------------------------|--------------------------|
|                  | Cells                     | Medium                   |
| 4°               | 100 ± 0 (6)               | 100 ± 0 (6)               |
| 22°              | 89 ± 1 (3)                | 98 ± 3 (4)                |
| 30°              | 70 ± 10 (4)               | 96 ± 2 (5)                |
| 33°              | 43 ± 5 (5)                | 82 ± 4 (6)                |
| 35°              | 0 ± 0 (1)                 | ND                       |
| 37°              | 0 ± 0 (5)                 | 41 ± 6 (6)                |

Values are expressed as the percentage of hyaluronidase activity remaining after a 3-h incubation at neutral pH at the indicated temperatures. After this preincubation at neutral pH, samples were acidified with formate buffer to pH 3.7, incubated for 8 or 16 h with hyaluronate substrate, and hyaluronidase activity was determined colorimetrically as indicated in Materials and Methods. Each value represents the mean ± standard error. The number of experimental samples is indicated in parentheses. Each sample was assayed in duplicate.

ND, Not determined.

### TABLE I

Thermal Stability at Neutral pH of Cell- and Medium-derived Hyaluronidase

| Preincubation temperature | Muscle fibroblasts | Skin fibroblasts |
|---------------------------|--------------------|------------------|
| 4°                        | 100 ± 0 (6)        | 100 ± 0 (6)      |
| 22°                       | 89 ± 1 (3)         | 98 ± 3 (4)       |
| 30°                       | 70 ± 10 (4)        | 96 ± 2 (5)       |
| 33°                       | 43 ± 5 (5)         | 82 ± 4 (6)       |
| 35°                       | 0 ± 0 (1)          | ND               |
| 37°                       | 0 ± 0 (5)          | 41 ± 6 (6)       |

|                  | Cells | Medium | Cells | Medium |
|------------------|-------|--------|-------|--------|
| 4°               | 100 ± 0 (6) | 100 ± 0 (6) | 100 ± 0 (4) | 100 ± 0 (4) |
| 22°              | 89 ± 1 (3)  | 82 ± 3 (4)  | 92 ± 0 (2)  | 94 ± 3 (3)  |
| 30°              | 70 ± 10 (4) | 96 ± 2 (5)  | 68 ± 6 (3)  | 93 ± 2 (3)  |
| 33°              | 43 ± 5 (5)  | 82 ± 4 (6)  | 41 ± 13 (3) | 76 ± 6 (4)  |
| 35°              | 0 ± 0 (1)   | ND        | 0 ± 0 (1)   | ND        |
| 37°              | 0 ± 0 (5)   | 41 ± 6 (6) | 0 ± 0 (4)   | 38 ± 9 (4) |
The greater thermal stability at neutral pH of the secreted, medium-derived hyaluronidase is further illustrated in Fig. 1. At a preincubation temperature of 33°C, 50% of the cell-associated enzyme activity was lost within 3 h, but a 50% loss in medium-derived hyaluronidase occurred only after 6–9 h, under these incubation conditions. At 37°C, a 50% loss of the cell-associated hyaluronidase activity occurred within 30 min, but an incubation time of ~90 min was required for a comparable decrease in medium-derived hyaluronidase activity to occur. In contrast to these results, preincubation of either cell- or medium-derived enzyme at pH 3.7 for up to 6 h at 37°C did not result in any loss of activity (Table II).

Inclusion of a battery of protease inhibitors (either alone or in combination) in cell-derived hyaluronidase samples failed to prevent loss of hyaluronidase activity at neutral pH and 37°C (Table III). Although inclusion of some protease inhibitors appeared to result in a partial reduction of hyaluronidase activity, preincubation for 2–3 h at 37°C and neutral pH of cell-associated hyaluronidase preparations in the presence or absence of protease inhibitors always resulted in complete loss of enzyme activity.

Mixing experiments also indicated that the higher thermal instability at neutral pH of the cellular form of hyaluronidase is not caused by proteases. These experiments also ruled out the presence of inhibitors in the cell-derived preparations. In these experiments, known aliquots of cell- and medium-derived hyaluronidase preparations were incubated at neutral pH and 37°C alone or together, and then assayed for hyaluronidase activity. Although under these conditions cell-de-
-derived hyaluronidase samples lost all activity, the mixed samples, containing both cell and medium hyaluronidase, had the same activity as the medium samples alone, at the end of the incubation at neutral pH (Table IV).

**Chromatographic Behavior of Cell- and Medium-derived Hyaluronidase on CMC**

Comparison of the chromatographic behavior of cell- and medium-derived hyaluronidase preparations on CMC demonstrated that the medium form of the enzyme is more acidic than the cellular form. With a step gradient, under conditions of acidic pH (3.7), >90% of the cellular form of hyaluronidase eluted from CMC at 0.2 M NaCl (Fig. 2A), whereas most of the medium-derived enzyme (60–90%) eluted at 0.1 M NaCl (Fig. 2B), with the remainder eluting at 0.2 M NaCl. When a linear gradient was used for elution, it was again found that the cell-derived hyaluronidase eluted at a higher ionic strength than the medium-derived enzyme (Fig. 3). The 0.1- and 0.2-M NaCl eluates from the step gradient of the medium enzyme (Fig. 2B) were also chromatographed in the linear gradient. The 0.2-M fraction gave an elution pattern almost identical to that of the cell preparation. The 0.1-M fraction, however, eluted in the same manner as the whole medium preparation, except that less overlap with the cell enzyme elution pattern was observed (Fig. 3). This suggests that the medium preparation contained a mixture of a small amount of the form of the enzyme obtained from the cells and another more acidic form that elutes at a lower ionic strength from the CMC columns and that comprises the bulk of the medium preparations.

**Effects of Neuraminidase and Alkaline Phosphatase on Medium-derived Hyaluronidase**

After neuraminidase digestion of the medium-derived hyaluronidase that elutes from CMC at 0.1 M NaCl, 70–100% of the enzyme eluted in 0.2 M NaCl (Fig. 4), the elution position of the cell-

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**Table III**

| Inhibitor | Controls (4°C) | Incubation at 37°C, 2 h |
|-----------|---------------|-------------------------|
| None      | 100           | 0                       |
| 3 mM PMSF | 76            | 3                       |
| 1 mM PMSF + 1 mM PCMB | 55 | 0  |
| 100 mM e-NH₂-caproic acid, 5 mM benzamidine-HCl, + 10 mM EDTA | 45 | 0  |
| 0.1% SBTI | 97            | 0                       |
| 0.1% e-NH₂-caproic acid | 96 | 0  |
| 2 mM NEM  | 85            | 0                       |
| 0.1% SBTI + 0.1% e-NH₂-caproic acid | ND | 0  |
| 0.1% SBTI, 2 mM NEM | ND | 0  |
| 0.1% SBTI, 2 mM NEM, + 0.1% e-NH₂-caproic acid | ND | 0  |
| 0.1% e-NH₂-caproic acid + 2 mM NEM | ND | 0  |
| ND, Not determined. |

**Table IV**

| Sample                | exp 1 | exp 2 |
|-----------------------|-------|-------|
|                       | 4°C   | 37°C  | 4°C   | 37°C  |
| μg GNAc               |       |       |       |       |
| Muscle fibroblasts    |       |       |       |       |
| Cells                 | 2.1   | 0.2   | 3.3   | 0.7   |
| Medium                | 5.7   | 2.7   | 7.2   | 4.7   |
| Cells and medium      | 7.6   | 3.2   | 9.4   | 5.6   |
| Skin fibroblasts      |       |       |       |       |
| Cells                 | 2.5   | 0.4   | 2.4   | 0.7   |
| Medium                | 2.9   | 1.7   | 7.3   | 4.3   |
| Cells and medium      | 5.0   | 2.6   | 8.7   | 5.2   |

100-μl aliquots from cell extracts and media were preincubated alone or together for 3 h at 4° or 37°C, at neutral pH. After the preincubation period, samples were acidified to pH 3.7 with formate buffer at a final concentration of 0.1 M Na formate, 0.15 M NaCl, and then incubated with hyaluronate substrate for 16 h at 37°C, and the products of the digestion were measured colorimetrically as described in Materials and Methods. Hyaluronidase activity is expressed as μg of N-acetylglucosamine (GNAc) released per sample aliquot.
associated hyaluronidase. Addition of 1 mg/ml sialyllactose, a substrate for neuraminidase, to these reaction mixtures largely protected the hyaluronidase from digestion by the neuraminidase, preventing the shift in elution position on CMC (84% of the enzyme eluted in 0.1 M NaCl, 16% in 0.2 M NaCl) (Fig. 4). Control samples, incubated in the absence of neuraminidase or sialyllactose, also eluted primarily in 0.1 M NaCl (89% of the enzyme eluted in 0.1 M NaCl, 11% in 0.2 M NaCl) (Fig. 4).

In some experiments, the neuraminidase was first inactivated by boiling before incubation with hyaluronidase-containing samples to eliminate the possibility that nonenzymatic contaminants of the neuraminidase preparations (see reference 33) might alter the hyaluronidase. Medium-derived hyaluronidase samples treated with heat-inactivated neuraminidase did not shift the position of elution on CMC, i.e., the enzyme continued to elute at 0.1 M NaCl.

In addition to shifting the elution profile on CMC of the medium-derived hyaluronidase toward that of the cell-associated enzyme, neuraminidase digestion of the medium form of hyaluronidase also decreased its thermal stability at neutral pH. Comparative temperature curves in which the temperature range was varied between 4° and 37°C demonstrated that the thermal stability of the neuraminidase-treated, medium-derived hyaluronidase preparations lies intermediate between that of cell-derived hyaluronidase and that of the untreated medium-derived hyaluronidase (Fig. 5).

In contrast to the effects of neuraminidase on the medium-derived form of hyaluronidase, treatment of the medium enzyme with alkaline phosphatase did not significantly affect its elution profile on CMC.

DISCUSSION

Cultures of chick embryo-derived fibroblasts, prepared from two different tissue sources, synthesize and secrete hyaluronidase (23). The cell-associated and secreted, medium-associated forms of this enzyme have specific characteristics in common, including acidic pH optima, substrate preference, and the products of digestion of hyaluronate substrate (23). We report here, however, that the hyaluronidase present within cells and that secreted into the medium represent two distinct forms of this enzyme.

The distinguishing feature of the secreted, me-
FIGURE 3  CMC chromatography of chick embryo fibroblast cell- and medium-derived forms of hyaluronidase in the presence of a linear gradient of NaCl. Samples were either directly applied to CMC or first isolated by step gradient elution from CMC (see Fig. 2), and then reapplied. Samples were loaded in 0.02 M sodium formate, 0.03 M NaCl, pH 3.7. A linear gradient of NaCl, from 0.03 to 0.28 M, in 0.02 M sodium formate, pH 3.7, was applied as indicated, and 4.5-ml fractions were collected. Aliquots of individual fractions were assayed for hyaluronidase activity, as indicated in the text. Hyaluronidase activity is expressed as units/fraction. (○) Medium-derived hyaluronidase, previously eluted at 0.1 M NaCl by step gradient CMC chromatography. (●) Medium-derived hyaluronidase, previously eluted at 0.2 M NaCl by step gradient CMC chromatography. (×) Medium-derived hyaluronidase applied directly to CMC. (∆) Cell-derived hyaluronidase, previously eluted at 0.2 M NaCl by step gradient CMC chromatography.

The presence of the additional sialic acid residues in the medium form appears to account for the two characteristics found to differ between the cell- and medium-derived forms, i.e., increased thermal stability at neutral pH and more acidic behavior on CMC chromatography. Treatment of the medium form of hyaluronidase with neuraminidase resulted in both a decrease in its thermal stability at neutral pH and a shift in its elution position on CMC toward that of the cellular form of the enzyme. That these changes resulted from neuraminidase action rather than contamination of the neuraminidase preparations by other enzymes or by nonenzymatic substances, e.g., merthiolates (see reference 33), was demonstrated in control experiments in which: (a) sialyllactose, a substrate for neuraminidase, prevented the change, and (b) boiled neuraminidase preparations were shown to have no effect on the hyaluronidase samples. In a study of the hyaluronidase present in extracts of rat skin, Cashman et al. (7) also observed a shift from more acidic to more basic forms of hyaluronidase after treatment with neuraminidase.

Additional experiments confirmed that the decreased thermal stability of the cell-associated enzyme is intrinsic to this form of the enzyme rather than caused by degradation by proteases or inhibition by other factors in the preparations. Thus, protease inhibitors failed to prevent the loss of cell-associated hyaluronidase activity under conditions of neutral pH and 37°C, and mixing experiments demonstrated that inhibitors of hyaluronidase were not present intracellularly, as the addition of cell-associated enzyme preparations to medium enzyme preparations did not cause a decrease in activity of the medium-derived hyaluronidase. Although the medium enzyme is more stable than the cellular form, it still loses activity relatively rapidly at neutral pH and 37°C, the half time of loss of activity in the absence of cells being.
The effect of neuraminidase treatment on the chromatographic behavior of chick embryo fibroblast medium-derived hyaluronidase on CMC. Hyaluronidase-containing samples, previously eluted from CMC with 0.1 M NaCl, were separated into equal volumes and one-third of them were treated with neuraminidase, one-third with neuraminidase and sialyllactose, and one-third without additives, as indicated in Materials and Methods. Treated and untreated samples were then individually reapplied to CMC, and each was eluted with successive step gradients of 0.1 and 0.2 M NaCl. 

\[ \text{No additives.} \]

The secreted forms of a number of lysosomal exoglycosidases have been shown to contain specific recognition sites that bind to cell surface receptors and mediate active pinocytosis of these enzymes, followed by incorporation into secondary lysosomes (11, 12, 19, 25, 31). In the case of human fibroblasts, the recognition marker appears to be a phosphomannosyl moiety for \( \beta \)-glucuronidase (11, 13), \( \alpha \)- and \( \beta \)-N-acetylgalcosaminidase (12, 13), \( \alpha \)-L-iduronidase (12, 19), \( \alpha \)-mannosidase (13), and \( \beta \)-galactosidase (12). These observations have led to a hypothesis for lysosomal enzyme action in which these enzymes are secreted and reinternalized, via the specific binding sites, before function within secondary lysosomes (19). An alternative hypothesis has recently been proposed in which the phosphomannosyl recognition marker is involved in segregating lysosomal enzymes from secretory products by retaining the former bound to the internal wall of exocytic vesicles during eversion, fusion with the plasma membrane, and secretion of unbound materials (26). According to this hypothesis, plasma membrane-bound lysosomal enzymes would then be reinternalized by endocytosis. Also, the presence of these enzymes with attached recognition markers in cell culture media would be caused by dissociation from the media.

\[ \text{No additives.} \]
plasma membrane receptors under the culture conditions.

In the present study, we have not found evidence for phosphate groups attached to the secreted form of hyaluronidase as judged by the lack of change of behavior on ion exchange chromatography after treatment with alkaline phosphatase. Several other specific receptor-mediated recognition systems for glycoproteins, including lysosomal exoglycosidases, have been described in addition to that mediated by phosphomannosyl moieties. These involve galactose (5), N-acetylglucosamine (1, 14, 18, 27), and mannose (1, 2, 6, 34) moieties. To date, however, no demonstration of involvement of sialic acid as a specific recognition marker has been made. On the contrary, removal of terminal sialic acid groups is necessary for recognition of the penultimate galactose in glycoproteins cleared from blood by liver cells (5). Terminal sialic acid residues have also been reported present in a number of exoglycosidases, isolated from a soluble fraction prepared from rat kidney lysosomes (10).

Treatment of these enzymes with neuraminidase was reported to convert them to more basic forms, resembling those found in the bound fraction of these lysosomal preparations (10). In studies on I-cell disease, in which lysosomal exoglycosidases accumulate extracellularly, Vladutiu (32) has proposed that inappropriate addition or failure to remove sialic acid residues, possibly resulting from a sialidase deficiency in these cells (28, 29), might block the phosphomannosyl recognition markers on these defective lysosomal enzymes. It has also been noted that enzymes secreted into culture media by I-cell fibroblasts are more thermally stable than their normal counterparts (see reference 26). Possibly, this difference is caused by the higher sialic acid content of the secreted I-cell exoglycosidases, as observed here for hyaluronidase.

The role of the additional sialic acid moieties present in the secreted form of hyaluronidase has yet to be resolved. It is unlikely that they are involved in modifying enzyme action, as both forms of enzyme are very similar in this respect. They both display activity over a narrow acidic pH range with no activity at neutral pH, have the same substrate preference for hyaluronate, and give rise to a similar size range of oligosaccharide digestion products (23). These findings strongly suggest that this enzyme does not act extracellularly but that its action would be restricted to lysosomes. The possible role of the sialic acid groups with respect to recognition and reuptake, as discussed above, will be clarified by experiments designed to test the relative efficiency of uptake of the two forms of hyaluronidase. A final consideration arises from the nature of the transport pathway of lysosomal enzymes from the Golgi apparatus to the lysosomes. These enzymes are believed to be transported from the Golgi apparatus to the plasma membrane, then either secreted and reinternalized (19) or briefly exposed to the extracellular milieu while bound to the plasma membrane and then endocytosed (26). Thus it would follow that these enzymes are exposed temporarily to a neutral pH environment before their incorporation into the acidic environment of the secondary lysosome. A possible function for the additional sialic acid residues may therefore be to protect lysosomal enzymes from loss of activity during this brief period.

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lases: conversion of acidic to basic forms by neuraminidase. FEBS (Fed. Eur. Biochem. Soc.) Lett. 13:68-72.

11. KAPLAN, A., D. T. ACHORD, and W. S. SLY. 1977. Phosphohexosyl components of a lysosomal enzyme are recognized by pinocytosis receptors on human fibroblasts. Proc. Natl. Acad. Sci. U. S. A. 74:2026-2030.

12. KAPLAN, A., D. FISCHER, D. ACHORD, and W. SLY. 1977. Phosphohexosyl recognition is a general characteristic of pinocytosis of lysosomal glycosidases by human fibroblasts. J. Clin. Invest. 66:1088-1090.

13. KAPLAN, A., D. FISCHER, and W. S. SLY. 1978. Correlation of structural features of phosphomannans with their ability to inhibit pinocytosis of human β-glucohydrolase by human fibroblasts. J. Biol. Chem. 253:647-650.

14. KAWASAKI, T., and G. ASHWELL. 1977. Isolation and characterization of an avian hepatic binding protein specific for N-acetylglucosamineterminated glycoproteins. J. Biol. Chem. 252:6536-6543.

15. LAMBERG, S. L, and A. DORFMAN. 1973. Synthesis and degradation of hyaluronic acid in the cultured fibroblasts of Marfan's disease. J. Clin. Invest. 52:2428-2433.

16. LOWRY, O. H. 1957. Micromethods for the assay of enzymes. II. Specific procedures: alkaline phosphatase. Methods Enzymol. 4:371-372.

17. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, and R. J. RANDALL. 1951. Protein measurements with the Folin phenol reagent. J. Biol. Chem. 193:265-275.

18. LOWRY, O. H., and G. ASHWELL. 1976. A hepatic receptor of avian origin capable of binding specifically modified glycoproteins. Proc. Natl. Acad. Sci. U. S. A. 73:341-343.

19. NEUFELD, E. F., G. N. SANDO, A. J. GARVIN, and L. H. ROME. 1977. Evidence for lysosomal enzyme recognition by human fibroblasts via a phosphorylated carbohydrate moiety. Biochem. J. 170:657-665.

20. ORKIN, R. W., and B. P. TOOLE. 1980. Isolation and characterization of hyaluronidase from cultures of chick embryo skin- and muscle-derived fibroblasts. J. Biol. Chem. 255:647-650.

21. ORKIN, R. W., and B. P. TOOLE. 1980. Two forms of hyaluronidase. Two Forms of Hyaluronidase 257