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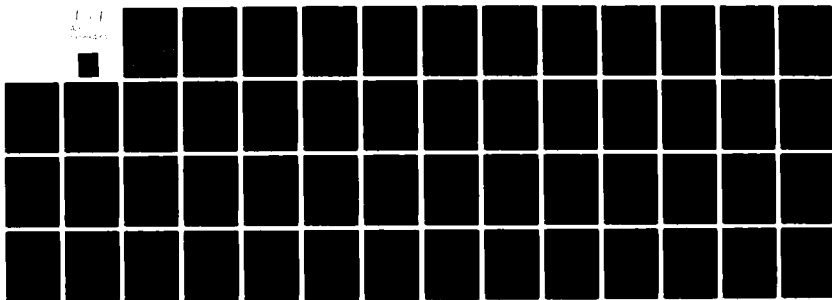
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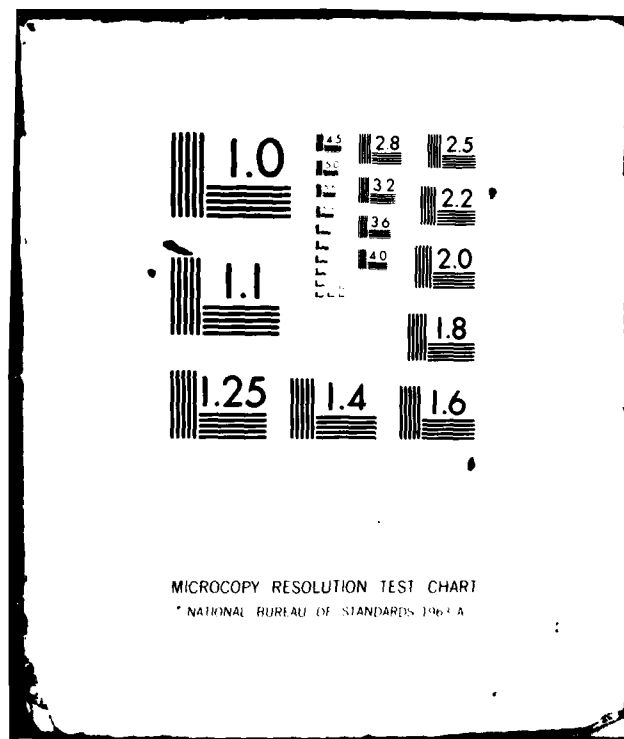
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| 19. KEY WORDS (Continue on reverse side if necessary and identify by block number)<br>Universal Blood Donor<br>$\alpha$ -N-acetylgalactosaminidase<br>$\alpha$ -D-galactosidase                                                                                                                                                                                                                                                     |                                       |                                                                                         |
| 20. ABSTRACT (Continue on reverse side if necessary and identify by block number)<br>We had previously reported on the purification of the $\alpha$ (1+3)-N-acetyl-<br>galactosaminidase from <u>C1. perfringens</u> (8000 fold) and of the $\alpha$ (1+3)-gal-<br>alactosidases, B-zyyme, from <u>C1. sporogenes</u> (2500 fold). Both enzymes were,<br>nonetheless demonstrated to be still impure by polyacrylamide gel electro- |                                       |                                                                                         |

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phoresis. The purest preparation of the A-zyme was still contaminated with related glycosidases and they even co-migrated on polyacrylamide gel electrophoresis. This, together with other observations, implicated a multi-enzyme complex involving SH  $\leftrightarrow$  S-S interactions.

The presence of such interactions was demonstrated in the A-zyme and the other glycosidases, co-purifying with it, as well as with the B-zyme. With this knowledge, it was possible to exploit these properties to purify both the A-zyme and B-zyme.

The applicability of immuno-affinity chromatography to the purification and separation of the exoglycosidases was also explored. A measure of success was attained. The development of this approach was arrested by the greater success we had by the above mentioned methods.

The action of the exo-glycosidases;  $\alpha(1\rightarrow3)$ -D-galactosidase,  $\alpha(1\rightarrow3)$ -N-acetyl-D-galactosaminidase and  $\alpha(1\rightarrow2)$ L-fucosidase, on the blood group substances and oligosaccharides appear to be regulated by steric hindrance. There is a distinct sequence of cleavage observed. The A-zyme and B-zyme must act first before the  $\alpha(1\rightarrow2)$ L-fucosidase can release the fucosyl residue.

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(6) PREPARATION OF A UNIVERSAL BLOOD DONOR TYPE.

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A. PERSONNEL

George N. Lowrie III, who was with the project since July 1977, has now left our group. He was responsible for the takeover of the purification of the B-zyne from Dr. Tadahisa Kogure. We had hoped to replace Mr. Lowrie with an immunologist with specific training in hybridoma methodology. Several such candidates were available on the market, but were rapidly taken up by industry.

The program limped for a while, but by parcelling out the project into smaller subunits, it was possible to utilize part-time workers to explore the effectiveness of some of our approaches.

Since then, we were able to recruit an appropriate team, and we are now forging ahead.

B. A-ZYME FROM CLOSTRIDIUM PERFRINGENS1. A-zyne Producing and Non-Producing Strains of *Cl. perfringens*

The A+ and A- strains of *Cl. perfringens*, that we had reported on previously (1), were investigated in greater detail in order to identify the bacterial strains involved. The biochemical reactions of the two strains, Table I, as well as the fingerprints obtained from the gas chromatographic analysis, Table II, strongly implicate the A+ strains as being *Cl. perfringens*, and the A- strain as *Cl. sporogenes*. The origin of this contaminant remains an intriguing mystery, since it contains all the glycosidases found in *Cl. perfringens* with the A-zyne manifesting the least activity, Table III. Further work on this aspect of the work has temporarily been halted.

2. Improvement in the Basic Approach to the Purification of A-zyne

We had previously reported on the purification of A-zyne, which achieved

a final product that was 8000 fold pure, but with a yield of 17%, (1,2) see Table IV. We wished to build up stocks of A-zyne for the more extensive purification to be described below and so developed a quick and more effective procedure as outlined in Table V.

Possible use of acetone as a step in the fractionation was also explored. It offers an alternative approach to ammonium sulfate as a precipitin and at the same time serves as a solvent for hydrophobic membrane components. The rationale for its use is to disrupt the possible multi-enzyme complex held together by bacterial membrane and to release the individual enzymes. The enzymes thus released would be more amenable to subsequent separation.

The ideal sequence for fractionation was found to be the introduction of the acetone fractionation step after the Sephacryl S-200, and before the DEAE step, as shown in Table VI. A comparison of ammonium sulfate and acetone fractionation procedures indicates that in both cases the recovery of enzyme is good, 98% with acetone, and 83% with ammonium sulfate.

### 3. Iso-electric Precipitation of A-zyne and its Effect on the Other Glycosidases

In our previous report (1) we had mentioned that the A-zyne could be purified by iso-electric precipitation at pH 4.5 and that it provided a useful step. It removed extraneous protein giving us an almost 3-fold purification step as well as removing other glycosidases e.g. 50% of the sialidase and  $\beta$ -Gal'ase, and almost 92% of the  $\beta$ -GlcNac'ase.

We considered it desirable to re-examine the effect of pH on the various glycosidases. The enzyme solution contained in five different dialysis chambers was equilibrated each with a different 0.1M acetate buffer at a ratio of 1:125 for 24 hr. with one change in buffer. The buffers ranged in pH from 4.0, 4.25, 4.50, 4.75 and to 5.0. At the end of the equilibration, the precipitates that developed were removed by centrifugation

and dissolved in buffer at pH 6.3. The supernatants were likewise adjusted to pH 6.3 before determining the activities of the various glycosidases present. Table VII shows the results obtained. It is apparent that the stability of the four enzymes vary at the low pH of 4.0. The A-zyme is most stable and the sialidase the least stable. At pH 4.25, 53% of A-zyme is to be found in the precipitate, with 39-40% of the initial  $\beta$ -Gal'ase and  $\beta$ -GlcNAc'ase still remaining. At pH 5.0 the A-zyme appears to be stable, while the supernatant shows only 15 and 40% of original activity of  $\beta$ -Gal'ase and  $\beta$ -GlcNAc'ase respectively.

In view of this difference in stability of A-zyme as compared to the other glycosidases, the following experiment was performed. The enzyme preparation was dialysed against buffer at pH 5 for twice as long a period. The enzymatic activities were followed and found to be as shown in Table VIII. The A-zyme was most stable and  $\beta$ -Gal'ase least.

#### 4. Molecular Weight of Glycosidases from *Cl. perfringens*

Sephacryl S-300 (73 ml) was prepared in a Pharmacia column of dimensions 2.6 x 100 cm. The column was calibrated with Dextran blue and a number of standard proteins of known molecular weight; cytochrome C 12,400, ovalbumin 45,000, bovine serum albumin 67,000 and catalase 232,000 daltons. A partially purified preparation of A-zyme was applied to the column at pH 5.0, and the 1 ml fractions collected used to determine the distribution of activities of the various enzymes:- A-zyme,  $\beta$ -Gal'ase,  $\beta$ -GlcNAc'ase by the PNP-glycoside assays and sialidase with OSM as substrate and the sialic acid released determined by the TBA method (3). The pattern of activities is shown in Fig 1.

The experiment was then repeated with the column equilibrated at pH 8.0, to give a similar pattern as at pH 5.0 for most of the enzymes with the exception of GlcNAc'ase, Fig 2.

The molecular weights computed from the elution data are shown in Table IX. While there is an appreciable error, 20%, under the conditions used. The results nonetheless indicate that most of these glycosidases have a large molecular weight and are most likely oligomeric proteins. We had previously reported 200,000 daltons for the  $\alpha(1\rightarrow2)\text{L}$ -fucosidase found in Cl. perfringens (4).

5. Involvement of SH  $\neq$  S-S in the Glycosidases of Cl. perfringens

a. Reasons for suspecting the involvement of SH  $\neq$  S-S.

i) Schiff (5) had previously demonstrated the need for a reducing agent, such as ascorbic acid, to reactivate and maintain activity of A-zyme from Cl. welchii. We have confirmed this observation and were able to achieve the same objective using DTT instead of ascorbic acid (1,2). ii) The various glycosidases persistently co-purified as a multi-enzyme complex with only an occasional hint of separation (1). iii) In our previous report (1) we had indicated that the A-zyme was inhibited by  $\text{HgCl}_2$ . iv) As demonstrated above, the molecular weights of the glycosidases vary from  $170 - 450 \times 10^3$  daltons, which is indicative of oligomeric proteins.

b. Ability of  $\text{HgCl}_2$  to inhibit all the glycosidases to varying extent.

We therefore predicted that the other glycosidases of Cl. perfringens would also be inhibited like the A-zyme. The ability of  $\text{HgCl}_2$  to inhibit the activities of the various glycosidases is shown in Fig 3. The inhibition over the concentration range of  $1 \times 10^{-4}$  to  $1 \times 10^{-3}$  was re-determined in order to demonstrate the sensitivities of the different glycosidases to  $\text{HgCl}_2$ .

c. Reactivation of A-zyme inhibited with 0.5mM HgCl<sub>2</sub> by use of DTT and EDTA

Table X demonstrates that the 0.5mM HgCl<sub>2</sub> inhibition is readily reversed, 92%, by the lowest concentration of DTT and completely reversed, 100%, by 1.0mM DTT. EDTA at low concentration can reverse the inhibition of A-zyme, on the other hand, at higher concentrations it inhibits the enzyme. A mixture of the two reagents indicates that the EDTA effect predominates suggesting that an important metal may be required for the enzyme to function effectively.

d. Effect of a redox potential gradient on the activities of  $\beta$ -Galactosidase

The activity of  $\beta$ -galactosidase was investigated in the presence of varying amounts of different redox reagents;- DTT, reduced glutathione, cysteine and oxidized glutathione. Fig 4 shows the results obtained, indicating that DTT has the ability to enhance the activity of  $\beta$ -Gal'ase whilst the other reagents inactivate it. The extent of inactivation depends on the concentration of the reagent. Oxidized glutathione appears to strongly inactivate the  $\beta$ -galactosidase, Fig 5, requiring approximately 90% of reductant, cysteine or GSH to reactivate it. Dithiothreitol is effective in reversing this inactivation by GSSG starting from a concentration of 60% upwards, Fig 5.

The effect of 40mM DTT on the various glycosidase activities was tested on two different preparations of A-zyme. Table XI shows the results obtained and indicates that  $\beta$ -GlcNAc'ase behaves differently from the A-zyme and  $\beta$ -Gal'ase, showing some inactivation rather than activation in the presence of DTT.

6. The Separation of Glycosidases on Sephacryl S-200 Before and After Treatment with Dithiothreitol

In the absence of DTT, the three enzymes  $\beta$ -Gal'ase,  $\beta$ -GlcNAc'ase and A-zyme bundle together as an apparent "multi-enzyme" complex, Fig 6A. Pretreatment with 20mM DTT followed by their filtration through an S-200 column equilibrated with DTT, at the same concentration, results in a distinct separation of the "macromolecule" into 3 peaks, Fig 6B. Pool I, consisting of a) large high molecular weight peak containing all 3 enzymes, and b) a similar lower molecular weight peak containing  $\beta$ -Gal'ase and  $\beta$ -GlcNAc'ase. Pool II, with an appreciably lower molecular weight, appears to be pure  $\beta$ -GlcNAc'ase. The analytical data on the starting enzyme preparation and those recovered in the two pools, I and II, are summarized in Table XII.

7. The Separation of Glycosidases on a Covalently Immobilized Thiol Sepharose Gel

An approximately 1 ml column of a covalently bound thiol gel (pharmacia) was used for this experiment. Four ml of enzyme were applied to the column and 0.5 ml fractions collected. The column was then washed with buffer to remove unadsorbed material and then eluted with 10mM DTT. The eluates were assayed for protein (Lowry) as well as for the various enzymatic activities (PNP-glycosides). The results are shown in Fig 7.

8. The Purification of  $\alpha$ -N-Acetylgalactosaminidase by Adsorption and Elution from Mercury Phenyl Agarose Gel

Mercury phenyl agarose beads (50 $\lambda$ ) were placed at the bottom of a tube and a solution of the A-zyme preparation in 0.05M acetate buffer pH 5.0 was added in 500 $\lambda$  aliquots and agitated with the beads for a period of 10 min. This was centrifuged rapidly and the supernate removed

for the assay of A-zyyme activity and protein content. Successive 500 $\lambda$  batches of A-zyyme solution were added, repeating the process until it became apparent that the gel was saturated with the A-zyyme. The gel was then rinsed with two batches of 500 $\lambda$  of buffer. Elution of enzymes was then initiated using progressively increasing concentrations of  $\text{HgCl}_2$  as shown in Table XIII. The inhibiting effect of  $\text{HgCl}_2$  on the A-zyyme was overcome by the addition of excess DTT.

A sample of eluate, fraction #6 Table XIII was subjected to polyacrylamide gel electrophoresis and stained for protein by the Coomassie staining method, while the A-zyyme and  $\beta$ -Gal'ase activities were detected by staining with the appropriate PNP-glycosides (1). A sample of the crude enzyme prior to the mercury phenyl agarose column was also run in parallel. The results, Table XIV, indicate that most of the protein contaminants have been removed. All of the three  $\beta$ -galactosidase isozymes present in the preparation are still detectable in the mercury phenyl agarose eluate. On the other hand one of the two isozymes of the A-zyyme had been lost in the process.

#### C. B-ZYME FROM CLOSTRIDIUM SPOROGENES (MAEBASHI)

##### 1. Involvement of SH $\rightleftharpoons$ S-S in the B-zyyme from Cl. sporogenes

The assay for the B-zyyme is a two step assay, involving the release of galactose and then the quantitation of the galactose released with galactose-dehydrogenase, GDH. We, therefore, first checked on the effect of  $\text{HgCl}_2$  and DTT on the GDH assay, Table XV.

Having demonstrated that the inhibition of GDH by  $\text{HgCl}_2$  can be reversed by excess DTT we then tested the effect of  $\text{HgCl}_2$  on the B-zyyme activity itself. The B-zyyme was inactivated by the  $\text{HgCl}_2$ , Table XVI. This inactivation, in contrast to that of A-zyyme, apparently could not be reversed

by DTT or DTT plus EDTA. The problem was not with the galactose-dehydrogenase, since subsequent addition of galactose to the incubation mixture could be quantitatively accounted for by the GDH assay, Table XVI.

## 2. Purification of B-zyne by the Use of a Thiol-Sepharose Column.

Since the B-zyne appeared to be irreversibly inactivated by  $\text{HgCl}_2$ , we did not anticipate success with the use of a mercury phenyl column. We therefore explored the possible use of a thiol-bound-sepharose column. B-zyne prepared as previously described (1) was used in these studies. A solution of the B-zyne was passed through a column of covalently bound thiol-sepharose. Most of the activity (96%) came through unadsorbed. The elution pattern is shown in Fig 8. Contents of tubes 3-7 were pooled and concentrated with simultaneous dialysis in an Amicon dialyzer with an XM50 filter. Since there was some loss of activity, the diffusate was also examined and indeed was found to contain 17% of the activity, Table XVII.

Application of the indiffusible material to a Sephacryl S-200 column now showed a clear separation of B-zyne activity from the contaminating (280 nm absorbing) protein, Fig 9. This treatment resulted in an overall 2.5 fold purification and represents a significant step forward.

## D. IMMUNOAFFINITY CHROMATOGRAPHY

### 1. As Applied to Enzymes from *Clostridium perfringens*

#### a) Antibodies to an isozyme of $\beta$ -galactosidase

We have repeatedly observed a number of isozymes of  $\beta$ -Gal'ase in the various preparations of A-zyne isolated from *Cl. perfringens*. The slowest migrating isozyme showed no contamination with other proteins or glycosidase and was considered suitable for the production of antibodies.

Two objectives were contemplated. First, to explore the possibility of using the resulting antibody as an immuno-affinity column to remove not only the slowest but all the isozymes of  $\beta$ -Gal'ase from the initial preparation of A-zyme. Second, to use the antibodies generated to develop a quantitative enzyme-neutralization assay for the initiation of the hybridoma project.

The successful immunization of a rabbit with the slowest isozyme of  $\beta$ -Gal'ase was demonstrated by the precipitin bands obtained in double diffusion analysis in agarose, and by the ability of the antiserum to neutralize  $\beta$ -Gal'ase activity as measured by the PNP- $\beta$ -galactoside colorimetric assay.

b) Quantitation of the Enzyme-Neutralization Assay

It has been repeatedly emphasized that the success of the hybridoma technique to separate monoclonal antibody-producing cells is dependent upon a quick and quantitative assay to detect the antibodies produced. Since we were planning to use the hybridoma technique to isolate our glycosidases in large quantities (1) we considered it expedient to develop a sensitive assay to detect antibody-producing cells by their ability to neutralize the activity of the glycosidases.

The appearance of a publication on the production of monoclonal antibodies against  $\beta$ -Gal'ase from E. coli (6) put a temporary halt to these investigations. Monoclonal antibodies were isolated by these workers which showed the entire spectrum of capabilities, from the complete neutralization of the  $\beta$ -Gal'ase activity to that of no ability to inhibit the enzymatic activity. Some of the isolated antibodies even enhanced the enzymatic activity. The screening procedure we were developing would thus identify only one type of antibody at one end of that spectrum. It thus became apparent that it would be necessary to develop a more

comprehensive assay for screening of hybridomas. This project was therefore temporarily halted in light of the greater success we were having with the procedures involving the sulfhydryl properties of the glycosidases.

## 2. As Applied to B-zyme from *Clostridium sporogenes*

The best preparation of B-zyme, as previously described (1) still contained several contaminating proteins as demonstrated by polyacrylamide gel electrophoresis. The band with B-zyme activity, was separated from the contaminating protein bands by slicing. This further purified B-zyme was used to immunize a rabbit.

The production of antibodies to the B-zyme containing gels was demonstrated by immunodiffusion bands appearing with the rabbit post-but not pre-immunization serum. The rabbit antiserum was purified by salting out of the gamma globulin with ammonium sulfate precipitation (0-33% saturation) followed by a DEAE fractionation procedure.

The purified antibody thus obtained was then coupled to sepharose using the cyanogen bromide technique (7). Excess cyanogen bromide sites were masked by interaction with ethanolamine.

The immobilized anti-"pure"-B-zyme was now ready to use as an immuno-affinity column with a crude B-zyme preparation. The B-zyme activity readily adsorbed to the affinity column with 51% of the total protein unadsorbed, Table XVIII. A number of chaotropic reagents, namely NaSCN, urea and guanidine HCl, were used in an attempt to elute the B-zyme (8). All were successful in eluting protein from the affinity column, but the eluates were enzymatically inactive. Re-examination of the problem revealed that the chaotropic agents, at the concentrations used, 2M, inactivated the B-zyme. At a concentration of 0.2M NaSCN, the enzyme

showed almost full activity. However, when used as an eluant, the 0.2M NaSCN eluted most of the adsorbed protein. Again, the eluate was inactive.

Resorting to a linear gradient of NaCl, 0-2M NaCl in 0.01M pH 7.0 phosphate buffer, we succeeded in accounting for 73% of the total protein and 56% of the initial activity, and a 2-3 fold purification, Table XVIII. This method shows promise and is worth developing.

#### E. PLACENTAL GLYCOSIDASES

Prof. Ray Brown from Wayne State Medical School has had extensive experience in the separation of proteins of similar properties by the use of iso-electric focusing. He recently undertook the challenge of assisting us in the separation of the A-zyme from the other contaminating glycosidases from Cl. perfringens. He experienced as much difficulty and frustration as we had.

Recently he has switched over to working with placentas as a source of the glycosidases. These glycosidases are of the lysosomal type, with a pH optimum of 4.5.

Dr. Brown gave us a sample of the enzyme at the early stages of purification. The enzyme was compared to our partially purified A-zyme and B-zyme, Tables XIX and XX. We are eagerly awaiting more highly purified preparations of the placental enzymes for determination of their action on blood group substances and their effects on the viability of erythrocytes.

#### F. STEREOSPECIFIC INHIBITION OF GLYCOSIDASES INVOLVED IN THE ABO BLOOD GROUP SYSTEM

In the previous report (1) we had indicated that only 20% of all the fucose could be released from A<sup>+</sup> PSM, as compared to 100 % from H<sup>+</sup> PSM.

Addition of A-zyme resulted in the release of all the fucose. A clarification of the results became evident when individual oligosaccharides isolated from the initial glycoprotein by the  $\beta$ -elimination procedure (9), were used as substrates. The tetrasaccharide readily released all its fucose, but the pentasaccharide did not release any, Table XXI. Addition of A-zyme resulted in the cleavage of the terminal non-reducing N-acetyl-galactosaminy1 residue and the molecule now became susceptible to fucosidase, Table XXI.

In like manner, the oligosaccharide alditols, obtained by the  $\beta$ -elimination method from B<sup>+</sup> ovarian cyst and used as substrate for our B-zyme assay, released 22% of its total fucose with the  $\alpha(1\rightarrow2)$ -L-fucosidase alone. The amount of fucose released by the fucosidase could be raised to 40% by the addition of B-zyme from Cl. sporogenes, itself free of fucosidase, Table XXI. Thus indicating that in this unseparated mixture of B<sup>+</sup> oligosaccharide alditols the terminal non-reducing  $\alpha$ -galactosyl residue, the B-determined, is also effective in stereospecifically inhibiting the action of the fucosidase. The results were further confirmed by the use of chemically synthesized A- and B-active trisaccharides bound to a high molecular weight complex, Table XXII.

It is appropriate to reiterate at this point that both  $\alpha(1\rightarrow3)$ -GalNAc and  $\alpha(1\rightarrow3)$ -Gal residues inhibit the release of fucose by  $\alpha(1\rightarrow2)$ -L-fucosidase. The converse, however, is not true.  $\alpha(1\rightarrow2)$ -Fucosyl residues do not interfere in the action of A-zyme or B-zyme.

The availability of the corresponding synthetic disaccharide derivatives enabled us to establish, Table XXII, that both A-zyme and B-zyme can act on the corresponding disaccharides even in the absence of the  $\alpha(1\rightarrow2)$ -L-fucosyl residue.

ABBREVIATIONS

|                     |                                                                                                             |
|---------------------|-------------------------------------------------------------------------------------------------------------|
| a-OSM               | Asialo-ovine submaxillary mucin                                                                             |
| aRBC                | Asialo-erythrocytes                                                                                         |
| A-zyme              | ( $\alpha 1 \rightarrow 3$ ) <u>N</u> -acetyl- <u>D</u> -galactosaminidase, enzyme that destroys A activity |
| $\beta$ -Gal'ase    | $\beta$ -galactosidase                                                                                      |
| $\beta$ -GlcNAc'ase | $\beta$ - <u>N</u> -acetylglucosaminidase                                                                   |
| B-zyme              | ( $\alpha 1 \rightarrow 3$ ) <u>D</u> -galactosidase, enzyme that destroys B activity                       |
| CPD                 | Citrate Phosphate Dextrose                                                                                  |
| DTT                 | Dithiolthreitol                                                                                             |
| EDTA                | Ethylene diamine tetra-acetate                                                                              |
| GalNAc              | <u>N</u> -acetyl- <u>D</u> -galactosamine                                                                   |
| GDH                 | Galactose Dehydrogenase                                                                                     |
| GSH                 | Reduced Glutathione                                                                                         |
| GSSG                | Oxidized Glutathione                                                                                        |
| H <sup>+</sup> -PSM | H-active porcine submaxillary mucin                                                                         |
| OSM                 | Ovine Submaxillary mucin                                                                                    |
| PAGE                | Polyacrylamide gel electrophoresis                                                                          |
| PNP                 | Paranitrophenyl-                                                                                            |
| R <sub>e</sub>      | Relative electrophoretic mobility                                                                           |
| SDS                 | Sodium dodecyl sulfate                                                                                      |
| TBA                 | Thiobarbituric Acid                                                                                         |

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Fig 1

ELUTION PATTERN OF GLYCOSIDASES FROM SEPHACRYL S-300,  
AT PH 5.0 AND WITHOUT DTT

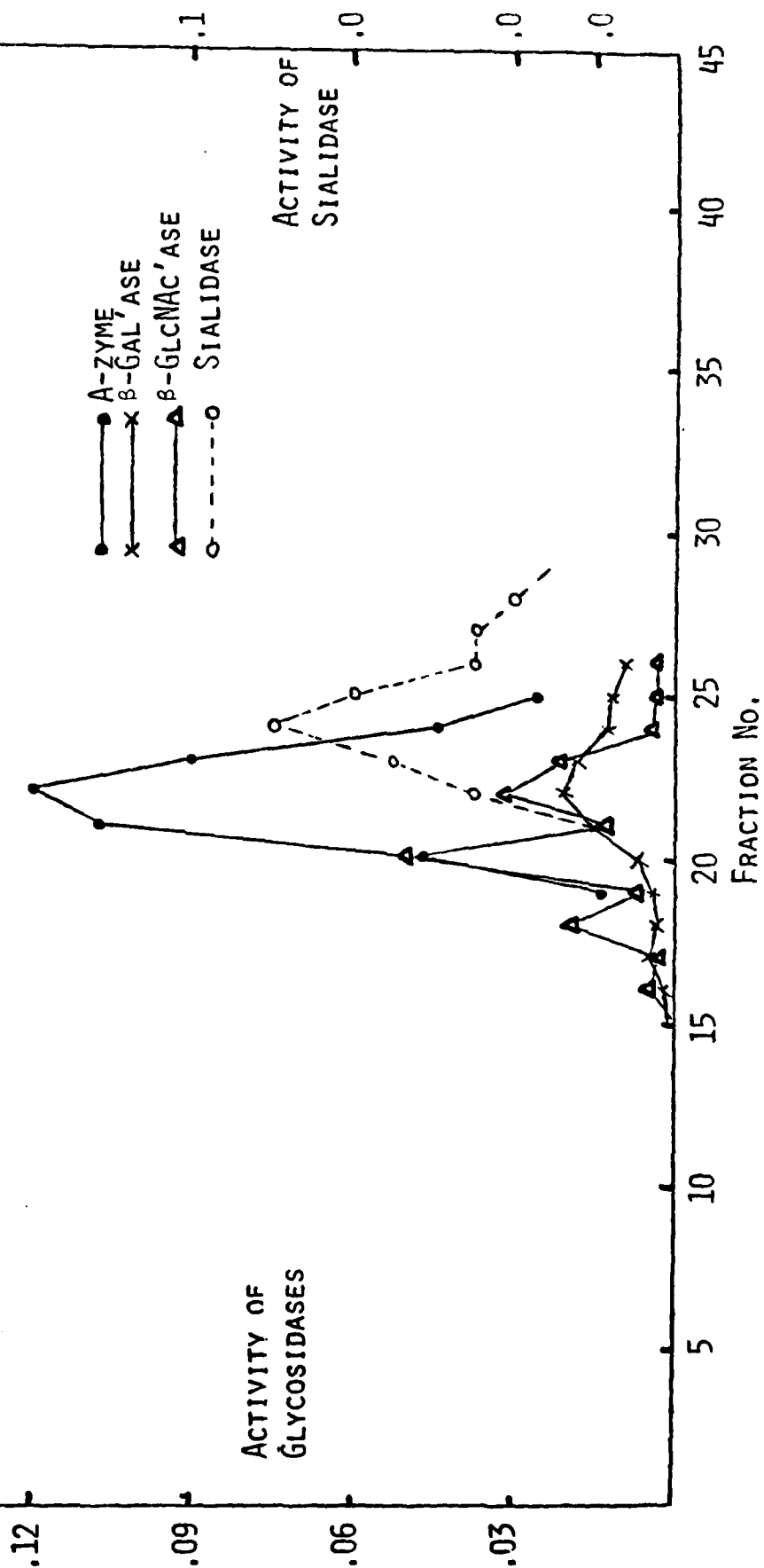


FIG 2

ELUTION PATTERN OF GLYCOSIDASES FROM SEPHACRYL S-300  
AT PH 8.0 AND WITHOUT DTT

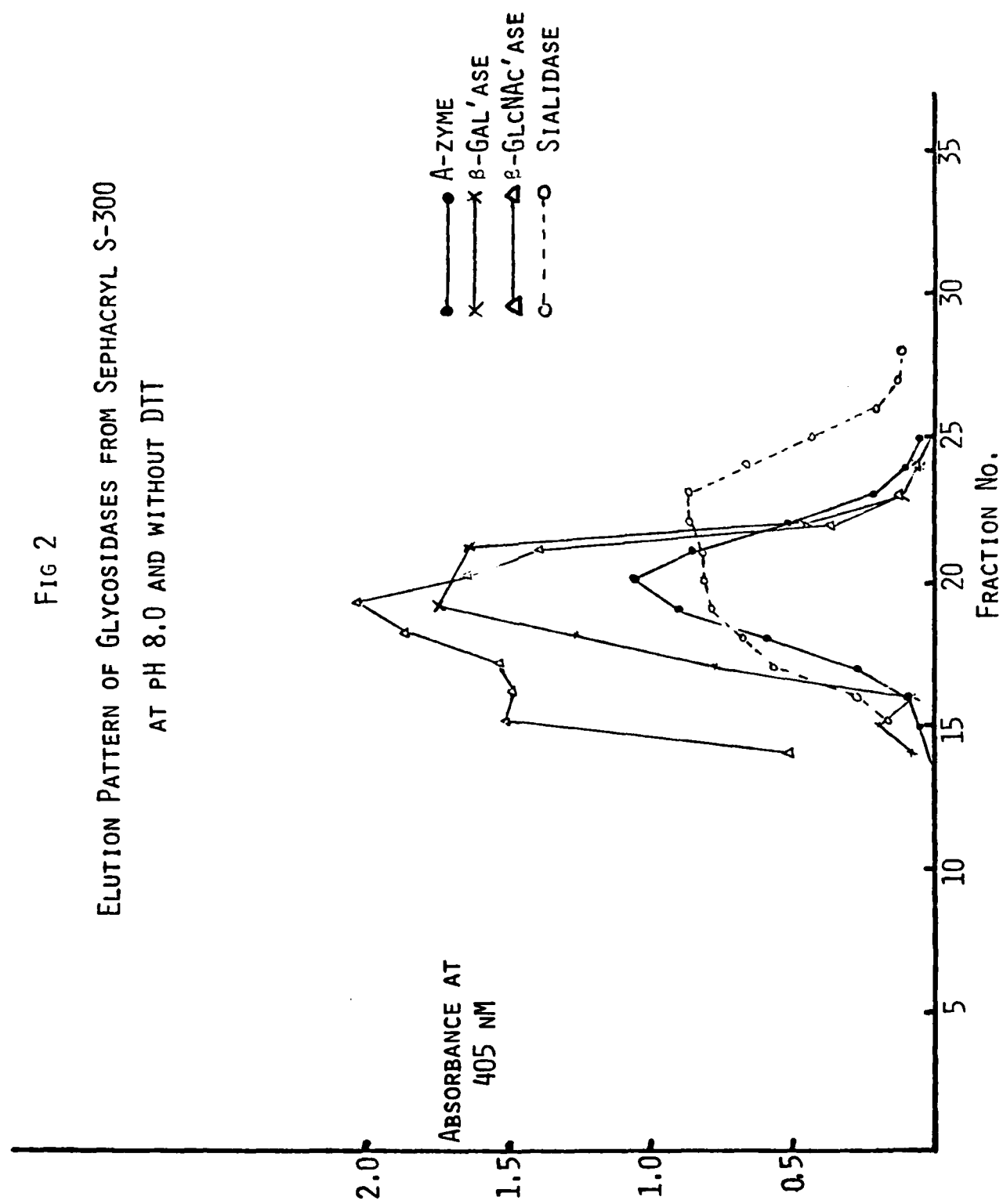


FIG 3  
EFFECT OF  $Hg^{++}$  ON ACTIVITY OF GLYCOSIDASES

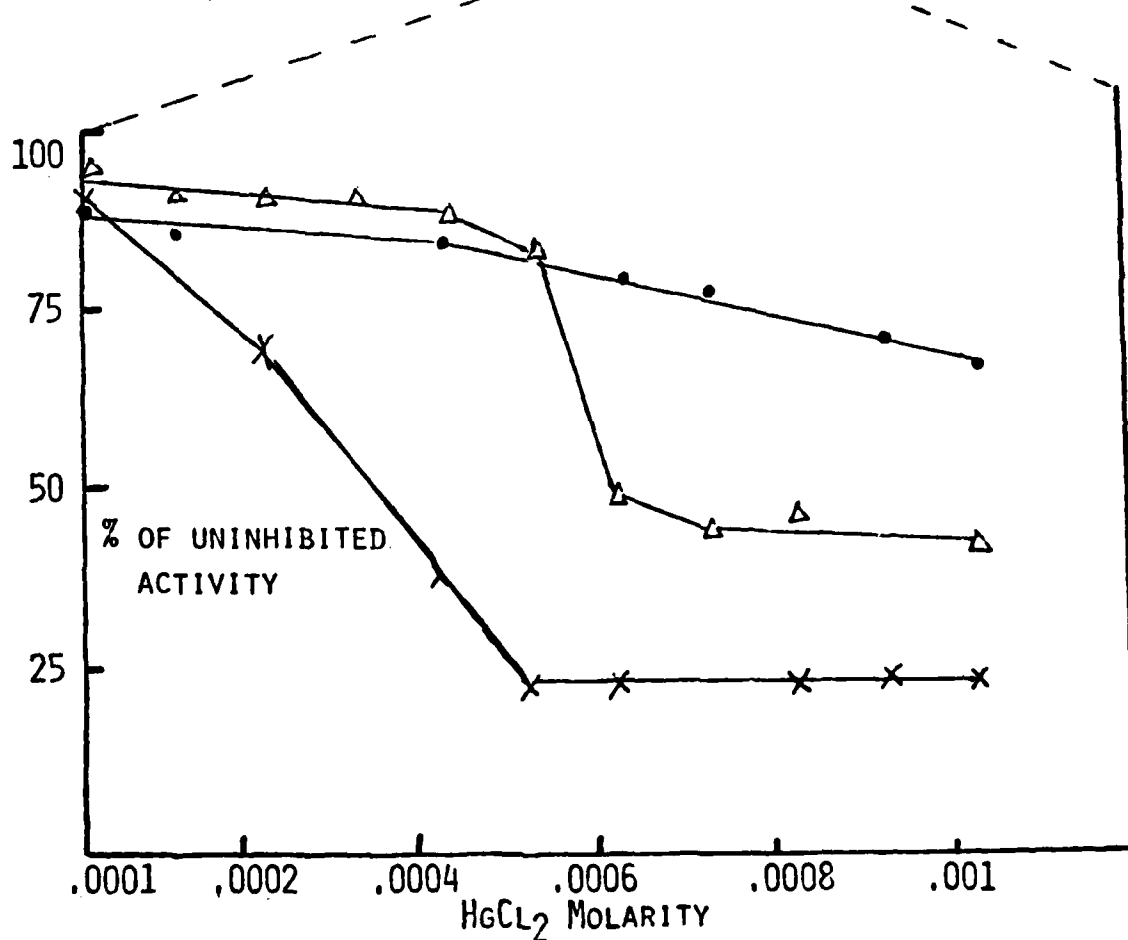
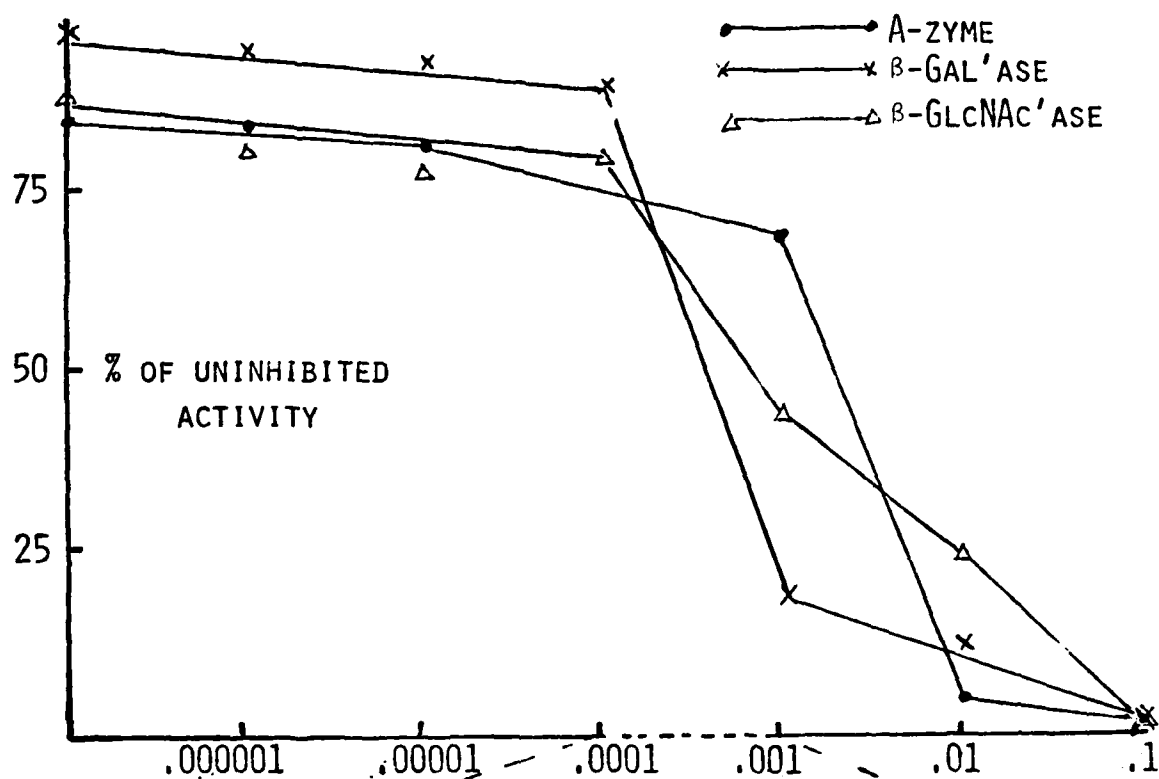


FIG 4

RESPONSE OF  $\beta$ -GALACTOSIDASE  
TO REDOX REAGENTS

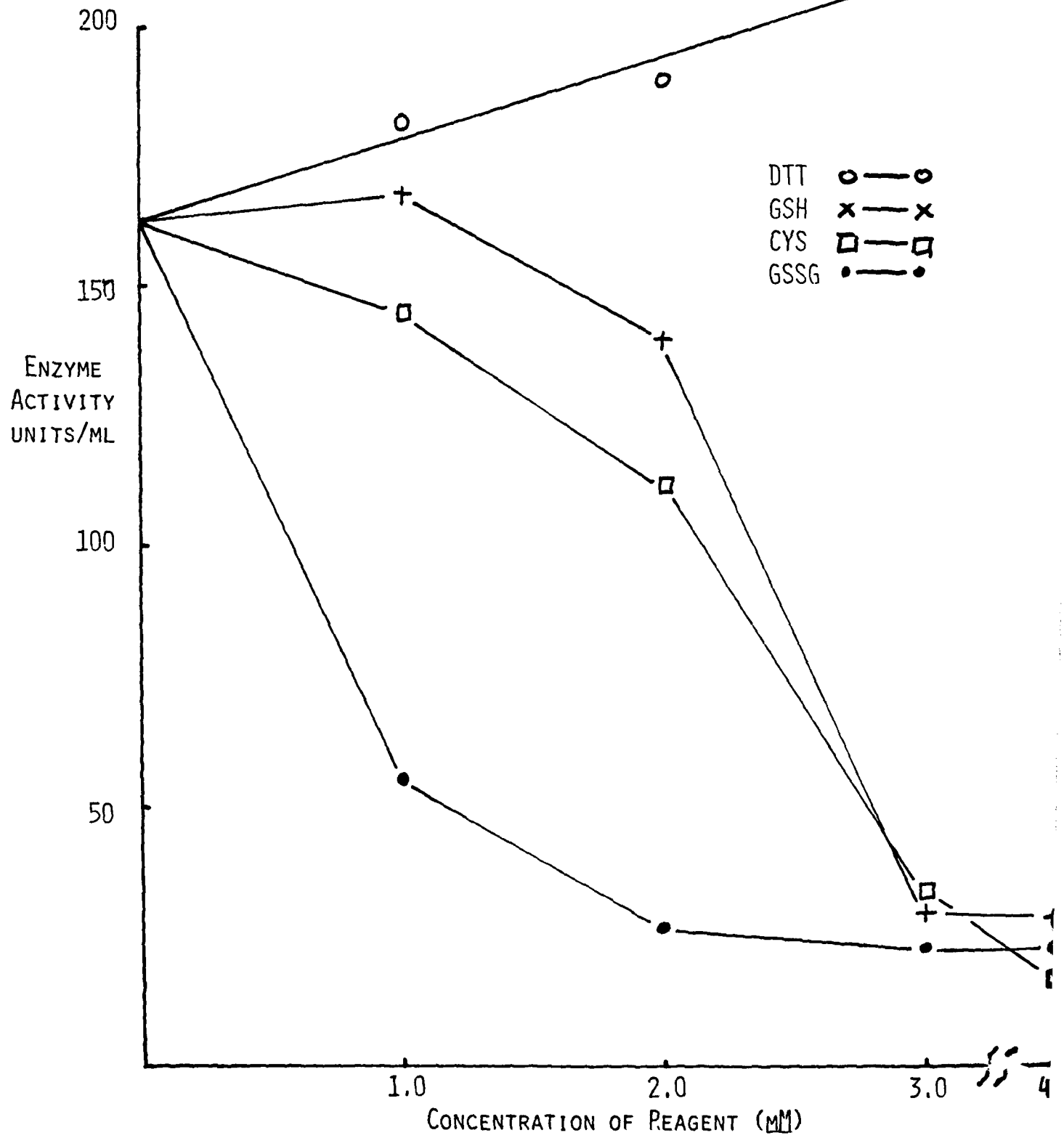


FIG 5  
ACTIVITY OF  $\beta$ -GALACTOSIDASE IN THE PRESENCE OF  
DECREASING PERCENTAGE OF OXIDANT GSSG (2MM)  
AND INCREASING AMOUNTS OF REDUCTANTS;-

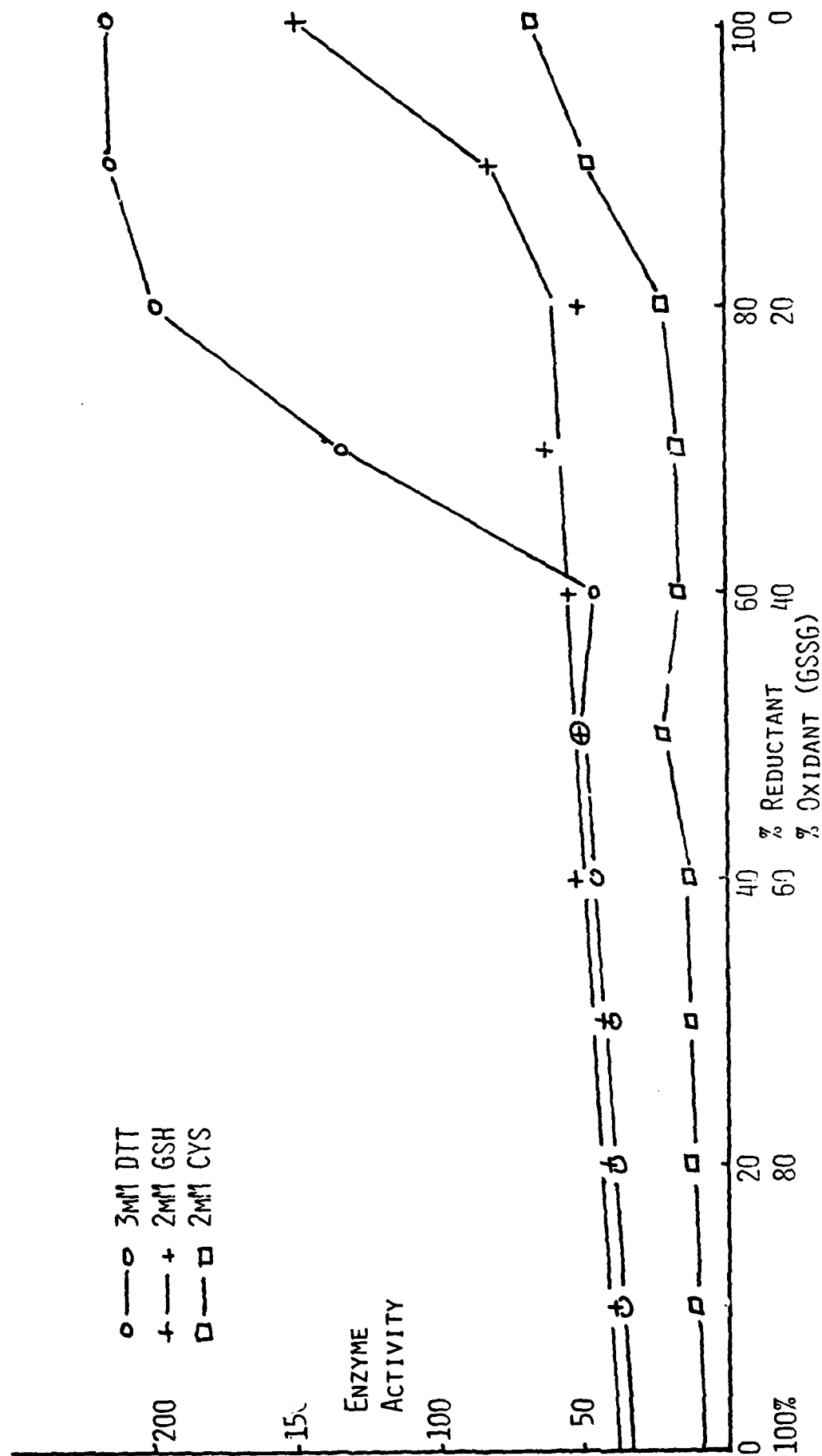


FIG 6A

SPECIFIC ACTIVITIES OF GLYCOSIDASES  
SEPARATED ON SEPHACRYL S-200

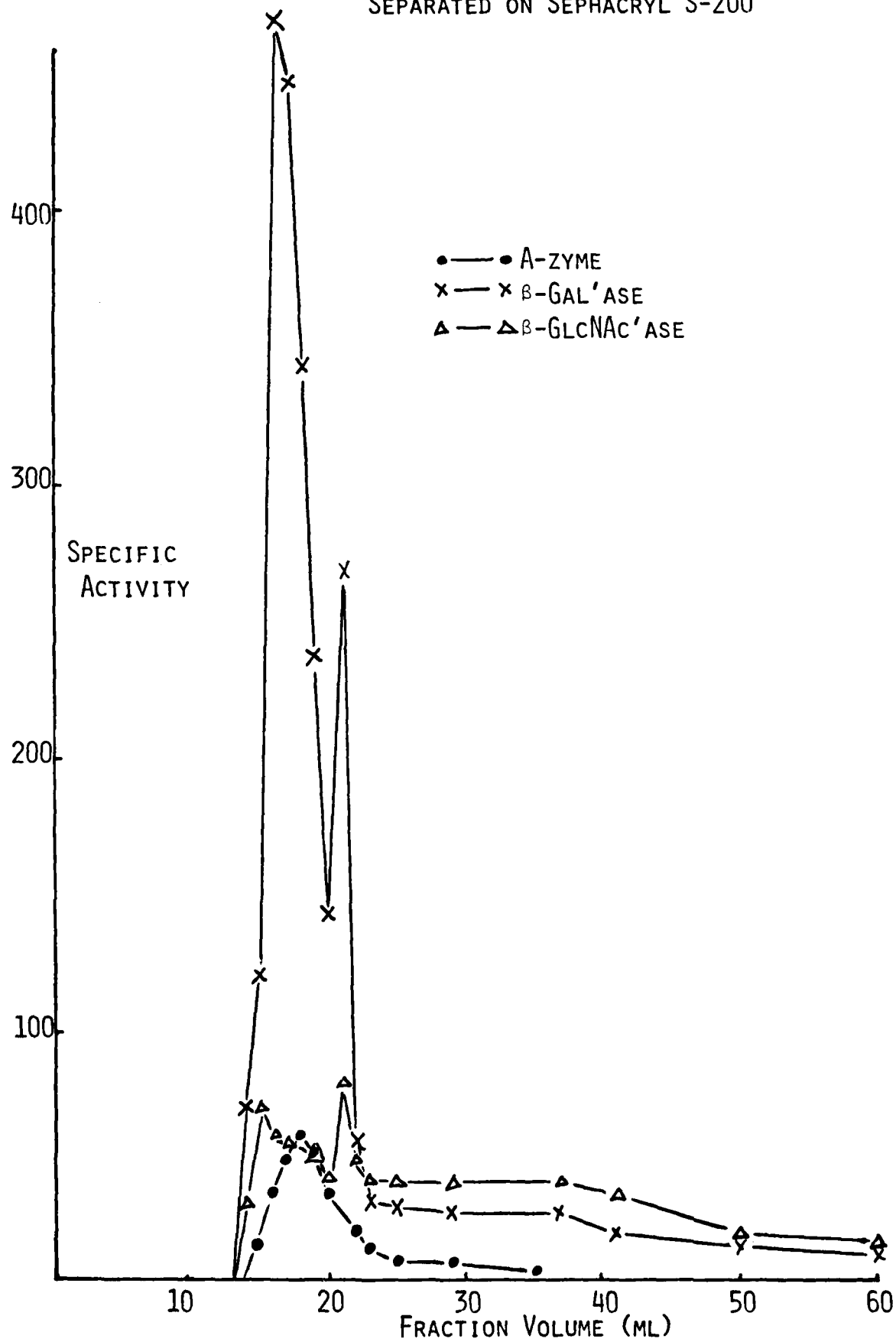
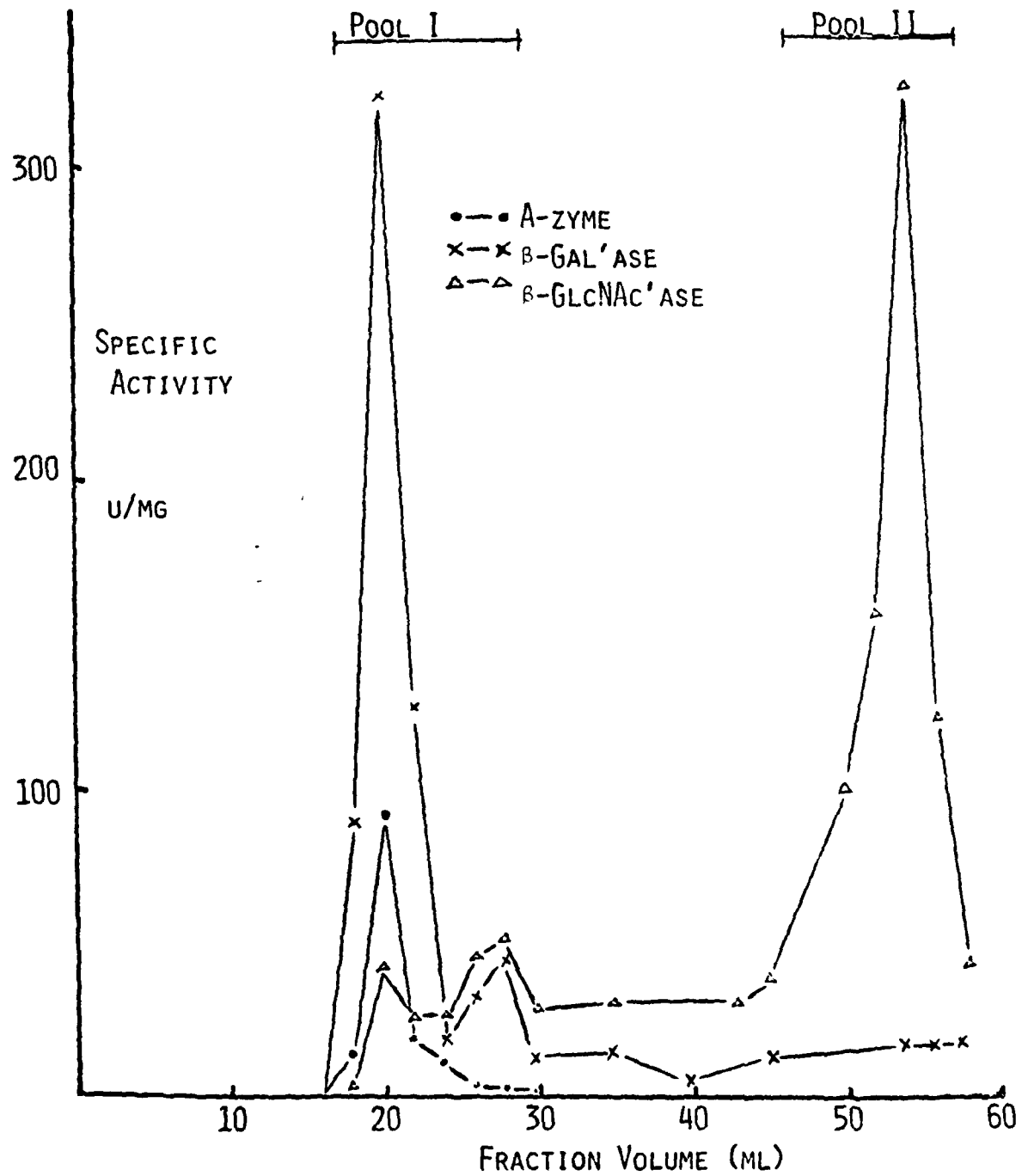


FIG 6B

SPECIFIC ACTIVITIES OF GLYCOSIDASES FROM  
S-200 AFTER DTT TREATMENT (20 mM)



# ADSORPTION AND ELUTION OF A-ZYME AND OTHER GLYCOSIDASES FROM A COVALENT THIOL COLUMN

FIG 7

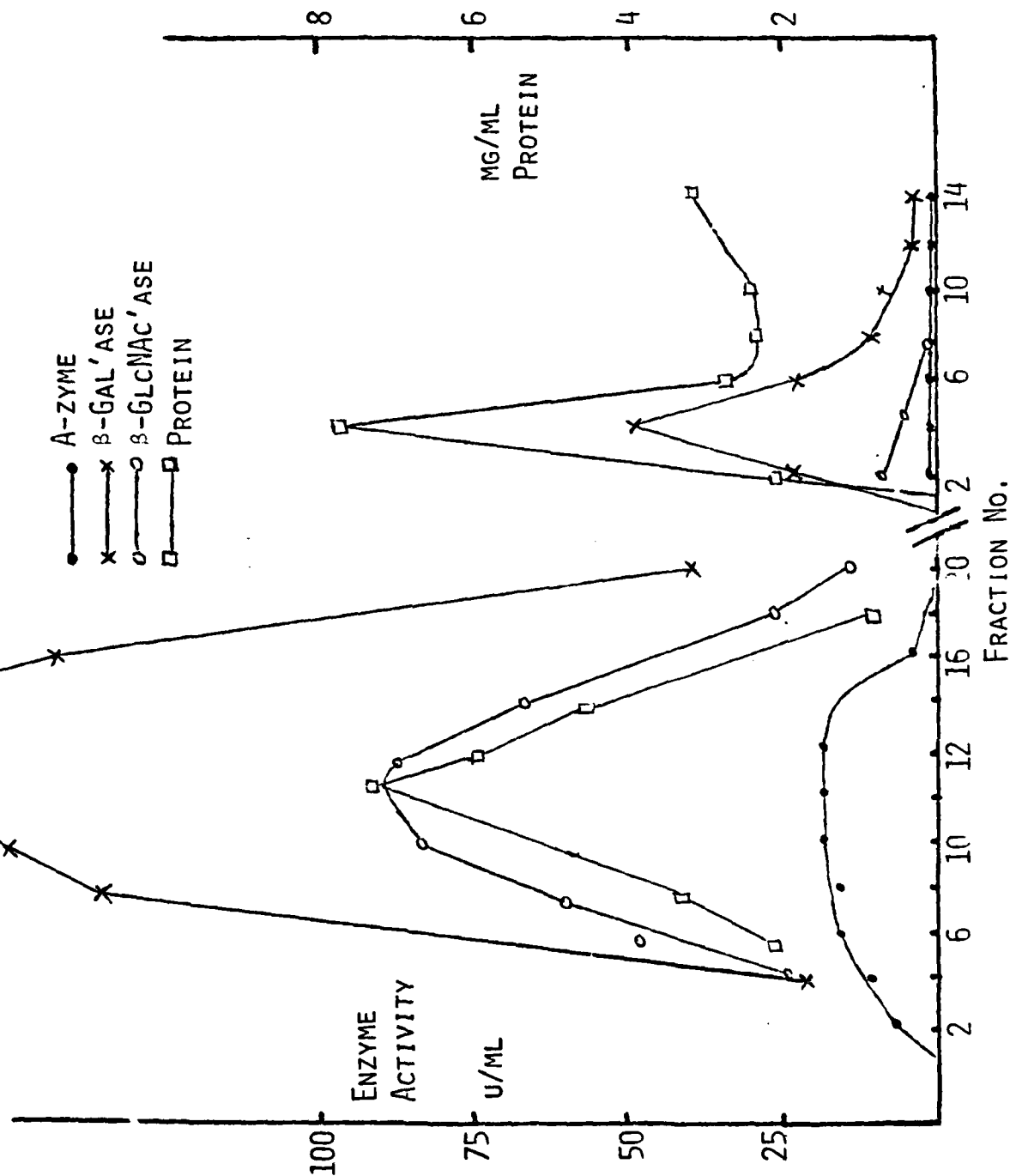


FIG 8  
ELUTION PATTERN OF B-ZYME  
FROM THIOL - SEPHAROSE 4B

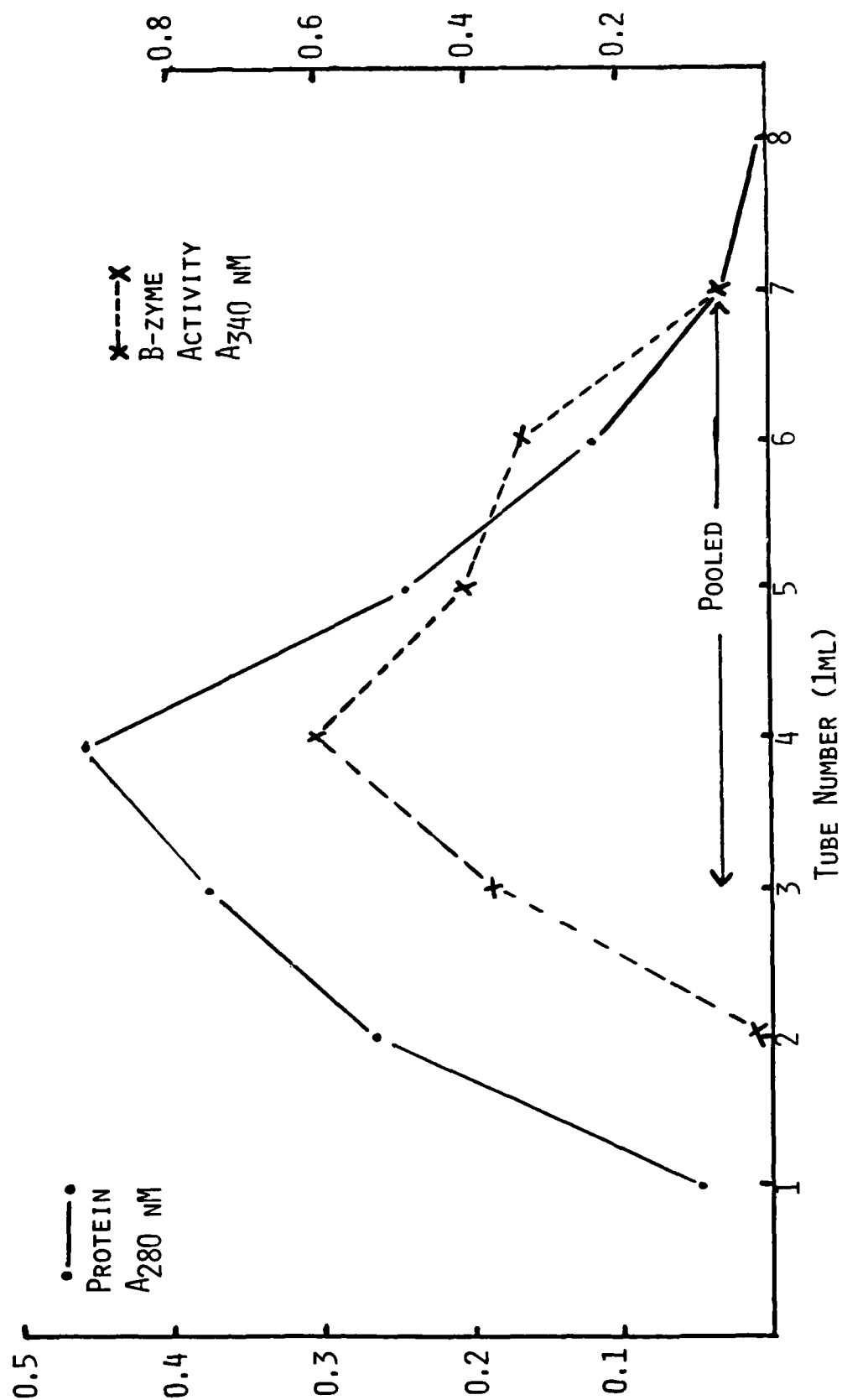


Fig 9  
 SEPHACRYL S-200  
 ELUTION PATTERN OF B-ZYME AFTER  
 THIOL - SEPHAROSE TREATMENT

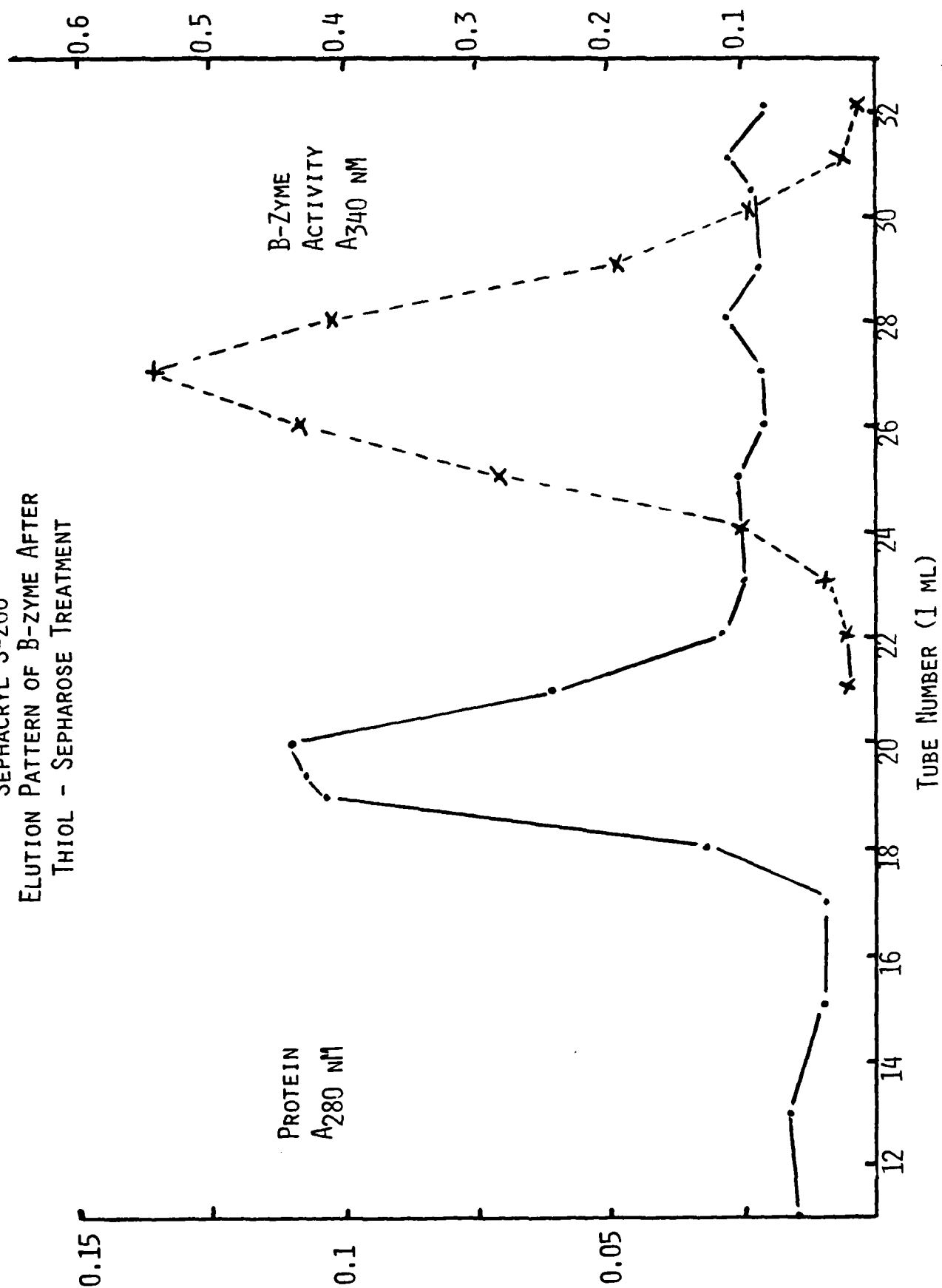


TABLE I

BIOCHEMICAL REACTIONS OF A<sup>+</sup>, A<sup>-</sup>

|             | A <sup>+</sup> | A <sup>-</sup> | PERFR. | SPOROG. |
|-------------|----------------|----------------|--------|---------|
| NITRATE     | +              | -              | +      | -       |
| INDOLE      | -              | -              | -      | -       |
| UREA        | -              | -              | -      | -       |
| GLUCOSE     | A              | A              | A      | V       |
| MANNITOL    | A              | -              | -/+    | -       |
| LACTOSE     | A              | -              | A      | -       |
| SACCHAROSE  | A              | -              | A      | -       |
| MALTOSE     | A              | A              | A      | V       |
| SALICIN     | A              | -              | -/+    | -       |
| XYLOSE      | -              | -              | -      | -       |
| ARABINOSE   | -              | -              | -      | -       |
| GELATIN     | +              | +              | +      | +       |
| ESCULIN     | -              | +              | -/+    | +       |
| GLYCEROL    | -              | -              | V      | -       |
| CELLOBIOSE  | -              | -              | -      | -       |
| MANNOSE     | -              | -              | A      | -       |
| MELEZITOSE  | -              | -              | -      | -       |
| RAFFINOSE   | A              | -              | -/A    | -       |
| SORBITOL    | -              | -              | -      | -       |
| RHAMNOSE    | A              | -              | -      | -       |
| TREHALOSE   | A              | -              | +/A    | -/+     |
| LECITHINASE | +              | -              | +      | -       |
| LIPASE      | -              | +              | -      | +       |

LIST OF SYMBOLS: + TEST RESULTS GENERALLY POSITIVE  
 - TEST RESULTS GENERALLY NEGATIVE  
 +/- TEST RESULTS MORE OFTEN POSITIVE  
 BUT NEGATIVES OCCUR  
 -/+ TEST RESULTS MORE OFTEN NEGATIVE  
 BUT POSITIVES OCCUR  
 A ACID REACTION  
 V TEST RESULT VARIABLE

TABLE II

CHROMATOGRAPHIC ANALYSIS OF  
METABOLIC PRODUCTS OF  $A^+$ ,  $A^-$ 

| $A^+$        | $A^-$         | PERFR.      | SPOROG.      |
|--------------|---------------|-------------|--------------|
| 1. ACETIC    | 1. ACETIC     | ACETIC      | ACETIC       |
| 1. BUTYRIC   | s. BUTYRIC    | BUTYRIC     | BUTYRIC      |
| s. PROPIONIC | s. ISOBUTYRIC | (PROPIONIC) | (ISOBUTYRIC) |
|              | s. PROPIONIC  |             | (PROPIONIC)  |
|              | s. ISOVALONIC |             | (ISOVALERIC) |

LIST OF SYMBOLS: 1. LARGE  
s. SMALL  
( ) VARIABLE

TABLE III

COMPARISON OF GLYCOSIDASE ACTIVITIES OF  
A+ AND A- STRAINS GROWN UNDER IDENTICAL CONDITIONS

| <u>GLYCOSIDASE</u>  | <u>SPECIFIC ACTIVITY</u> |           | <u>ACTIVITIES IN A-</u><br><u>AS % OF A+</u> |
|---------------------|--------------------------|-----------|----------------------------------------------|
|                     | <u>A+</u>                | <u>A-</u> |                                              |
| A-ZYME              | 0.15                     | 0.0015    | 1                                            |
| $\beta$ -GAL'ASE    | 4.50                     | 0.34      | 7.6                                          |
| $\beta$ -GLCNAc'ASE | 3.7                      | 0.93      | 25.1                                         |
| SIALIDASE           | 96                       | 42        | 42.7                                         |

TABLE IV

PURIFICATION OF  $\alpha$ -N-ACETYL GALACTOSAMINIDASE (A-ZYME)

| STEP                                                                                                      | Sp. Act.<br>U/MG | YIELD<br>% | FOLD<br>PURIFICATION | A-ZYME<br>SIALIDASE | A-ZYME<br>$\beta$ -GAL'ASE | A-ZYME<br>$\beta$ -GLCNAC'ASE |
|-----------------------------------------------------------------------------------------------------------|------------------|------------|----------------------|---------------------|----------------------------|-------------------------------|
| 1. CRUDE FILTRATE                                                                                         | 0.13             | 100        | 1                    | 0.12                | 0.06                       | 0.04                          |
| 2. AMMONIUM SULFATE<br>0-67% SAT.                                                                         | 0.48             | 76         | 3.7                  | 0.12                | 0.06                       | 0.05                          |
| 3. SEPHACRYL S-200                                                                                        | 3.8              | 61         | 29                   | 0.12                | 0.08                       | 0.08                          |
| 4. DEAE-SEPHACEL                                                                                          | 55               | 35         | 425                  | 5.3                 | 0.18                       | 0.41                          |
| 5. ISOELECTRIC PRE-<br>CIPITATION                                                                         | 149              | 34         | 1125                 | 9.0                 | 2.9                        | 4.7                           |
| 6. NEGATIVE ADSORPTION<br>WITH TYPE O RBC<br>FOLLOWED BY:<br>A) SEPHAROSE 6B<br>B) ISOELECTRIC PPTN. 1043 |                  | 17         | 8025                 | 542                 | 53                         | 79                            |

TABLE V

## PURIFICATION OF A-ZYME BY RAPID/EFFICIENT PROCEDURE

| STEP                   | VOL<br>ML. | UNITS<br>U/ML | SPECIFIC<br>ACTIVITY<br>U/MG | YIELD<br>% | FOLD<br>PURIFICATION |
|------------------------|------------|---------------|------------------------------|------------|----------------------|
| CRUDE FILTRATE         | 6640       | 0.35          | 0.025                        | 100        | 1                    |
| MILLIPORE CONC.        | 191        | 12.1          | 0.22                         | 100        | 8.8                  |
| 40-65% $\text{AmSO}_4$ | 38.5       | 53            | 0.88                         | 87         | 35                   |
| SEPHACRYL S-200        | 255        | 6.7           | 3.9                          | 85         | 160                  |
| DEAE                   | 100        | 16            | 30.0                         | 70         | 1200                 |

|      | A-ZYME<br>SIALIDASE | A-ZYME<br>$\beta$ GAL'ASE | A-ZYME<br>$\beta$ GLCNAC'ASE |
|------|---------------------|---------------------------|------------------------------|
| DEAE | 19.5                | 0.57                      | 1.1                          |

TABLE VI

USE OF ACETONE TO PURIFY THE A-ZYME  
(PURIFICATION FACTOR AT EACH STEP SHOWN IN PARENTHESIS)

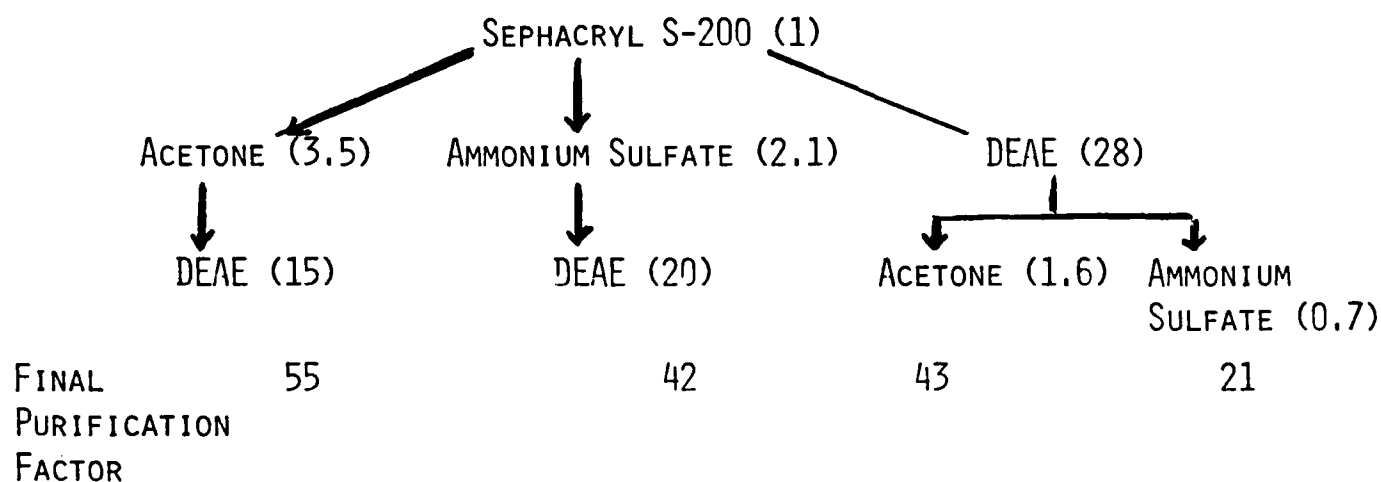


TABLE VII

EFFECT OF PH ON SOLUBILITY AND STABILITY OF BACTERIAL  
A-ZYME,  $\beta$  GALACTOSIDASE AND  $\beta$  NACETYLGLUCOSAMINIDASE

| ENZYME              | FRACTION         | pH  |      |      |      |     |
|---------------------|------------------|-----|------|------|------|-----|
|                     |                  | 4.0 | 4.25 | 4.50 | 4.75 | 5.0 |
| A ZYME              | % IN SUPERNATANT | 46  | 22   | 50   | 92   | 107 |
|                     | % IN PPT.        | 14  | 53   | 36   | 7.4  | 2.9 |
|                     | % RECOVERED      | 60  | 75   | 86   | 100  | 110 |
| $\beta$ GLC'NAC'ASE | % IN SUPERNATANT | 11  | 5.8  | 17   | 33   | 40  |
|                     | % IN PPT.        | 18  | 39   | 36   | 21   | 6.2 |
|                     | % RECOVERED      | 29  | 45   | 53   | 54   | 46  |
| $\beta$ GAL'ASE     | % IN SUPERNATANT | 5.6 | 2.1  | 3.4  | 8.5  | 15  |
|                     | % IN PPT.        | 27  | 40   | 39   | 26   | 3   |
|                     | % RECOVERED      | 33  | 42   | 42   | 34   | 18  |
| SIALIDASE           | % IN SUPERNATANT | 0   | 5.6  | 42   | 58   | 78  |
|                     | % IN PPT.        | 0   | 5.6  | 5    | 3    | 4   |
|                     | % RECOVERED      | 0   | 11.0 | 47   | 61   | 82  |

TABLE VIII

REPEATED TREATMENT OF GLYCOSIDASES AT pH 5.0  
AND ITS EFFECT ON ACTIVITIES OF A-ZYME,  
 $\beta$ -NACETYLGLUCOSAMINIDASE AND  $\beta$ -GALACTOSIDASE

| % ACTIVITY<br>OF SUPERNATANT RELATIVE TO UNTREATED ENZYME |                  |                                   |                                   |
|-----------------------------------------------------------|------------------|-----------------------------------|-----------------------------------|
|                                                           | <u>UNTREATED</u> | <u>1ST EXPOSURE<br/>TO pH 5.0</u> | <u>2ND EXPOSURE<br/>TO pH 5.0</u> |
| A-ZYME                                                    | 100              | 87 $\pm$ 2                        | 86 $\pm$ 2                        |
| $\beta$ -GLCNAc'ASE                                       | 100              | 77 $\pm$ 3                        | 55 $\pm$ 5                        |
| $\beta$ -GAL'ASE                                          | 100              | 37 $\pm$ 1                        | 36 $\pm$ 4                        |

TABLE IX

MOLECULAR WEIGHTS OF SOME GLYCOSIDASES  
FROM CLOSTRIDIUM PERFRINGENS FILTRATES

| <u>ENZYME</u>       | <u>APPROXIMATE MOL. WT. AT pH 5.0</u> |
|---------------------|---------------------------------------|
| A-ZYME              | 220,000                               |
| $\beta$ -GAL'ASE    | 230,000                               |
| $\beta$ -GLCNAC'ASE | 170,000 - 450,000                     |
| SIALIDASE           | 50 - 100,000                          |

TABLE X

REACTIVATION OF A-ZYME BY DTT AND EDTA  
AFTER INHIBITION BY 0.5 mM HgCl<sub>2</sub>

| <u>I</u> | <u>DTT</u> | <u>EDTA</u> | <u>DTT &amp;<br/>EDTA</u> |
|----------|------------|-------------|---------------------------|
| .02M     | 100        | 21          | 23                        |
| .001M    | 100        | 46          | 39                        |
| .0005M   | 98         | 40          | 21                        |
| .00025M  | 92         | 43          | 41                        |

TABLE XI

## EFFECT OF DITHIOTHREITOL (DTT) ON THE GLYCOSIDASES

|                        | <u>UNITS/ML</u>       |                         |           |
|------------------------|-----------------------|-------------------------|-----------|
|                        | <u>ABSENCE OF DTT</u> | <u>PRESENCE OF DTT*</u> | <u>%Δ</u> |
| <u>ENZYME PREP. I</u>  |                       |                         |           |
| α-GALNAC'ASE           | 17.0                  | 18.0±0.1                | 5.9±0.6   |
| β-GAL'ASE              | 64.6±0.3              | 71.8±0.2                | 11.0±0.8  |
| β-GLCNAC'ASE           | 9.7±0.2               | 8.7±0.1                 | -10.0±1.0 |
| <u>ENZYME PREP. II</u> |                       |                         |           |
| β-GALNAC'ASE           | 16.2±0.1              | 19.3                    | 19.0      |
| β-GAL'ASE              | 132.0±3.0             | 149.0±1.0               | 13.0±3    |
| β-GLCNAC'ASE           | 34.0±0.4              | 31.7±0.2                | -6.8±2    |

\* 40MM DTT USED TO ASSURE MAXIMUM ACTIVITIES OF THE α-GALNAC'ASE.

TABLE XII

THE SEPARATION OF GLYCOSIDASES ON SEPHACRYL S-200  
AFTER TREATMENT WITH DITHIOTHREITOL (20mM)

| ENZYME               | TOTAL<br>UNITS<br>APPLIED | SPEC.<br>ACT. | UNITS<br>RECOVERED |         | % RECOVERED |         | SPEC. ACT. |         | PURIFICATION<br>FACTOR |         |
|----------------------|---------------------------|---------------|--------------------|---------|-------------|---------|------------|---------|------------------------|---------|
|                      |                           |               | POOL I             | POOL II | POOL I      | POOL II | POOL I     | POOL II | POOL I                 | POOL II |
| $\alpha$ -GALNAC'ASE | 40.3                      | 32.5          | 39.5               | 0       | 98.0        | 0       | 43.7       | 0       | 0.1                    | 0       |
| $\beta$ -GAL'ASE     | 232.5                     | 187.5         | 161.0              | 0.4     | 69.2        | 2.0     | 176.0      | 15.4    | 0.9                    | 0.1     |
| $\beta$ -GLCNAC'ASE  | 51.0                      | 41.1          | 29.2               | 3.5     | 57.0        | 6.8     | 31.9       | 133.5   | 0.8                    | 325     |

TABLE XIII

PURIFICATION OF A-ZYME BY ADSORPTION AND ELUTION FROM  
MERCURY-PHENYL AGAROSE

| <u>FRACTION</u>                       | <u>PROTEIN</u><br><u>MG/ML</u> | <u>TOTAL</u><br><u>UNITS</u> | <u>SPECIFIC</u><br><u>ACTIVITY</u><br><u>U/MG</u> | <u>FOLD</u><br><u>PURIFICATION</u> |
|---------------------------------------|--------------------------------|------------------------------|---------------------------------------------------|------------------------------------|
| ENZYME PREPARATION<br>1200 X PURIFIED | 13.4                           | 3.75                         | 0.28                                              | 1                                  |
| <u>ELUTION FRACTIONS</u>              |                                |                              |                                                   |                                    |
| 1. 1MM HgCL <sub>2</sub>              | .17                            | 0.12                         | .72                                               | 2.6                                |
| 2. 1MM HgCL <sub>2</sub>              | .34                            | 0.32                         | .95                                               | 3.4                                |
| 3. 2MM HgCL <sub>2</sub>              | .11                            | 0.32                         | 2.87                                              | 10.2                               |
| 4. 5MM HgCL <sub>2</sub>              | .08                            | 0.31                         | 3.84                                              | 13.7                               |
| 5. 10MM HgCL <sub>2</sub>             | .07                            | 0.31                         | 4.45                                              | 16.0                               |
| 6. 20MM HgCL <sub>2</sub>             | .05                            | <u>0.32</u>                  | 6.48                                              | 23.1                               |
| TOTAL                                 |                                | 1.7                          |                                                   |                                    |
| %                                     |                                | 45                           |                                                   |                                    |

ENZYMATIC ACTIVITY RESTORED BY INCUBATION WITH DTT

TABLE XIV

PAGE GELS OF A-ZYME FROM  
MERCURY-PHENYL AGAROSE COLUMN

| <u>STAIN</u>     | <u>RE</u><br><u>CRUDE ENZYME</u> | <u>RE</u><br><u>ELUATE</u>                   |
|------------------|----------------------------------|----------------------------------------------|
| A-ZYME (P-NP)    | I. .392                          |                                              |
|                  | II. .255                         | I. .255                                      |
| B-GAL'ASE (P-NP) | I. .413                          | I. .390                                      |
|                  | II. .298                         | II. .326                                     |
|                  | III. .250                        | III. .255                                    |
| COOMASSIE BLUE   | I. .475                          | INSUFFICIENT<br>PROTEIN FOR<br>VISIBLE BANDS |
|                  | II. .365                         |                                              |
|                  | III. .315                        |                                              |
|                  | IV. .245                         |                                              |
|                  | V. .225                          |                                              |
|                  | VI. .205                         |                                              |

TABLE XV

## GALACTOSE DEHYDROGENASE ASSAY FOR FREE GALACTOSE

| INCUBATION MIXTURE                                            | % ACTIVITY |
|---------------------------------------------------------------|------------|
| GALACTOSE DH + GALACTOSE                                      | 100        |
| GALACTOSE DH + 12.5MM DTT + GALACTOSE                         | 98±2       |
| GALACTOSE DH + 0.62MM HgCL <sub>2</sub> + GALACTOSE           | <1         |
| GALACTOSE DH + 1MM HgCL <sub>2</sub> + 12.5MM DTT + GALACTOSE | 100±4      |

NOTE: WITH GALACTOSE DH AND GALACTOSE KEPT CONSTANT

TABLE XVI

## THE B-ZYME ASSAY

| <u>INCUBATION MIXTURE</u>                                              | <u>ADDITION OF<br/>EXTRA DTT TO<br/>GDH ASSAY</u> | <u>% ACTIVITY</u> |
|------------------------------------------------------------------------|---------------------------------------------------|-------------------|
| ENZYME + SUBSTRATE                                                     | 5mM                                               | 100               |
| ENZYME + SUBSTRATE + 1.1mM HgCl <sub>2</sub>                           | 5mM                                               | <1                |
| ENZYME + SUBSTRATE + 28mM DTT                                          | 5mM                                               | 95±6              |
| ENZYME + SUBSTRATE + 1.1mM HgCl <sub>2</sub><br>+29mM DTT              | 5mM                                               | <1                |
| ENZYME + SUBSTRATE + 38mM EDTA                                         | 5mM                                               | 89±2              |
| ENZYME + SUBSTRATE + 0.57mM HgCl <sub>2</sub><br>+29mM DTT + 29mM EDTA |                                                   | 6±3               |

SUBSEQUENT ADDITION OF GALACTOSE IS READILY DETECTED

| <u>INCUBATION MIXTURE</u>                                    | <u>GALACTOSE<br/>DETECTED</u> |
|--------------------------------------------------------------|-------------------------------|
| GALACTOSE                                                    | 100                           |
| GALACTOSE 0.57mM HgCl <sub>2</sub> + 29mM DTT                | 100±4                         |
| GALACTOSE 0.57mM HgCl <sub>2</sub><br>+ 29mM DTT + 29mM EDTA | 100±1                         |

TABLE XVII

PURIFICATION OF B-ZYME BY PASSAGE THROUGH  
A THIOL-SEPHAROSE AND SEPHACRYL S-200 COLUMNS

| <u>STEP</u>                                           | <u>TOTAL ENZ.</u><br><u>UNITS</u> | <u>%</u><br><u>RECOVERY</u> | <u>SPECIFIC</u><br><u>ACTIVITY</u> | <u>FOLD</u><br><u>PURIFICATION</u> |
|-------------------------------------------------------|-----------------------------------|-----------------------------|------------------------------------|------------------------------------|
| 1. STARTING FRACTION                                  | 3.3                               | 100                         | 8.3                                | 1                                  |
| 2. THIOL-SEPHAROSE PERCOLATE                          | 3.2                               | 96                          | 9.4                                | 1.1                                |
| 3. AMICON FILTRATION (XM50)                           | 2.0                               | 64                          | 8.2                                | 0.9                                |
| DIFFUSATE                                             | 0.53                              | 17                          | 6.0                                |                                    |
| 4. SEPHACRYL S-200 - POOL<br>OF ENZ. ACTIVE FRACTIONS | 1.8                               | 91                          | 10.0                               | 1.2                                |
| 5. AMICON FILTRATION (UM2)                            | 1.5                               | 85                          | 21.0                               | 2.5                                |
| DIFFUSATE                                             | 0.05                              | 3                           | 1.0                                |                                    |

NET RECOVERY OF PUREST PRODUCT WAS 46%

TABLE XVIII

ADSORPTION AND ELUTION OF B-ZYME FROM AN IMMUNO-AFFINITY COLUMN  
PREPARED FROM AN ANTIBODY TO PURE B-ZYME

| FRACTION                    | ENZYME         |               | PROTEIN |      | SPECIFIC<br>ACTIVITY | PURIF.<br>FACTOR |
|-----------------------------|----------------|---------------|---------|------|----------------------|------------------|
|                             | TOTAL<br>UNITS | %<br>ACTIVITY | TOTAL   | %    |                      |                  |
| CRUDE B-ZYME                | 1.4            | 100           | 16      | 100  | 0.098                | 1                |
| UNADSORBED PROTEIN          | 0              | 0             | 7.12    | 50.7 |                      |                  |
| B-ZYME ELUTED WITH 2M NaCl; |                |               |         |      |                      |                  |
| A)                          | 0.48           | 34            | 2.4     | 15.0 | 0.20                 | 2.3              |
| B)                          | 0.31           | 22            | 1.2     | 7.5  | 0.26                 | 3.0              |
| TOTAL                       |                | 56            |         | 73.2 |                      |                  |

TABLE XIX

ACTION OF PLACENTAL GLYCOSIDASES ON ARTIFICIAL SUBSTRATES  
AT OPTIMUM pH 4.5

| <u>PNP-SUBSTRATE</u> | <u>UNITS/ML</u> | <u>RATIO OF ACTIVITIES</u> |
|----------------------|-----------------|----------------------------|
| $\alpha$ GALNAC      | 5.60            | 1.00                       |
| $\alpha$ GLcNAC      | 0.06            | 0.06                       |
| $\beta$ GAL          | 0.74            | 0.13                       |
| $\alpha$ GAL         | 1.20            | 0.21                       |
| $\beta$ GALNAC       | 4.20            | 0.75                       |
| $\beta$ GLcNAC       | 13.10           | 2.34                       |

TABLE XX

COMPARISON OF PLACENTAL AND CLOSTRIDIAL GLYCOSIDASES  
ON A<sup>+</sup> AND B<sup>+</sup> SUBSTANCES

1. 38-52% AM. SULF. CUT OF PLACENTAL EXTRACT OBTAINED FROM DR. RAY BROWN
2. A-ZYME OBTAINED FROM CL. PERFRINGENS
3. B-ZYME OBTAINED FROM CL. SPOROGENES

A. COMPARISON OF PLACENTAL (1) WITH BACTERIAL A-ZYME (2)

SUBSTRATE=A<sup>+</sup> HOG SUBMAXILLARY GLYCOPROTEIN

|                               | <u>SUGAR RELEASED</u> |                |                    |               |
|-------------------------------|-----------------------|----------------|--------------------|---------------|
|                               | <u>NAC HEXOSAMINE</u> |                | <u>SIALIC ACID</u> |               |
|                               | <u>1 HR.</u>          | <u>24. HR.</u> | <u>1 HR.</u>       | <u>24 HR.</u> |
| 1. PLACENTAL ENZYME AT PH 4.5 | 0.43                  | 1.45           | 0                  | 0             |
| 2. BACTERIAL ENZYME AT PH 7.0 | 1.37                  | 10.70          | 0.78               | 5.07          |

B. COMPARISON OF PLACENTAL (1) WITH BACTERIAL B-ZYME (3)

SUBSTRATE=OLIGOSACCHARIDE ALDITOLS OBTAINED FROM  
 B<sup>+</sup> OVARIAN CYST FLUID

|                               | <u>SUGAR RELEASED</u> |                |               |               |
|-------------------------------|-----------------------|----------------|---------------|---------------|
|                               | <u>GALACTOSE</u>      |                | <u>FUCOSE</u> |               |
|                               | <u>1 HR.</u>          | <u>24. HR.</u> | <u>1. HR.</u> | <u>24 HR.</u> |
| 1. PLACENTAL ENZYME AT PH 4.5 | 0.36                  | 1.07           | 0.41          | 2.5           |
| 2. BACTERIAL ENZYME AT PH 6.3 | 119.4                 | 1066           | 0             | 0             |

TABLE XXI

ACTION OF  $\alpha(1\rightarrow2)$ L-FUCOSIDASE ON VARIOUS SUBSTRATES,  
ALONE AND WITH OTHER GLYCOSIDASES

| SUBSTRATE                                                                      | % OF TOTAL FUCOSE RELEASED FROM SUBSTRATE<br>BY |                                    |        |
|--------------------------------------------------------------------------------|-------------------------------------------------|------------------------------------|--------|
|                                                                                | FUCOSIDASE                                      | PLUS 16 HR FURTHER INCUBATION WITH |        |
|                                                                                | ALONE FOR<br>24 HR                              | A-ZYME                             | B-ZYME |
| H <sup>+</sup> PSM                                                             | 98                                              | -                                  | -      |
| A <sup>+</sup> PSM                                                             | 21                                              | 97                                 | -      |
| GALNAC <sup>α</sup> 3GAL <sup>β</sup> GALNACOL<br>α↑(1→3) ↑(2→6)<br>FUC NEUNGc | 0                                               | 97                                 |        |
| GAL <sup>β</sup> GALNACOL<br>α↑(1→3) ↑(2→6)<br>FUC NEUNGc                      | 100                                             | 100                                | -      |
| B <sup>+</sup> OVARIAN CYST<br>OLIGOSACCHARIDE ALDITOLS                        | 22                                              | -                                  | 40     |

TABLE XXII

## ENZYMATIC CLEAVAGE OF SYNTHETIC OLIGOSACCHARIDES

| SUBSTRATE                                                                                          | % SUGAR RELEASED              |            |                            |            |                                               |            |
|----------------------------------------------------------------------------------------------------|-------------------------------|------------|----------------------------|------------|-----------------------------------------------|------------|
|                                                                                                    | $\alpha$ -GALNAC'ASE<br>ALONE |            | $\alpha$ -FUC'ASE<br>ALONE |            | $\alpha$ -FUC'ASE AND<br>$\alpha$ -GALNAC'ASE |            |
|                                                                                                    | <u>GALNAC</u>                 | <u>FUC</u> | <u>GALNAC</u>              | <u>FUC</u> | <u>GALNAC</u>                                 | <u>FUC</u> |
| GALNAC( $\alpha$ 1 $\rightarrow$ 3)GAL $\beta$ $\rightarrow$ R<br>$\uparrow$ $\alpha$ (1,2)<br>Fuc | 48                            | 0          | 0                          | 0          | 100                                           | 100        |
| GALNAC( $\alpha$ 1 $\rightarrow$ 3)GAL $\beta$ $\rightarrow$ R                                     | 100                           | ND         | 0                          | ND         | 100                                           | ND         |

| SUBSTRATE                                                                                       | % SUGAR RELEASED           |            |                            |            |                                            |            |
|-------------------------------------------------------------------------------------------------|----------------------------|------------|----------------------------|------------|--------------------------------------------|------------|
|                                                                                                 | $\alpha$ -GAL'ASE<br>ALONE |            | $\alpha$ -FUC'ASE<br>ALONE |            | $\alpha$ -FUC'ASE AND<br>$\alpha$ -GAL'ASE |            |
|                                                                                                 | <u>GAL</u>                 | <u>FUC</u> | <u>GAL</u>                 | <u>FUC</u> | <u>GAL</u>                                 | <u>FUC</u> |
| GAL( $\alpha$ 1 $\rightarrow$ 3)GAL $\beta$ $\rightarrow$ R<br>$\uparrow$ $\alpha$ (1,2)<br>Fuc | 100                        | 0          | 0                          | 0          | 100                                        | 100        |
| GAL( $\alpha$ 1 $\rightarrow$ 3)GAL $\beta$ $\rightarrow$ R                                     | 100                        | ND         | 0                          | ND         | 100                                        | ND         |

ND, NOT DETERMINED