*Carbohydrate Research*, 205 (1990) 444–452
Elsevier Science Publishers B.V., Amsterdam

# Note

## Studies on cervical glycoproteins. Isolation and characterization of neutral oligosaccharides from Pronase-treated glycoproteins of bonnet monkey (*Macacca radiata*)*,†

Nasir-ud-Din, Syed Altaf Hussain,
*Institute of Biochemistry, University of Baluchistan, Quetta (Pakistan)*

Roger W. Jeanloz,
*Laboratory for Carbohydrate Research, Massachusetts General Hospital, Charlestown, Massachusetts 02129 and Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115 (U.S.A.)*

and Evelyn Walker-Nasir
*Laboratoires Sauter S.A., CH-1211 Geneva 28 (Switzerland)*

(Received December 29th, 1989; accepted for publication April 16th, 1990)

In the human cervix the columnar and gbolet cells[2], or only the columnar cells[3], perform the function of mucus secretion, whereas in the bonnet monkey only columnar cells are involved[4]. The cervical mucus of the bonnet monkey was chosen for glycoprotein studies because it is produced by a homogenous, single type of cells in copious amounts, and because the bonnet monkey is phylogenetically closely related to man[5–10]. Cervical mucus is a complex mixture of epithelial secretions exhibiting several rheological properties, such as viscosity, flow elasticity, "spinnbarkeit", and stickiness, which are regulated by ovarian functions. The alteration of these properties during the menstrual cycle is accompanied by alterations of the carbohydrate composition[11] and the chemical structure of the glycoproteins[6]. Thus, it is of interest to relate the carbohydrate structure of the glycoprotein with the biological functions and the morphological setting of the mucus. In a previous communication[10], one of us described the chemical structure of seven acidic oligosaccharides isolated from mucus of the periovulatory phase after purification by Pronase treatment. We describe herein the structure of five neutral oligosaccharides isolated at the same phase after an identical treatment.

* Dedicated to Professor Leslie Hough in the year of his 65th birthday.

† This is publication No. 1057 of the Robert W. Lovett Memorial Group for the Study of Diseases Causing Deformities, Harvard Medical School and Massachusetts General Hospital, Boston, Massachusetts. This investigation was supported, in part, by grants AM-03564 and HD-12431 (to R.W.J.) from the National Institutes of Health. For a preliminary communication, see ref. 1.

0008-6215/90/$03.50 

EXPERIMENTAL

*Materials.* — Bio-Gel P-200, P-6, P-4, and P-2 gels, and AG 50W-X8 (100–200 mesh) and AG 1-X2 (100–200 mesh) ion-exchange resins were purchased from Bio-Rad Laboratories, Richmond, CA. Sepharose 2B was purchased from Pharmacia LKB Biotechnology Inc., Piscataway, NJ, and insolubilized Pronase (Enzite protease) from Miles Inc., Kankakee, IL.

*Collection of cervical mucus.* — The cervical mucus from the bonnet monkey was collected by aspiration with a suction pump at the estrogen-stimulated phase. The secretion was promptly frozen and maintained in the frozen state until use.

*General methods.* — The hexose content of the column-eluate fractions was determined by the phenol–$H_2SO_4$ method[12], and the protein content by measuring the absorbance at 278 nm. Quantitative analysis of carbohydrates by g.l.c. was performed according to the procedure of Reinhold[13]. 2-Acetamido-2-deoxythreitol was determined by g.l.c. as described previously[6]. G.l.c.–m.s. was performed with an analytical system consisting of a Varian MAT 731 instrument fitted with a combined c.i., e.i., and f.d. ion source. Columns of Bio-Gel P-200 and Sepharose 2B were washed with 50mM sodium phosphate (pH 7.0) containing 0.2% $NaN_3$. Chromatography on columns of Bio-Gel P-4 and P-6 was performed in 50mM pyridine–acetic acid, pH 5.4, and the column eluates were examined by counting tritium radioactivity or by the phenol–$H_2SO_4$ procedure for hexoses[12]. Chromatography on DEAE-Sephadex columns was performed as previously described[10].

*Purification, Pronase treatment, and alkali–borohydride treatment of mucus glycoproteins.* — The glycoproteins were purified on a Bio-Gel P-200 column, the eluted glycoprotein was treated with Pronase, and then purified by gel filtration on Sepharose 2B as described earlier[8]. The purified glycoprotein (30 mg) was treated with 2M $NaBH_4$ in 50mM NaOH according to the method of Iyer and Carlson[14], and with sodium [$^3$H]borohydride as previously described[10]. The neutral oligosaccharides were separated from the acidic oligosaccharides on a column of AG 1-X2 anion-exchange resin ($AcO^-$). The column was washed successively with water, 5mM acetic acid, and a gradient of 50mM–0.2M acetic acid. The water and 5mM acetic acid eluates contained the neutral oligosaccharides, which were further purified by chromatography on a Bio-Gel P-6 column (100–200 mesh), and subsequent paper chromatography or paper electrophoresis[10]. Paper chromatography (p.c.) was performed in *A*, 1:2:1 (v/v) butanol–propanol–acetic acid, and *B*, 7:7:6 (v/v) pentanol–pyridine–water.

*Enzymic degradation of oligosaccharides.* — Oligosaccharides were digested with the following enzymes: (*a*) α-L-fucosidase (EC 3.2.1.51) from beef epididymis (Sigma Chemical Co., St. Louis, MO), in 10mM sodium citrate buffer, pH 6.0, for 48 h at 37°; (*b*) α-D-galactosidase (EC 3.2.1.22) from *Aspergillus niger* (Sigma), in 50mM sodium citrate, pH 5.0, for 48 h at 37°; (*c*) β-D-galactosidase (EC 3.2.1.23) from *Charonia lampas* (Miles), in 50mM sodium citrate, pH 4.0, 48 h at 37°; (*d*) β-D-galactosidase from *Escherichia coli* (Boehringer Mannheim Corp., Indianapolis, IN), in 50mM sodium phosphate, pH 7.0, 48 h at 37°; (*e*) *N*-acetyl-β-D-glucosaminidase (EC 3.2.1.30) from

jack bean (Sigma), in 50mM sodium citrate, pH 4.5, 48 h at 37°; (*f*) *N*-acetyl-α-D-galactosaminidase (EC 3.2.1.49) from *C. lampas* (Miles), in McIlvaine sodium citrate–phosphate buffer, pH 4.1, 42 h at 37°; and (*g*) *N*-acetyl-β-D-glucosaminidase, also referred as *N*-acetyl-β-D-hexosaminidase (EC 3.2.1.30; Sigma), from jack bean, sodium citrate, pH 4.0, 76 h at 37°, to act also as *N*-acetyl-β-D galactosaminidase.

*Methylation analysis and periodate oxidation–sodium borohydride reduction (Smith degradation) of oligosaccharides.* — Neutral oligosaccharides (as alditols) were methylated by Hakomori's procedure[15], and the methylated oligosaccharides processed as previously described[10]. The methylated oligosaccharides were hydrolyzed with trifluoroacetic acid, and the methylated sugars identified by g.l.c.–m.s.[10].

Reduced, neutral oligosaccharides (1–2 mg) were treated with $NaIO_4$, and subsequently reduced with $NaBH_4$, treated with acid, and processed as described earlier[10].

## RESULTS

Alkali–borohydride treatment of the Pronase-treated, purified glycoprotein gave a mixture of oligosaccharide alditols. The neutral oligosaccharides were separated on a column of Bio-Gel P-6 into seven fractions, of which five contained oligosaccharides (Table I and Fig. 1), and the remaining two fractions glycopeptides.

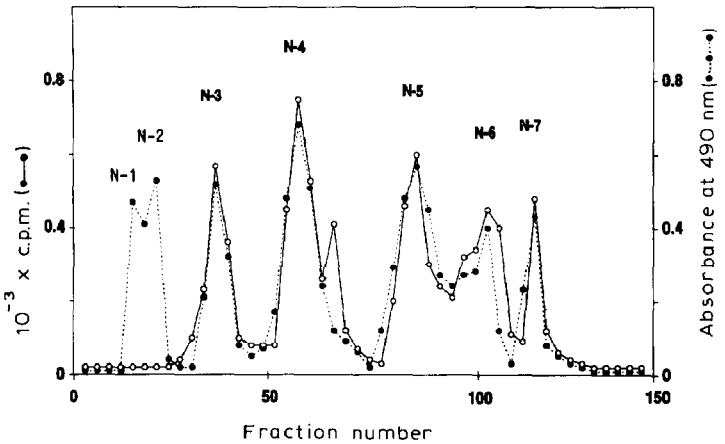


Fig. 1. Fractionation of neutral oligosaccharides on a column (125 × 1.2 cm) of Bio-Gel P-6. Two glycopeptides (N-1 and N-2) and five oligosaccharides (N-3, 5.0 mg; N-4, 4.0 mg; N-5, 3.0 mg; N-6, 4.6 mg; and N-7, 3.0 mg) were obtained. Hexoses were monitored by the phenol–sulfuric acid procedure (o–o) and 2-acetamido-2-deoxy-galactitol by counting tritium radioactivity (●·····●).

*Oligosaccharide fraction N-3.* — This fraction was further purified by Bio-Gel P-6 chromatography and was homogeneous on p.c. in solvents *A* and *B*. Methylation of the oligosaccharide showed the presence of a terminal 2-acetamido-2-deoxy-D-galactopyranosyl group and of 4-linked 2-acetamido-2-deoxy-D-glucopyranosyl, 3- and 4-linked D-galactopyranosyl, and 3,6-linked 2-acetamido-2-deoxy-D-galactitol residues (Table II). Treatment of the oligosaccharide with *N*-acetyl-α-D-galactosaminidase and sub-

TABLE I

Sugar composition of purified neutral oligosaccharides

| *Fraction* | *Component* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Fucose* | | *Galactose* | | *2-Acetamido-2-deoxyglucose* | | *2-Acetamido-2-deoxygalactose* | | *2-Acetamido-2-deoxygalactitol* | |
| | % | *MR*[a] | % | *MR*[a] | % | *MR*[a] | % | *MR*[a] | % | *MR* |
| N-3 | | | 25.4 | 1.95 | 16.4 | 1.05 | 31.2 | 1.95 | 16.0 | 1.00 |
| N-4 | | | 32.0 | 2.0 | 20.5 | 1.05 | 19.8 | 1.0 | 20.0 | 1.00 |
| N-5 | 15.6 | 1.0 | 33.0 | 1.9 | 21.8 | 1.0 | | | 20.9 | 1.00 |
| N-6 | 15.5 | 1.0 | 34.2 | 2.0 | 22.0 | 1.0 | | | 21.0 | 1.00 |
| N-7 | | | 41.0 | 2.05 | 27.4 | 1.1 | | | 25.0 | 1.00 |

[a] Molar ratio relative to 2-acetamido-2-deoxygalactitol.

sequent methylation showed the presence of the same group and residues, whereas a prolonged treatment with *N*-acetyl-β-D-hexosaminidase, followed by methylation, showed the appearance of a terminal D-galactopyranosyl group. Finally, sequential treatment of the oligosaccharide with *N*-acetyl-β-D-hexosaminidase and β-D-galactosidase, and subsequent methylation indicated the appearance of a terminal 2-acetamido-2-deoxy-D-glucosyl group, and the disappearance of the subterminal 4-linked D-galactosyl residue (Table II).

Periodate oxidation followed by borohydride reduction and subsequent acid hydrolysis removed the 2-acetamido-2-deoxy-D-galactosyl and partly the D-galactosyl residues, and converted the 2-acetamido-2-deoxy-D-galactitol into a 2-acetamido-2-deoxy-D-threitol residue, while 2-acetamido-2-deoxy-D-glucose was recovered unchanged. These results suggest that the oligosaccharide of fraction N-3 is a hexasaccharide having structure **1**. The anomeric configuration of the terminal 2-acetamido-2-deoxy-D-galactosyl group was not clearly established, as *N*-acetyl-β-D-hexosaminidase, which also has *N*-acetyl-β-D-galactosaminidase activity, only partly removed this group.

*Oligosaccharide fraction N-4.* — This oligosaccharide fraction was further purified by gel filtration on Bio-Gel P-4, and ion-exchange chromatography on DEAE-Sephadex, and was homogeneous on p.c. in solvents *A* and *B*. Methylation of the oligosaccharide and identification of the methylation products by g.l.c.–m.s. showed the presence of terminal, nonreducing 2-acetamido-2-deoxy-D-galactosyl and D-galactosyl groups, and 3-linked D-galactosyl, 4-linked 2-acetamido-2-deoxy-D-glucosyl, and 3,6-linked 2-acetamido-2-deoxy-D-galactitol residues (Table II). Treatment of the oligosaccharide with *N*-acetyl-α-D-galactosaminidase and subsequent methylation showed the disappearance of the 2-acetamido-2-deoxy-D-galactosyl nonreducing group and the 3-linked D-galactosyl group (Table II). Smith degradation of this oligosaccharide removed the D-galactosyl (in part) and 2-acetamido-2-deoxy-D-galactosyl groups and converted the 2-acetamido-2-deoxy-D-galactitol into a 2-acetamido-2-deoxy-D-threitol

TABLE II

*O*-Methyl derivatives obtained by acid hydrolysis of methylated oligosaccharides before and after enzyme treatment

| *Oligo-saccharides* | *Enzyme treatment*[a] | *O-Methyl derivatives* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Fuc* | *Gal* | | | | *GlcNAc* | | *GalNAc* | *GalNAc-ol* | |
| | | *2,3,4* | *2,3,6* | *2,4,6* | *3,4,6* | *2,3,4,6* | *3,6* | *3,4,6* | *3,4,6* | *1,4,5* | *1,3,4,5* |
| N-3 | None | | + | + | | | + | | + | + | |
| | α-GalNAc-ase | | + | + | | | + | | + | + | |
| | β-HexNAc-ase | | + | | | + | + | | + | + | |
| | β-HexNAc-ase, then β-Gal-ase | | | + | | | | + | + | + | |
| N-4 | None | | | + | | + | + | | + | + | |
| | α-GalNAc-ase | | | | | + | + | | | + | |
| N-5 | None | + | | | + | + | + | | | + | |
| | β-Gal-ase | + | | | + | | | + | | + | |
| | β-Gal-ase, then α-Fuc-ase | | | | | + | + | | | + | |
| N-6 | None | + | | | + | + | + | | | + | |
| | α-Fuc-ase | | | | | + | + | | | + | |
| | β-Gal-ases | + | | | + | + | + | | | + | |
| | α-Fuc-ase, then β-Gal-ase | | | | | [b] | | + | | [b] | + |
| N-7 | None | | | | | + | + | | | + | |
| | β-Gal-ase | | | | | + | | + | | + | |

[a] α-GalNAc-ase, *N*-acetyl-α-D-galactosaminidase; β-HexNAc-ase, *N*-acetyl-β-D-hexosaminidase; β-Gal-ase, β-D-galactosidase; and α-Fuc-ase, α-L-fucosidase.
[b] Trace.

residue, whereas 2-acetamido-2-deoxy-D-glucose was unchanged. The results of these experiments suggest that the oligosaccharide of fraction N-4 is a pentasaccharide having structure **2**.

*Oligosaccharide fraction N-5.* — This oligosaccharide was purified by chromatography on Bio-Gel P-4 and was homogeneous on p.c. in solvents *A* and *B*, and on paper electrophoresis. Methylation of the oligosaccharide of fraction N-5 and characterization of the products of hydrolysis by g.l.c.–mass spectrometry showed the presence of terminal L-fucosyl and D-galactosyl groups, and 2-linked D-galactosyl, 4-linked 2-acetamido-2-deoxy-D-glucosyl, and 3,6-linked 2-acetamido-2-deoxy-D-galactitol residues (Table II).

Treatment of the oligosaccharide with β-D-galactosidase and subsequent methylation of the enzyme-treated oligosaccharide showed the presence of terminal, nonreducing L-fucosyl and 2-acetamido-2-deoxy-D-glucosyl groups, and 2-linked D-galactosyl and 3,6-linked 2-acetamido-2-deoxy-D-galactitol residues. α-L-Fucosidase degradation of the β-D-galactosidase-treated oligosaccharide showed, by methylation analysis (Table II), the presence of terminal, nonreducing D-galactosyl and 2-acetamido-2-deoxy-D-glucosyl groups, and a 3,6-linked 2-acetamido-2-deoxy-D-galactitol residue.

Periodate oxidation–borohydride reduction of the oligosaccharide removed the terminal nonreducing L-fucosyl and D-galactosyl groups and the internal D-galactosyl residue, and converted the 2-acetamido-2-deoxy-D-galactitol into a 2-acetamido-2-deoxy-D-threitol residue, but left 2-acetamido-2-deoxy-D-glucose unchanged. On the basis of these experiments, the structure of pentasaccharide **3** is proposed for the oligosaccharide of fraction N-5.

*Oligosaccharide fraction N-6.* — This fraction was purified to homogeneity by chromatography on Bio-Gel P-4. Methylation analysis of the oligosaccharide showed the presence of terminal, nonreducing L-fucosyl and D-galactosyl groups, and 2-linked D-galactosyl, 4-linked 2-acetamido-2-deoxy-D-glucosyl, and 3,6-linked 2-acetamido-2-deoxy-D-galactitol residues (Table II). Treatment of the oligosaccharide with α-L-fucosidase followed by methylation analysis showed the presence of terminal, nonreducing D-galactosyl groups, a 4-linked 2-acetamido-2-deoxy-D-glucosyl, and a 3,6-linked 2-acetamido-2-deoxy-D-galactitol residue (Table II). Extensive treatment of the oligosaccharide with β-D-galactosidases from *C. lampas* and *E. coli* indicated only a partial removal of the D-galactosyl groups as shown by methylation (Table II), but sequential treatment of the oligosaccharide with α-L-fucosidase and β-D-galactosidase removed both the L-fucosyl and β-D-galactosyl groups. Methylation analysis of this enzyme-treated oligosaccharide showed the presence of a terminal, nonreducing 2-acetamido-2-deoxy-D-glucosyl group and a trace of D-galactosyl group, and 6- and traces of 3,6-linked 2-acetamido-2-deoxy-D-galactitol residues (Table II).

Smith degradation of the oligosaccharide completely removed the terminal, nonreducing L-fucosyl and D-galactosyl groups and the internal D-galactosyl residue, and converted the 2-acetamido-2-deoxy-D-galactitol into a 2-acetamido-2-deoxy-D-threitol residue, while 2-acetamido-2-deoxy-D-glucose was unchanged. These results

suggested pentasaccharide structure **4** for the oligosaccharide of fraction N-6.

*Oligosaccharide fraction N-7.* — This oligosaccharide was homogeneous after gel filtration through Bio-Gel P-2 (p.c. in solvents *A* and *B*). Carbohydrate analysis of the purified oligosaccharide suggested a tetrasaccharide structure. Methylation analysis (Table II) showed the presence of terminal, nonreducing D-galactosyl groups, and 4-linked 2-acetamido-2-deoxy-D-glucosyl and 3,6-linked 2-acetamido-2-deoxy-D-galactitol residues. β-D-Galactosidase treatment of the oligosaccharide, followed by methylation analysis of the enzyme-treated remainder, showed the presence of terminal, nonreducing 2-acetamido-2-deoxy-D-glucosyl and D-galactosyl groups, and a 3,6-linked 2-acetamido-2-deoxy-D-galactitol residue (Table II).

Smith degradation removed the terminal, nonreducing D-galactosyl groups and converted the 2-acetamido-2-deoxy-D-galactitol into a 2-acetamido-2-deoxy-D-threitol residue, while 2-acetamido-2-deoxy-D-glucose was unchanged. These results suggested structure **5** for the oligosaccharide of fraction N-7.

D-Gal*p*NAc-(1→4)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→6 or 3)-D-GalNAc-ol
3 or 6
↑
1

**1** D-Gal*p*NAc-(1→3)-β-D-Gal*p*

β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→6 or 3)-D-GalNAc-ol
3 or 6
↑
1

**2** α-D-Gal*p*NAc-(1→3)-β-D-Gal*p*

β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→6 or 3)-D-GalNAc-ol
3 or 6
↑
1

**3** α-L-Fuc*p*-(1→2)-β-D-Gal*p*

α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→6 or 3)-D-GalNAc-ol
3 or 6
↑
1

**4** β-D-Gal*p*

β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→6 or 3)-D-GalNAc-ol
3 or 6
↑
1

**5** β-D-Gal*p*

Chart 1. Proposed structures for oligosaccharides of fractions N-3–N-7.

## DISCUSSION

In the present study, mucus was collected from several monkeys at the time of estrogen surge. The mucus was purified, and the resulting glycoproteins were treated with Pronase and purified to homogeneity on Sepharose 2B, as shown by chromatography and electrophoresis[10]. It is likely that, after Pronase treatment, these purified glycoproteins differed only in molecular size. On treatment of the glycoproteins with alkali–borohydride, a mixture of neutral and acidic oligosaccharides was obtained that was separated by ion-exchange chromatography[10], and the neutral oligosaccharides were further fractionated on Bio-Gel P-6 (Fig. 1). Each oligosaccharide obtained from this chromatography was subsequently purified by paper chromatography or electrophoresis to provide five neutral oligosaccharide fractions (N-3–N-7). The structure of each oligosaccharide was studied by chemical and enzymic procedures. The main limitations of this study were the availability of specific enzymes and the amounts of available oligosaccharides. Consequently, it was not possible to ascertain definitely the anomeric configurations of the 2-acetamido-2-deoxy-D-glucosyl and -D-galactosyl residues linked to the 2-acetamido-2-deoxy-D-galactitol residue, and they were attributed in analogy with the results obtained for the acidic oligosaccharides[9,10]. In the case of oligosaccharide fraction N-3, the anomeric configuration between the terminal, nonreducing 2-acetamido-2-deoxy-D-galactosyl group and the D-galactosyl residue was not definitely established, as *N*-acetyl-α-D-galactosaminidase did not split off this group, but *N*-acetyl-β-D-hexosaminidase was also not able to completely remove it. These results suggested that either the latter enzyme cleaves off some 2-acetamido-2-deoxy-β-D-galactosyl groups with difficulty, or that some α-D-anomeric groups were also present. 2-Acetamido-2-deoxy-β-D-galactopyranosyl groups have been characterized in the acidic oligosaccharides of the intact glycoprotein[9], and therefore it is probable that they are also present in the neutral oligosaccharides.

The precise linkages of the penultimate 2-acetamido-2-deoxy-β-D-glucosyl and D-galactosyl residues to the 2-acetamido-2-deoxy-D-galactosyl residue involved in the carbohydrate–protein linkage were also not completely defined, as the methods used could only indicate that both O-3 and O-6 of this residue are substituted. As the 2-acetamido-2-deoxy-β-D-glucose residue is linked (1→6) and the β-D-galactosyl group is linked (1→3) to the 2-acetamido-2-deoxy-D-galactose residue in the acidic oligosaccharides of the bonnet monkey glycoprotein[9], it is very likely that identical linkages exist in the neutral oligosaccharides of the periovulatory-phase mucus. These two linkages are also the preponderant ones in mucins from other sources[16,17]. The oligosaccharides presently characterized bear chemical structures similar to those of the acidic oligosaccharides[9,10], all having the core structure, β-D-Gal*p*-(1→3)-[β-D-Glc*p*NAc-(1→6)]-D-GalNAc. The same common core was identified in the partial chemical structures of four carbohydrate chains obtained from human cervical glycoproteins[18]. The great heterogeneity of the carbohydrate chains of cervical mucus glycoproteins, as compared with those of other mucins, may be related to the changing physiological functions and biophysical properties of the mucus.

REFERENCES

1 Nasir-ud-Din, R. W. Jeanloz, S. A. Hussain, E. Walker-Nasir, and I. Khan, *Abstr. Int. Symp. Glycoconjugates, IXth*, (1987) A-87.
2 E. S. E. Hafez, in M. Elstein, K. S. Moghissi, and R. Borth (Eds.), *Cervical Mucus in Human Reproduction*, Scriptor, Copenhagen, 1973, pp. 23–32.
3 E. S. E. Hafez, in M. Elstein and D. V. Park (Eds.), *Mucus in Health and Disease*, Plenum Press, New York, 1977, pp. 19–38.
4 J. Ovadia, J. W. McArthur, L. Kopito, and H. Uldfelder, *Biol. Reprod.*, 5 (1971) 127–145.
5 V. B. Hatcher, G. O. H. Schwarzmann, R. W. Jeanloz, and J. W. McArthur, *Biochemistry*, 16 (1977) 1518–1524.
6 Nasir-ud-Din, R. W. Jeanloz, V. N. Reinhold, and J. W. McArthur, *Carbohydr. Res.*, 75 (1979) 349–356.
7 Nasir-ud-Din, R. W. Jeanloz, P. Roussel, and J. W. McArthur, *Biol. Reprod.*, 28 (1983) 1189–1199.
8 Nasir-ud-Din, R. W. Jeanloz, J. R. Vercelotti, and J. W. McArthur, *Biochim. Biophys. Acta*, 678 (1981) 483–496.
9 Nasir-ud-Din, R. W. Jeanloz, G. Lamblin, P. Roussel, H. van Halbeek, J. H. G. M. Mutsaers, and J. F. G. Vliegenthart, *J. Biol. Chem.*, 261 (1986) 1992–1997.
10 Nasir-ud-Din, *Carbohydr. Res.*, 159 (1987) 95–107.
11 S. Iacobelli, N. Garcea, and C. Angeloni, *Fertil. Steril.*, 22 (1971) 727–734.
12 M. F. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 350–356.
13 V. N. Reinhold, *Methods Enzymol.*, 25 (1972) 244–249.
14 R. N. Iyer and D. M. Carlson, *Arch. Biochem. Biophys.*, 142 (1971) 101–105.
15 S. Hakomori, *J. Biochem. (Tokyo)*, 55 (1964) 205–207.
16 H. van Halbeek, L. Dorland, J. F. G. Vliegenthart, W. E. Hull, G. Lamblin, M. Lhermitte, A. Boersma, and P. Roussel, *Eur. J. Biochem.*, 127 (1982) 7–20.
17 I. Carlstead, J. K. Sheenan, A. P. Corfield, and J. T. Gallagher, *Essays Biochem.*, 20 (1985) 40–71.
18 E. C. Yurewicz and K. S. Moghissi, *J. Biol. Chem.*, 256 (1981) 11 895–11 904.