Multiglycosyltransferase System of Canine Respiratory Tissue

URIDINE DIPHOSPHATE GALACTOSE: MUCIN GALACTOSYLTRANSFERASE*

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SUMMARY

A galactosyltransferase has been found in a particulate preparation prepared from normal canine respiratory tissue which catalyzes the transfer of galactose from its uridine diphosphate derivative to N-acetylgalactosamine residues. The transfer to sialidase-treated ovine submaxillary mucin was dependent upon Mg** and was stimulated several-fold by Triton X-100. The optimal pH was between 6 and 7. The K_m was 7 x 10^{-4} M for UDP-galactose and 1 x 10^{-2} M for sialidase-treated ovine submaxillary mucin. Removal of the N-acetylgalactosamine residues on this acceptor reduced the incorporation of galactose. The enzyme was present throughout the airway passage with some evidence of higher activity in the lower portion of the trachea and the extrapulmonary primary bronchi.

Fetuin from which sialic acid and galactose were removed was also an acceptor (K_m = 0.9 x 10^{-3} M). When the terminal N-acetylglucosamine residues were removed from this acceptor, the incorporation of galactose was reduced. No competition was demonstrated when this acceptor was mixed with sialidase-treated ovine submaxillary mucin; this suggests that there are at least two separate galactosyltransferases present in canine respiratory tissue or two catalytic sites on the same molecule.

UDP-Gal + GalNAc → protein
Gal + GalNAc → protein (+UDP)

EXPERIMENTAL PROCEDURE

Materials—UDP-[U-14C]galactose and [U-14C]galactose were purchased from New England Nuclear, and the corresponding nonradioactive substances obtained from Calbiochem were used to adjust the specific activity. [U-14C]Galactose 1-phosphate was purchased from International Chemical and Nuclear Corporation. UDP-N-acetyl[1-14C]glucosamine and nonradioactive UDP-N-acetylgalactosamine were obtained from New England Nuclear and Boehringer Mannheim, respectively. Fresh-frozen submaxillary glands were obtained from Pentex, Inc., Kanakee, Illinois, and fresh-frozen canine tracheas were

1 R. Havez and M. Biserte, Abstracts of papers presented at the Colloque International de Pathologie Thoracique, Lille, France, September 27 to 29, 1968, p. 43.
2 C. Voisin, Abstracts of papers presented at the Colloque International de Pathologie Thoracique, Lille, France, September 27 to 29, 1968, p. 23.

Glycoproteins are the major macromolecular components of the secretion elaborated by the mucus-producing structures of the tracheobronchial tree. Those isolated from both human and canine respiratory secretions have the characteristic structural patterns of other mucin-type glycoproteins. The small polypeptide units glycosidically linked to the protein core constitute about 40% of the molecular weight and include sialic acid, fucose, galactose, and N-acetylgalactosamines. The studies of Gerner-Rieux et al. (2) indicate that in human bronchial mucins, a basic unit made up of galactose, N-acetylgalactosamine, and N-acetylgalactosamine residues is attached to the polypeptide chain, and superimposed on this basic structure are fucose, and either N-acetylneuraminic acid, or sulfate groups, or both. The relative amounts of these three moieties serve as the basis for classifying tracheobronchial mucins into three distinct types (2, 3). The presence of these three types of mucins in physiological proportions presumably provides the necessary viscoelasticity and adhesion to allow the secretion to perform its vital role (or roles). Pathological conditions of the respiratory tract are usually characterized by a hypersecretion of mucus with altered physicochemical properties. These changes in the secretion itself probably contribute significantly to the disease state and may be attributable to abnormal proportions of the three types of epithelial glycoproteins (4). Presumably, changes in the proportions of the various glycoproteins should be a reflection of changes in the activity of the enzymes involved in their biosynthesis or alterations in physiological control mechanisms regulating that biosynthesis. In order to investigate these relationships, information concerning the biosynthesis of these complex molecules of tracheobronchial secretions in normal tissue must be available. It is toward this goal that our present efforts are concentrated. In general, the assembly of the carbohydrate units on glycoproteins involves a series of glycosyltransferases that function to transfer monosaccharides, in a stepwise manner, to growing oligosaccharide chains (5). This paper describes a transferase present in normal canine tracheobronchial tissue which catalyzes the following reaction.

* A preliminary report of this work was reported at the meeting of the American Society of Biological Chemists in April 1970 (1).

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purchased from Rockland. Adult male standard laboratory beagles that were normal by clinical and laboratory studies were supplied by White Eagle Farm. Ovine, bovine, and porcine submaxillary mucins were isolated essentially by the procedure of Hashimoto, Hashimoto, and Pigman (6) as modified by De-Salegui and Pigman (7). Pig submaxillary glands were pooled according to their ability to inhibit human A-anti A hemagglutination (8). Fetuin was prepared as previously described by Spiro (9). Sialic acid residues were removed (over 95%) by digestion with neuraminidase (Workington Biochemical) as described by Carlson, McGuire, and Jourdian (10). N-Acetylgalactosaminidase and galactosidase residues were removed by digestion with N-acetylgalactosaminidase and galactosidase, respectively, obtained from a strain of Clostridium perfringens (11). The exact experimental details for the preparation of these glycosidases were kindly supplied by Mr. S. Chipowsky and Dr. E. McGuire. The enzymes were purified between 500- and 1000-fold and were free of any proteolytic activity. Approximately 50 mg of the glycoprotein, dissolved in 12 ml of 0.1 M phosphate buffer, pH 6.0, were treated with approximately 1 unit of the appropriate glycosidase. Over 95% of the protein-bound N-acetylgalactosamine residues were removed, whereas only 70% of the galactose residues could be removed from Fet(-NAN).4 Unless stated differently, the concentrations of all glycoproteins are expressed as the number of sugar residues released by the respective glycosidases. Methyl 2-acetamido-2-deoxy-a-D-galactopyranoside was generously supplied by Dr. Lee Griggs. All other chemicals were reagent grade and were used without further purification.

Gas Chromatography—Carbohydrate analyses by gas chromatography were performed as described by Griggs (12). The alditol acetates of the neutral and amino sugars were employed in this study.

Paper Chromatography—Descending paper chromatography was performed on Whatman No. 1 paper in Solvent A, ethyl acetate-glacial acetic acid-water, 140:30:30 (v/v); or Solvent B, 1-butanol-pyridine-water, 6:4:3 (v/v). Radioautography using Kodak Royal Blue x-ray film was used to visualize chromatograms for radioactivity.

Preparation of Particulate Enzyme—All procedures in the preparation of the particulate enzyme were performed at 0–4°C. The inner mucosal lining of fresh-frozen canine tracheas were removed by scraping with a scalpel, and were homogenized in a Virtis homogenizer in 8 volumes of 0.25 M sucrose containing 0.001 M EDTA and 0.01 M 2-mercaptoethanol at pH 7.4. The homogenate was centrifuged at 12,000 × g for 15 min, yielding a supernatant which was subsequently centrifuged at 105,000 × g for 1 hour. The resulting particles were washed twice with the sucrose solution, using 5 volumes per g of original scrapings, and centrifuged at 105,000 × g for 1 hour after each wash. The washed particles were resuspended in the sucrose solution (0.2 ml per g of original scrapings) and stored at -20°C. The high speed supernatants had no enzymatic activity. These preparations were stable to freezing and thawing, and over a period of several months there was no significant loss of activity.

Assay of Enzymatic Activity—The galactosyltransferase assay was performed at 37°C in a final volume of 0.05 ml, containing 0.25 μmoles of sialidase-treated ovine submaxillary mucin, 0.05 μmoles of UDP-galactose (106 dpm per pmole), 2.5 μmoles of 2-(N-morpholino)ethanesulfonic acid, pH 6.5, 0.01 ml of 5% Triton X-100, 1.5 μmoles of MnCl2, and enzyme. After the mixture was incubated for 60 min, 0.01 ml of 0.3 mM EDTA was added to terminate the reaction. An aliquot of the incubation mixture was subjected to paper electrophoresis on Whatman No. 3MM paper saturated with tetraborate, pH 9.0, for 30 min (13). The labeled substrate and its degradation products migrated rapidly, whereas the product remained at the origin. Areas of the paper at the origin were counted by liquid scintillation methods (Packard Tri-Carb), using a tolune system prepared by adding 74 ml of the concentrated liquid scintillator, Spectrafluor butyl-PBD (Amersham-Searle), to 1 liter of toluene. The efficiency for counting 14C under these conditions was approximately 75%.

Protein Determination—Protein was estimated by the method of Lowry et al. (14) using crystalline bovine serum albumin as a standard.

Estimation of Km and Vmax—Km and Vmax were determined by a computer-generated linear regression analysis by the method of Wilkinson (15).

RESULTS

Requirements of Enzymatic Activity—The requirements for the incorporation of galactose into OSM(-NAN) are shown in Table I. Mn²⁺ was essential for activity, while other divalent cations tested showed either little or no activity. The reaction was stimulated approximately 4-fold by Triton X-100. The low activity in the absence of OSM(-NAN) suggested that only a small amount of endogenous acceptors was present in the particulate enzymatic preparation.

Kinetic Studies—The transfer of galactose was linear with time for at least 4 hours and was proportional to protein concentration from 0.65 to 3.4 μg per ml of assay mixture. The enzyme displayed optimum activity in 2-(N-morpholino)ethanesulfonic buffer between pH 6 and 7 (Fig. 1). Transfer of galactose to OSM(-NAN) as a function of nucleotide concentration is illustrated in Fig. 2. An approximate Km value of 7 × 10⁻⁴ M was determined for UDP-galactose. The effect of increasing the concentration of OSM(-NAN) on the enzymatic activity is summarized in Fig. 3. An approximate Kmax value for OSM(-NAN) was calculated to be 1 × 10⁻³ M. Fig. 4 shows the dependence of

| Mineral | Vmax (μmoles/mg protein/hr) |
|---------|-----------------------------|
| Mn²⁺   | 2                           |
| Mn²⁺ plus Mg²⁺ | 7                       |
| Mn²⁺ plus Ca²⁺  | 7                       |
| Mn²⁺ plus Zn²⁺  | 2                       |
| Triton X-100  | 38                      |

Table I

Requirements of canine tracheal UDP-galactose: mucin galactosyltransferase

The standard assay as described in the text was used, using 96 μg of enzyme protein.

Incubation mixture | Galactose transferred (μmoles/mg protein/hr)
|-------------------|-----------------------------|
| Completo          | 165                        |
| Minus OSM(-NAN)   | 3                           |
| Minus enzyme      | 2                           |
| Minus Mn²⁺        | 2                           |
| Minus Mn²⁺ plus Mg²⁺ | 7                       |
| Minus Mn²⁺ plus Ca²⁺  | 7                       |
| Minus Mn²⁺ plus Zn²⁺  | 2                       |
| Minus Triton X-100  | 38                      |
| Minus enzyme plus boiled enzyme | 2 |

The abbreviations used are: Fet(-NAN, Gal), sialidase-galactosidase-treated fetuin; OSM(-NAN), sialidase-treated ovine submaxillary mucin; NAN, N-acetyleneuraminic acid.
the centrifuged 105,000 x g for 1 hour and the supernatant volume of
lyophilized. The lyophilized residue was reconstituted in a small
fluid containing the 14C product was dialyzed against water and
(2 x 2 X ('III) ali11ed with water. The peak corresponding
and 5.9 mg of enzyme protein. The incubation mixture was
ethanesulfo~ic acid, pH 6.5, 150 pmoles; Triton X-100, 0.03
OSM(-NAN), 10 pmoles; MnCl2, 60 pmoles; 2-(N-morpholino)ethane
mucin. The incubations contained 96 p.L of enzyme protein, and
enzymatic activity on I h concentration. The optimal con-
formation of galactose into sialidase-treated ovine submaxillary
FIG. 2 (right). The effect of UDP-galactose concentration on the
incorporation of galactose into sialidase-treated ovine sub-
maxillary mucin. Each tube contained 48 pg of enzyme protein. Other
conditions were as described for the standard assay procedure.
The particulate enzymatic preparation prepared from canine
tracheal tissue was capable of transferring galactose to several
acceptors, as shown in Table II. A comparison of the Kmax values and Km values suggests that glycoproteins containing
terminal N-acetylgalactosamine or N-acetylglucosamine were the
best acceptors. When N-acetylgalactosamine and N-acetylglu-
coamine were incubated with OSM(-NAN) instead of UDP-[U-14C]-
galactose in the standard assay, there was no incorporation of the
labeled sugar.

The particulate enzymatic preparation prepared from canine
mucin product give only one radioactive spot at the origin
in 1% borate, pH 9.0. A portion of the [4C]mucin product was hydrolyzed in 2 n HCl at 100° for 90 min, the acid was removed, and the residue was re-
constituted in water. Aliquots were subjected to electrophoresis in 1% borate (pH 9.0) and chromatography in Solvents A and B, and in each case, only one radioactive spot was found which cor-
responded to galactose.

To characterize further the nature of the linkage between the
incorporated galactose and OSM(NAN), 2 pmoles of the [4C]-
mucin product was treated with alkaline borohydride (8). After
the mixture was neutralized with acetic acid, it was passed through a column of Dowex 50 X12, 200 to 400 mesh, H+ form,
to remove cations. The eluate was evaporated to dryness, and
borate was removed by repeated evaporation in the presence of
methanol. The residue containing the reduced oligosaccharide
was subjected to paper chromatography in Solvents A and B
electrophoresis in 1% borate, pH 9.0, and paper chromatography in Solvents A and B. Only one radioactive spot was detected which corresponded to galactose.

Carbohydrate analysis of the [4C] mucin product by gas chro-
matography showed the presence of only two sugar components.
The retention times of these components were identical with those of galactitol hexaacetate and N-acetylgalactosaminitol
eptaacetate.

Treatment of both the mucin and the reduced disaccha-
ride with galactosidase from C. perfringens, galactosidase
from Escherichia coli (Worthington Biochemical Corporation),
or galactosidase from Aspergillus niger (16) failed to release
galactose as evidenced by the electrophoretic pattern following
incubation. These enzymes have been shown to release galac-
tose in B(1-3), B(1-4), and a(1-6) linkages, respectively.

Specificity of Galactosyltransferase—When [U-14C]galactose,
[U-14C]galactose 1-phosphate, or UDP-N-acetyl[1-14C]gluco-
samine were incubated with OSM(-NAN) instead of UDP-[U-14C]-galactose in the standard assay, there was no incorporation of the
labeled sugar.

The "soluble enzyme" was still stimulated (1.7-
fold) by the presence of Triton X-100. The Kmax values for OSM-
(-NAN, Gal), respectively, the amount of galactose incorporated was markedly

reduced. Inactive acceptors were a- and /3-methyl-D-glucoside,
N-acetyl-o-mannosamine, o-methyl-o-mannoside, and porcine
submaxillary mucins (A+ and A-).

The effect of Mn2+ concentration on the incor-
poration of galactose was studied with OSM(-NAN), the following sub-
stances: N-acetyl-D-mannosamine, cr-methyl-n-mannoside, and porcine
tracheal tissue was capable of transferring galactose to several
acceptors, as shown in Table II. A comparison of the Vmax values and Km values suggests that glycoproteins containing
terminal N-acetylgalactosamine or N-acetylglucosamine were the
best acceptors. When N-acetylgalactosamine and N-acetylglu-
cosamine were removed from OSM(-NAN) and Fet(NAN, Gal), respectively, the amount of galactose incorporated was markedly
reduced. Inactive acceptors were a- and /3-methyl-D-glucoside,
N-acetyl-o-mannosamine, o-methyl-o-mannoside, and porcine
submaxillary mucins (A+ and A-).

Effect of Mixed Substrates on Enzymatic Activity—The ability
of the enzyme preparation to transfer galactose to N-acetyl-
galactosamine, N-acetylglucosamine, and glucose in the presence of @-lactalbumin suggests the presence of more than one
catalytic center. Mixed substrate experiments were performed
and the results are summarized in Table III. The transfer of

FIG. 3 (right). The effect of Mn2+ concentration on the incor-
poration of galactose into sialidase-treated ovine sub-
maxillary mucin. Each tube contained 48 pg of enzyme protein. Other
conditions were as described in the text. The specific activity of the galactosyltransferase after
this treatment. The "soluble enzyme" was still stimulated (1.7-
fold) by the presence of Triton X-100. The Kmax values for OSM-
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reduced. Inactive acceptors were a- and /3-methyl-D-glucoside,
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catalytic center. Mixed substrate experiments were performed
and the results are summarized in Table III. The transfer of

FIG. 4 (right). The effect of UDP-galactose concentration on
the incorporation of galactose into sialidase-treated ovine sub-
maxillary mucin. Each tube contained 48 pg of enzyme protein. Other
conditions were as described in the text.

Solubilization of Galactosyltransferase—After ultrasonic treat-
ment of the particles obtained from the 105,000 x g centrifuga-
sequence, approximately 86% of the membrane bound enzyme was present in the supernatant of a subsequent 105,000 x g centrifuga-
tion for 1 hour. However, there was not a pronounced increase in the specific and total activity of the galactosyltransferase after
this treatment. The "soluble enzyme" was still stimulated (1.7-
fold) by the presence of Triton X-100. The Kmax values for OSM-
(-NAN) and UDP-galactose for the soluble enzyme were the
same as those determined for the particulate enzyme.

Product Identification—In order to characterize the product
formed by the transferase with OSM(-NAN), the following sub-
stances were incubated at 37° for 30 hours in a total volume of
3.0 ml: UDP-galactose, 20 pmoles (2 x 106 dpm per pmoles); OSM(-NAN), 10 pmoles; MnCl2, 60 pmoles; 2-(N-morpholino)ethane sulfonic acid, pH 6.5, 150 pmoles; Triton X-100, 0.03
ml; and 5.9 mg of enzyme protein. The incubation mixture was
then centrifuged at 105,000 x g for 1 hour and the supernatant
fluid containing the 14C product was dialyzed against water and
lyophilized. The lyophilized residue was reconstituted in a small
volume of water and passed through a Sephadex G-50 column
(2 x 28 cm) and eluted with water. The peak corresponding
to the 14C-product was pooled and lyophilized. The residue will
be referred to as [4C]mucin product. Electrophoresis of this
[4C]mucin product gave only one radioactive spot at the origin
in 1% borate, pH 9.0.

A portion of the [4C]mucin product was hydrolyzed in 2 n HCl
at 100° for 90 min, the acid was removed, and the residue was re-
constituted in water. Aliquots were subjected to electrophoresis in 1% borate (pH 9.0) and chromatography in Solvents A and B, and in each case, only one radioactive spot was found which cor-
responded to galactose.

Carbohydrate analysis of the [4C] mucin product by gas chro-
matography showed the presence of only two sugar components.
The retention times of these components were identical with those of galactitol hexaacetate and N-acetylgalactosaminitol
eptaacetate.
Substrate specificity of canine tracheal galactosyltransferase

The standard assay as described in the text was used, using 96 µg of enzyme protein. When glucose was the acceptor, 0.25 pmol of UDP and 60 µg of α-lactalbumin were added to the incubation mixture.

| Acceptor                     | Galactose transferred | V_{max} | K_{m} |
|------------------------------|-----------------------|---------|-------|
| Bovine submaxillary mucin    |                       |         |       |
| Native                       | 66                    | 118     | 0.6   |
| NAN-free                     | 290                   | 339     | 0.6   |
| Ovine submaxillary mucin     |                       |         |       |
| Native                       | 23                    | 54      | 8.7   |
| NAN-free                     | 110                   | 155     | 1.3   |
| N-acetylglucosamine          | 90                    | 100     | 3.4   |
| N-acetylgalactosamine        | 15                    | 142     | 210   |
| Methyl-2-acetamido-2-deoxy-α-| 147                   | 170     | 3.4   |
| galactopyranoside            |                       |         |       |
| Fetuin                       | 2                     |         |       |
| Native                       | 3                     |         |       |
| NAN-free                     | 80                    | 141     | 0.9   |
| Galactose                    | 62                    | 73      | 6.3   |

Concentration is expressed as milligrams, dry wt, of the acceptor and not as acceptor site concentration.

Mixed substrate experiments

The standard assay as described in the text was used, using 127 µg of enzyme protein and the following amounts of acceptor molecules (micromoles): OSM(-NAN), 0.25; Fet(-NAN, Gal), 0.05; N-acetylgalactosamine, 0.45; N-acetylglucosamine, 22.5; glucose, 0.55. α-Lactalbumin concentration was 0.1%.

The mucin galactosyltransferase from canine trachea appears to be similar in many respects to the galactosyltransferase from porcine submaxillary gland (17, 18). Both have the same acceptor specificity. The transfer is dependent upon Mn²⁺ and is stimulated several-fold by Triton X-100. The kinetic constants of tracheal galactosyltransferase are similar to those reported for the submaxillary enzyme; differences are probably due to the particulate nature of both preparations. Unlike the galactosyltransferase from porcine submaxillary gland, the latter enzyme catalyzes the transfer of galactose to N-acetylgalactosamine but not as acceptor site concentration.

DISCUSSION

It is believed that the biosynthesis of the oligosaccharide side chain of mucin involves a series of glycosyltransferases which attach 1 monosaccharide residue at a time to the growing unit (5). A galactosyltransferase has been demonstrated in normal canine tracheal tissue which is consistent with such a hypothesis. The enzyme catalyzes the transfer of galactose residues to terminal N-acetylgalactosamine residues of mucin type molecules; removal of the N-acetylgalactosamine residues results in a marked decrease of enzymatic incorporation of galactose. Free N-acetylgalactosamine, as well as the α-methyl glucoside of this hexosamine, also served as acceptors.

Summary

The mucin galactosyltransferase from canine trachea appears to be similar in many respects to the galactosyltransferase from porcine submaxillary gland (17, 18). Both have the same acceptor specificity. The transfer is dependent upon Mn²⁺ and is stimulated several-fold by Triton X-100. The kinetic constants of tracheal galactosyltransferase are similar to those reported for the submaxillary enzyme; differences are probably due to the particulate nature of both preparations. Unlike the galactosyltransferase from porcine submaxillary gland, the latter enzyme catalyzes the transfer of galactose to N-acetylgalactosamine but not as acceptor site concentration.
corporated galactose was not susceptible to hydrolysis by a galactosidase which hydrolyzes β(1→3) linkages; nor was it susceptible to galactosidases which hydrolyze β(1→4) or α(1→6) linkages. This suggests that galactose is being incorporated by the tracheal enzyme onto terminal N-acetylgalactosamine residues of OSM(-NAN) by a linkage other than these three possibilities. Alternatively, the incorporated galactose may not be susceptible to hydrolysis by the available galactosidases because of steric hindrance caused by the protein core of ovine submaxillary mucin.

In addition to the mucin galactosyltransferase, mixed substrate experiments (Table III) suggest the presence of either another galactosyltransferase or an additional enzyme site. This enzyme is similar to the galactosyltransferase which is found in colostrum and tissue fractions and which utilizes terminal N-acetylglucosamine positions on glycoproteins (19, 20). Free N-acetylglucosamine is also an acceptor.

These results suggest that the following sequences are present in canine tracheal mucins: galactosyl-N-acetylgalactosamine and galactosyl-N-acetylglucosamine. Carbohydrate analyses have shown the presence of these sugars in canine tracheal mucins, but no structural studies have been done on the native molecules to confirm these speculated sequences. In addition, two other glycosyltransferases have been demonstrated in normal canine respiratory tissue (21). One is an N-acetylgalactosaminyltransferase which transfers N-acetylgalactosamine residues to a polypeptide, and the other is a sialyltransferase which transfers sialic acid to N-acetylgalactosamine residues.

Based on the composition of the oligosaccharide side chains, three fundamental types of glycoproteins have been characterized from tracheobronchial secretions (2). It appears that certain chronic obstructive lung diseases associated with hypersecretion are characterized, not by abnormal constituents, but by abnormal proportions of these three basic constituents (4). This would indicate an alternation of the relative activities of the glycosyltransferases of the multienzyme system present in respiratory tissue. An irritant-induced state which closely resembles the histopathological characteristics of human chronic bronchitis can be produced in animals by the inhalation of noxious materials (22); present studies are concerned with determining the activities of the glycosyltransferases in this experimentally induced disease state.

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REFERENCES

1. BAKER, A., and MUNRO, J. R., Fed. Proc., 29, 857 (1970).
2. GERNEZ-RIEUX, C., BISERTE, G., HAYEZ, R., VOISIN, C., ROUSBEL, P., and DPOUND, P., Prensa Med. Argent., 63, 426 (1966).
3. LAMB, D., and REID, L., J. Pathol., 98, 213 (1969).
4. REID, L., The lung, The Williams and Wilkins Company, Baltimore, 1967, p. 87.
5. ROSEMAN, S., in E. ROSSI and E. STOLL (Editors), Biochemistry of glycoproteins and related substances, Part 11, S. Karger, New York, 1968, p. 244.
6. HASHIMOTO, Y., HASHIMOTO, S., and PIGMAN, W., Arch. Biochem. Biophys., 104, 292 (1964).
7. DE SALLEGUI, M., and PIGMAN, W., Fed. Proc., 26, 608 (1967).
8. CARLSON, D. M., J. Biol. Chem., 243, 616 (1968).
9. SPIRO, R. G., J. Biol. Chem., 235, 2800 (1968).
10. CARLSON, D. M., McGUIRE, E., and JOUARDIAN, G. W., in E. F. NEUFELD and V. GINSBURG (Editors), Methods in enzymology, Vol. VII, Academic Press, New York, 1966, p. 361.
11. CHIPOWSKY, S., and McGUIRE, E. J., Fed. Proc., 26, 606 (1969).
12. GRIGGS, L., Anal. Biochem., in press.
13. ROSEMAN, S., in E. F. NEUFELD and V. GINSBURG (Editors), Methods in enzymology, Vol. VIII, Academic Press, New York, 1966, p. 354.
14. LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., and RANDALL, R. J., J. Biol. Chem., 193, 265 (1951).
15. WILKINSON, G. N., Biochem. J., 80, 324 (1961).
16. LEE, Y. C., and WACEK, V., Arch. Biochem. Biophys., 133, 264 (1970).
17. SCHACHTER, H., and McGUIRE, E. J., J. Biol. Chem., 273, 345 (1968).
18. McGUIRE, E. J., in D. AMINOFF (Editor), Blood and tissue antigens, Academic Press, New York, 1970.
19. McGUIRE, E. J., JOUARDIAN, G. W., CARLSON, D. M., and ROSEMAN, S., J. Biol. Chem., PC 240, 4112 (1965).
20. WATKINS, W. M., and HASSID, W. Z., J. Biol. Chem., 237, 1459 (1962).
21. BAKER, A., SAWYER, J., and MUNRO, J. R., Fed. Proc., 30, 1186 (1971).
22. REID, L., Brit. J. Exp. Pathol., 44, 437 (1963).
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