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Meconium Proteins and Mucoproteins in Meconium Ileus (32806)

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Information about the composition of meconium in meconium ileus (MI) is accumulating (1–4). An early report (1) indicated that MI meconium contained similar water, 13 times more trichloroacetic acid (TCA)-insoluble nitrogen, and 4 times less carbohydrate than is found in normal meconium. Other reports describe the presence of serum proteins by immunologic detection (2–4) and mucoids by isolation (4). Serum albumin was detected also in fibrocystic meconium (5).

Previous studies have not provided comprehensive and quantitative information about the protein and mucoprotein fraction of the MI meconium. The protein and mucoprotein fraction is probably of primary significance relative to the development of the pathophysiological state.

This report (a) presents a comprehensive quantitation of meconium proteins and mucoproteins and (b) correlates this quantitation with existing segments of information.

Methods and Materials. The individual meconium samples were frozen immediately and stored at −20°C for several days. Frozen raw meconium (1 gm) was suspended in chilled distilled water (10 ml; 4°C) and was homogenized (Vir-Tis model 23; speed setting of 30 for 4 min) in an ice-water bath. The homogenate was centrifuged at 1,500*g* for 30 min in a refrigerated centrifuge (2°C). The supernate was harvested and used without further processing. Solids were determined on the centrifugate and on an aliquot of the supernate.

The meconium supernates were electrophoresed on paper at 4°C by use of the Beckman/Spinco model R assembly. Beckman accessories and procedures were utilized. Protein was determined with the bromophenol blue method (Procedure B); and mucoprotein was determined with the periodic acid Schiff method (Beckman Technical Bulletin 6095A). All quantitation was by Analytrol scan with human serum albumin (4 times crystallized) and α-globulin (Cohn fraction IV-I) from the Nutritional Biochemicals Corp. as color standards for protein and mucoprotein, respectively.

All substances being measured in the protein and mucoprotein methods would not react identically to the standards, of course, on a weight basis. It is not possible to quantitate such a broad range of substances on an absolute basis, but the use of albumin and α-globulin as standards allows an approximate quantitation of the same types of substances

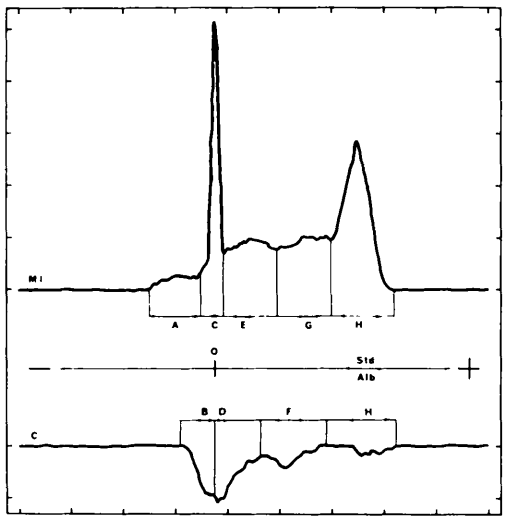

FIG. 1. Densitometric scans on the Analytrol of Proteins A through H in the meconium of meconium ileus (MI) and control (C) subjects. O, origin; Std Alb, human serum albumin mobility reference. The extent of the *concentrated* protein band is indicated by the arrowed horizontal inclusion; and the extent of the quantitation zone is indicated by the associated vertical lines. Indicated resolutions were obvious on the stained electrophoretograms although some are not apparent on the scan tracings. Sample volumes: 50 μl MI and 100 μl C meconium supernate.

in normal and abnormal samples. The protein quantitations are expressed as equivalents of albumin; the mucoprotein quantitations are expressed as if 5.0% of the α-globulin reference standard were reacting in the colorimetric scan densitometry. The latter standardization is based on the content of α_2-mucoprotein (and equivalent substances) in Cohn plasma protein fraction IV-I (6).

It is not known what degree of duplication might exist for substances measured by the protein and mucoprotein methods. None of the 6 mucoprotein zones corresponded with any of the 8 protein zones, i.e., these would seem to be 14 different substances. The impression gained from the visual evaluation of the stained electrophoretograms is that there is very little duplication.

The subjects of this study (1 meconium ileus and 1 control) were under the clinical care of Dr. Nancy Huang at St. Christopher's Hospital, Philadelphia.

Results and Discussion. The MI and control subjects had meconium water contents of 66.0 and 72.9%, respectively. The homogenate supernates contained 70.0 and 98.0% of the solids of the raw meconiums from MI and control subjects, respectively. The qualitative profiles of the meconium proteins and mucoproteins[1] are indicated in Figs. 1 and 2; and the quantitative profiles are indicated in Table I.

A total of 8 proteins and 6 mucoproteins were recognized on the paper strips loaded with the meconium extracts. H (human serum albumin) was the only protein and C and E were the only mucoproteins found in both meconiums, however, at different concentra-

TABLE I. Proteins and Mucoproteins in Meconium.

Constituent	% of Meconium solids	
	Mec. ileus	Control
Protein A	2.0	—
B	—[a]	3.6
C	11.5	—
D	—	3.4
E	12.4	—
F	—	2.1
G	12.9	—
H	22.2	0.4
Total	61.0	9.5
Mucoprotein A	3.4	—
B	—	2.0
C	6.5	1.3
D	2.4	—
E	2.8	2.6
F	—	1.4
Total	15.1	7.3

[a] Less than 0.5%.

[1] The nomenclature of mucosubstances is extremely variable. The following terms are used to designate saccharide–peptide substances in this paper. Protein, very small amounts of carbohydrate (e.g., <1%); glycoprotein, small amounts of carbohydrate (e.g., >1, <10%); mucoprotein, significant but minor amounts of carbohydrate (e.g., >1, <50%); mucoid, significant but minor amounts of protein (e.g., >1, <50%); mucopolysaccharide, small amounts of protein (e.g., <10%). We regret, for example, that Schachter and Dixon (4) describe a preparation with minor protein content (12–21%) as a mucoprotein.

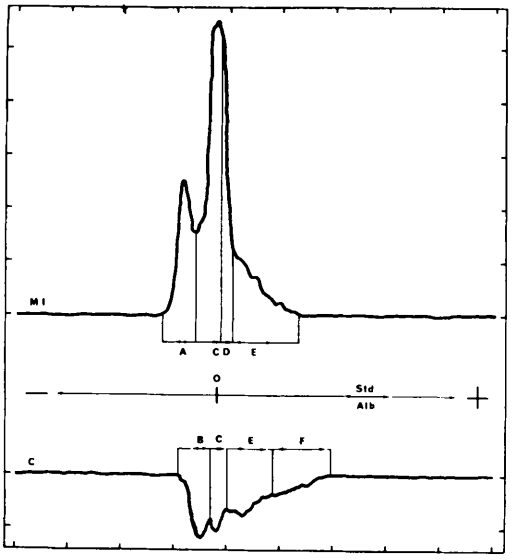

FIG. 2. Densitometric scans on the Analytrol of Mucoproteins A through F in the meconium of meconium ileus (MI) and control (C) subjects. See Fig. 1 for technical detail. Sample volumes: 100 μl meconium supernate for both MI and C.

tions. The abnormal meconium characteristically contained proteins A, C, E, and G and mucoproteins A and D. On the other hand, the abnormal meconium characteristically lacked proteins B, D, and F and mucoproteins B and F. The quantitative relationships are indicated in Table I.

Albumin,[2] detected previously qualitatively (2–5), was found in the MI meconium to the extent of 22.2 and 7.5% of meconium solids and raw meconium, respectively. Albumin[3] was found in control (normal) meconium to the extent of 0.4 and 0.1% of meconium solids and raw meconium, respectively. The α-, β-, and γ-globulins were found less than 4.4, 4.2, and 0.7%, respectively, in MI raw meconium and less than 0.2, 0.2, and 0.2%, respectively, in normal raw meconium.

It is now possible, with the results from this study and one published by Schachter and Dixon (4), to calculate an informative overview of the difference between MI and control meconium. This is presented in Table II. The various segments of data, although reported from different laboratories, come from reports based on meconium solids (from meconium samples which had water contents of comparable magnitude). It is notable that this logical compilation results in summation totals which approximate 100%. This tabulation gives emphasis to the outstanding difference between MI and normal meconium, namely, the major content of proteins and mucoproteins in MI meconium and the major content of mucoids, mucopolysaccharides, and other small units in normal meconium.

These studies indicate that there is a profoundly different pattern of proteins and mucoproteins in the meconiums of meconium ileus and control subjects. Perhaps information on (a) why these differences exist between meconium ileus and control subjects and (b) how a variety of meconium macromolecules is normally converted to a group of mucoid heteropolymers may lead to understandings about the metabolic defect in meconium ileus and in cystic fibrosis.

Summary. Qualitative and quantitative profiles were established for 14 proteins and mucoproteins in meconium from meconium ileus and control subjects. Three of these substances were found in both meconiums, at different concentrations. The abnormal meco-

TABLE II. Major Constituents of Meconium.

Item[d]	Constituent	% of Meconium solids: Mec. ileus	Controls
1	Ash	1.8	4.0
2	Carbohydrate, dialyzable	1.3	4.6
3	Lipid	9.7	12.3
4	Proteins and mucoproteins[a]	76.1	16.8
5	Mucoids[b]	3.5	26.0
6	Mucopolysaccharides, etc.[c]	8.8	41.2
	Totals	101.2	104.9

[a] Including glycoproteins.

[b] Described as a mucoprotein entity in Ref. (4); regarded here as a group of mucoid heteropolymers.

[c] Including polysaccharides, peptides, and amino acids.

[d] Items 1, 2, and 3 are from Ref. (1); item 4 is from this report; item 5 is from Ref. (4); and item 6 is calculated from TCA-soluble N times 6.25 plus nondialyzable carbohydrate of Ref. (1) minus item 5.

[2] Human serum albumin or a substance of identical mobility.

nium characteristically contained 6 and lacked 5 proteins and mucoproteins.

The authors are grateful to Dr. Nancy Huang for the supply of meconium samples and information about her patients.

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Alteration of Measles Virus Hemagglutinating Properties by Sonication* (32807)

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Hemagglutination titers of unfractionated measles virus pools may be enhanced up to 32-fold by the addition of salts such as $(NH_4)_2SO_4$ to the assay system. The degree of enhancement varies from one preparation to another and appears to be correlated with the presence of measles hemagglutinating particles with different salt requirements for adsorption and hemagglutination (1). One class of particle is dependent upon high concentrations of polyvalent anions for adsorption, and its hemagglutination titers increase with increasing ion concentration. This class is referred to as the salt-dependent agglutinin (SDA) and its physical and hemagglutinating properties have been described (1).

Measles pools also contain variable amounts of hemagglutinating particles which, unlike SDA, adsorb to red blood cells in isotonic saline (2), and which may or may not show enhanced hemagglutination titers following addition of salt to the diluent (1). Collectively, these particles have been well studied (3), although their differential response to salt concentration was unrecognized at the time.

It was the purpose of this study to distinguish between those particles which show enhanced hemagglutination titers in high salt concentrations (S-HA particles) and those which are not enhanced (HA particles). The results of these comparisons plus the finding that HA particles acquire S-HA properties following sonication suggested an interrelationship between particles with different hemagglutinating properties which is discussed. Application of these findings to the preparation of antigens for detection of measles hemagglutination-inhibiting (HI) antibodies is also discussed.

Methods. Preparation of virus pools, reagents, and experimental procedures have been described in detail (1). The S-HA titrations were done in the same way as SDA titrations, i.e., 0.8 *M* $(NH_4)_2SO_4$ in PBS (pH 7.3) was used for diluent except when specific ionic or pH requirements for enhancement were being determined.

Results and Conclusions. The data in Table I show that S-HA hemagglutination reactions are similar to SDA reactions and most differences are quantitative only. One striking difference was demonstrated, however. The S-HA titers increase with increasing Cl^- ion concentration to an optimum at about 1.0 *M* NaCl. On the other hand, SDA was not detectable by hemagglutination at any NaCl concentration tested (0.15–2.0 *M*).

In size and density, S-HA particles as a

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