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Note

# Immunoelectrophoretic analysis of human meconium antigens of non-plasma origin

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Meconium is the first stool passed after birth and represents the accumulation of intestinal contents during foetal life. The main constituent of meconium is the mucilaginous ground substance which is rich in blood group substances [1,2] but other macromolecules such as serum proteins [3] and enzymes of pancreatic and intestinal origin have also been characterized [4, 5]. Knowledge of the distribution of the meconium components may be of value in increasing our understanding of the biochemistry of the foetal gut and perhaps of more immediate importance, be of use when developing methods of screening for diseases of gut metabolism in the neonate [6]. Immunochemical methods such as electroimmunoassay and immunoelectrophoresis provide rapid and simple ways for the characterization and determination of macromolecules in biological fluids and may be adapted to the routine analysis of such components, an important criterion in a screening procedure. However, until now, the analysis of meconium by such methods has been limited to the study of its serum protein component [4, 7]. I wish to report here the results of a cross immunoelectrophoretic analysis of meconium antigens of non-plasma origin.

## EXPERIMENTAL

### *Meconium specimens and preparation of water soluble extract*

Meconium specimens were collected during the course of a routine screening programme from 50 healthy neonates born at full term. All specimens were lyophilized, bulked and stored at −20° before use. A water-soluble extracts was prepared as follows: 200 mg of the bulked, dried material was suspended in 10 ml of saline with the aid of a Potter's tissue homogeniser and the resultant sus-

pension spun at 84,000 *g* in a Beckman SW50 rotor ($r_{av}$ = 8.3 cm) for 30 min at 4°. The clear supernatant was collected and used in the subsequent electrophoretic analysis.

*Immunoelectrophoretic analysis*

Antisera to lactoferrin and secretory IgA were prepared in rabbits as previously described [8]. A similar procedure was used to raise antiserum against meconium proteins using a 1% suspension of meconium as an antigen source. After adsorption of the antiserum with gluteraldehyde-insoluble human serum [8] to remove antibodies to the serum proteins (SP), it was designated anti-meconium (—SP) serum. Antisera to human trypsin and chymotrypsin were obtained from Miles Labs. (Slough, Great Britain). Antiserum to carcino-embryonic antigen was obtained from DAKO-Immunoglobulins (Copenhagen, Denmark). Antiserum to human blood group A was a gift from the Welsh Transfusion Centre.

Quantitative cross immuno-electrophoresis of the meconium extract was carried out against the appropriate antisera as previously described [8]. After electrophoresis, the washed, dried plates were stained for protein with Coomassie blue [8], for carbohydrate by the periodic acid —NADI (naphthol-phenylenediamine) reaction [9] and for alkaline phosphatase, esterase, tryptic and chymotryptic activities by the methods described by Uriel [9]. Specific antiserum was used to identify arcs by the intermediate gel method [10]. The specific antiserum was incorporated into an intermediate gel interposed between the first dimension gel containing the separated antigens and the second dimension gel containing polyvalent antiserum (reference gel).

*Gel chromatography*

A 5-ml aliquot of the 2% meconium saline extract was separated on a column of Sepharose CL-4B (2.5 × 90 cm) with 0.05 *M* Tris—0.09 *M* acetic acid, pH 7.3, containing 0.3 *M* sodium chloride. The eluent was monitored at 280 nm and 5-ml fractions were collected. The elution profiles of the individual meconium antigens were then determined using the polyvalent antiserum with a "fused rocket" technique [11] and as the column had been previously calibrated with human serum proteins of known molecular weight, an approximate estimation of the molecular sizes of the major meconium antigens was made.

*Inhibition of haemagglutination*

Inhibition of haemagglutination was tested as follows: 50 μl of 2% washed group A red cells were mixed with 50 μl of the test solution and 50 μl of anti-A serum and agglutination assessed after standing for 30 min at room temperature. Serial dilution of the 2% meconium extract and chromatography fractions were studied for inhibition activity.

## RESULTS

Cross immunoelectrophoresis of 2% meconium extract against anti-meconium (—SP) developed 7 arcs designated Mec I to Mec VII (Fig. 1). The Mec I arc had a distinct peak but all the arcs were elongated suggesting each com-

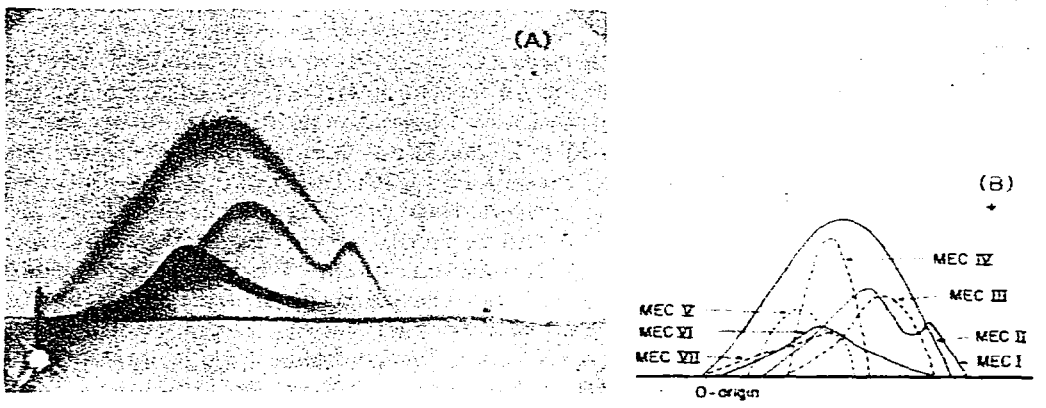


Fig. 1. Quantitative cross immunoelectrophoresis of meconium extract against anti-meconium serum adsorbed with human serum: antiserum (—SP). (A) 2.0% meconium extract against anti-meconium (—SP), immunoelectropherogram was stained with Coomassie blue. (B) Line diagram of meconium extract against anti-meconium (—SP). Mec III arc was due to carcinoembryonic antigen and Mec IV was due to alkaline phosphatase.

ponent had some degree of electrophoretic heterogeneity. Mec I, Mec II, Mec Vi and Mec VII stained for carbohydrate; Mec IV had alkaline phosphatase activity and the ascending anodal part of the Mec VI had chymotrypsin activity. None of the arcs had tryptic or carboxylesterase activity. Identification of antigens with specific antiserum was carried out using intermediate gel cross immunoelectrophoresis. An arc developed in the intermediate gel which contained antiserum to CEA (carcino-embryonic antigen) while the Mec III arc was absent from the polyvalent pattern that developed in the corresponding reference gel. No precipitation arcs developed in the intermediate gel when antiserum to chymotrypsin, trypsin, lactoferrin, secretory IgA or blood group substance A was used and there were no significant changes in the polyvalent patterns. A number of "high titre" anti-A sera were used but in no instance was it possible to develop a precipitation arc against the meconium extract although a meconium extract concentration of 0.02% could inhibit the haemagglutination of group A cells by all the anti-A sera tested. It was concluded that the anti-A activity titre was too low to be used in this immunoelectrophoretic system.

An approximate estimation of molecular weights of the unidentified meconium antigens was made by gel chromatography on a previously calibrated column of Sepharose CL-4B. Mec I had a molecular weight of about 100,000. Mec II was eluted in the void volume suggesting a molecular weight of 1,000,000—2,000,000. Mec VI had a molecular weight of about 500,000 and Mec VII demonstrated pronounced molecular polydispersity between 100,000 to 1,000,000. The Mec V arc was too faint and it was not possible to determine its elution profile. The inclusion of 6 *M* urea into the chromatography buffer had no effect on the elution profiles of the meconium antigens. Inhibition of haemagglutination activity was found throughout the column eluent although there was a peak of inhibitory activity in the void volume.

## DISCUSSION

Seven meconium antigens of non-plasma origin have been detected, two of which have been identified as carcinoembryonic antigen (Mec III) and alkaline phosphatase (Mec IV). Some tentative conclusion concerning the nature of the other antigens can also be made. Mec I, Mec II and Mec VI were glycoproteins with molecular weights of approximately 100,000; 1,000,000—2,000,000 and 500,000 respectively. None of the antigens were proteins commonly associated with exocrine secretions, lactoferrin and secretory piece or secretory IgA, nor were they pancreatic proteinases. Although part of the Mec VI arc had chymotryptic activity, its molecular size and its negative reaction with antiserum to chymotrypsin precluded the possibility that Mec VI was chymotrypsin. The fact that the Mec VI arc did not also have tryptic activity implied that the antigen had some specificity towards chymotrypsin adsorption. Although no precipitation arcs developed with the anti-A sera, the marked inhibition of haemagglutination by the meconium extract leaves the possibility that one or more of the antigen arcs were due to blood group substances.

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