Viscosity and Origin of Meconium in Meconium Ileus.* (24459)

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Meconium ileus, thought to be an early manifestation of the more generalized disease, mucoviscidosis, is a congenital disorder of the newborn in which abnormally viscous meconium obstructs the infant's intestinal tract. Until recently, little information has been available concerning the chemical nature of this abnormal meconium. In a comparative study of the composition of normal and abnormal meconium, Buchanan and Rapoport (1) found the latter to contain a greater amount of nitrogen, most of which was trichloroacetic acid (TCA) precipitable. While work was in progress in the authors' laboratory, Green *et al.*(2), reported results of paper electrophoretic analyses which demonstrated the presence of human serum albumin and what appear to be small amounts of alpha, beta, and gamma globulin in meconium from patients with meconium ileus. The present study was directed toward elucidation of the nature and origin of the viscous meconium in this disease.

Materials and methods. A sample of meconium was obtained, at surgery, from a 2-day-old infant suffering from meconium ileus. Soluble components of abnormal meconium were dissolved by stirring with water overnight at 5°C. The resulting suspension was centrifuged at 10,300 × *g* for 30 minutes. The supernatant solution (Sol. I) was clear and dark green. Addition of 10% TCA to Sol. I resulted in formation of a heavy precipitate.

Results. After dialysis of Sol. I against water, it was dialyzed against 2 l of pH 8.60, ionic strength 0.1, barbital buffer and subjected to electrophoresis with the Tiselius apparatus supplied by Frank Pearson Associates. Fig. 1 (Top) is a schlieren scanning diagram(3) of the boundaries after 172 min. at 5.23 v/cm. Planimetry of this pattern gives the following percentages of total pattern area, exclusive of salt boundary, for each component: A, 69.5%; B, 17.4%; and C, 13.1%. Semi-micro Kjeldahl analyses, performed on Sol. I and on supernatant solution obtained after precipitation of the protein in Sol. I with TCA, indicate 8.0% of dry weight of meconium to be water soluble, TCA precipitable nitrogen. If the protein present is 16% nitrogen by weight, it follows that 50% of dry weight of meconium is water soluble, TCA precipitable protein. In agreement with Green *et al.*(2), normal meconium was found to contain no TCA precipitable protein. Boundaries A, B, and C migrate in a fashion characteristic of albumin and alpha and beta globulin respectively in pH 8.60, ionic strength 0.1, barbital buffer. These results are in agreement with those reported elsewhere (2), but the present study does not demonstrate a boundary corresponding to gamma globulin.

Because it is known that the fetus swallows amniotic fluid at least as early as fifth month of intrauterine life(4), it was believed that proteins present in meconium may have originated from swallowed amniotic fluid. Accordingly, samples of amniotic fluid were obtained from apparently normal healthy females at parturition.‡ A small amount of insoluble material was removed by centrifugation at 25,500 × *g* for 20 minutes. The supernatant solution was then concentrated 5 times by pervaporation. After dialysis against water and then against pH 8.60, ionic strength 0.1, barbital buffer, the concentrated amniotic fluid was analyzed by electrophoresis. Fig. 1 (Bottom) shows the pattern after 95 min. at 7.85 v/cm. Planimetry of this pattern gives the following component percentages: D, 64.8%; E, 22.4%; and F,

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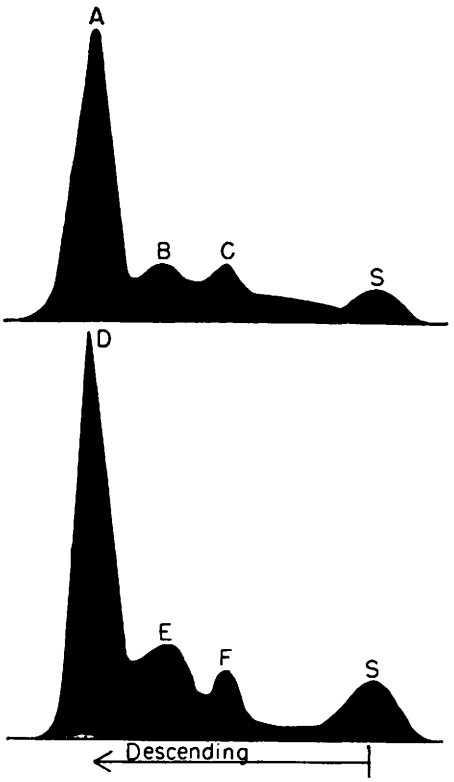

FIG. 1. (Top) Electrophoretic pattern of Sol. I. (Bottom) Electrophoretic pattern of amniotic fluid. *S* denotes salt boundary.

12.9%. Similar results have been reported elsewhere(5).

The 2 proteins forming boundaries A and D were purified from Sol. I and amniotic fluid by the technic of Schwert(6). The identity of these proteins with human serum albumin was indicated by their similar electrophoretic mobilities and sedimentaton constants and was confirmed by Ouchterlony technic(7) of double diffusion in agar employing solutions of these proteins and rabbit antibody to human serum albumin.

To determine the effect of added protein on plastic properties of normal meconium, a pooled sample of meconium from 2 apparently normal newborn infants was lyophilized and dry weight determined. The lyophilized meconium was then divided into 2 portions. To one was added crystalline human serum albumin (Cohn Fraction V) such that its concentration in normal meconium was that found in abnormal meconium. The other portion was retained as a control. The appropriate volume of water, based upon dry weight determinations of the abnormal meconium, was added to each. After thorough mixing, the 2 samples were compared with each other, and with samples of normal untreated meconium and with meconium from the patient with meconium ileus. The lyophilized sample with added albumin was much more viscous and tenacious than either control or untreated normal meconium, and appeared identical with meconium from the patient with meconium ileus.

Discussion. The theory that an abnormally viscous mucoprotein may be produced in patients with meconium ileus has been invoked to explain the viscous nature of meconium found in this disease(8). In view of the effect of albumin upon normal meconium, and that pancreatic digestive enzymes are either diminished or totally absent in patients with this disease(8), such an hypothesis would seem unnecessary. From present results, it appears that amniotic fluid is the source of proteins in abnormal meconium. Presumably proteins continually ingested in amniotic fluid are unable to be hydrolyzed, but are mixed with components of normal meconium and are concentrated by intestinal absorption of water to produce meconium characteristic of meconium ileus.

Summary. 1) Meconium from a patient with meconium ileus has been studied electrophoretically, revealing presence of albumin and of smaller amounts of other proteins with mobilities corresponding to α and β globulin. The identity of albumin was established by immunochemical and physical methods. Semimicro Kjeldahl analyses of the abnormal meconium indicate 50.2% of dry weight of meconium to be water soluble, TCA-precipitable protein. Addition of human serum albumin to lyophilized pooled samples of meconium from normal infants results in meconium grossly indistinguishable from abnormal meconium. 2) The electrophoretic pattern of human amniotic fluid is identical with that of meconium ileus meconium and it is suggested that amniotic fluid is the source of protein present in meconium from patients with meconium ileus.

1. Buchanan, D. J., Rapoport, S., *Pediatrics,* 1952, v9, 304.
2. Green, M. N., Clarke, J. T., Shwachman, H., *ibid.,* 1958, v21, 635.
3. Longsworth, L. G., *J. Am. Chem. Soc.,* 1939, v61, 529.
4. Reifferscheid, W., Schmiemann, R., *Zentralb. f. Gynak.,* 1939, v63, 146.
5. De Marco, C., Stirpe, F., *Giorn. Biochim.,* 1954, v3, 291.
6. Schwert, G. W., *J. Am. Chem. Soc.,* 1957, v79, 139.
7. Ouchterlony, O., *Arkiv. Kemi, Mineral Geol.,* 1949, B26 No. 14, 1.
8. Villee, C. A., in *Eighteenth Ross Pediatric Research Conf.,* (Columbus, Ross Laboratories, 1955), p20.

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Effect of Sonic Energy, Ultracentrifugation, and Phenol on *Neisseria gonorrhoeae* Endotoxin.* (24460)

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For certain endotoxin-antigen studies the natural cell-free complex is desired. For other studies the smallest active unit of the original biologic complex is necessary. The present investigations deal with both types of products.

Preparation of soluble nucleoprotein-bound endotoxin by physical methods. Cultivation of *N. gonorrhoeae*† has been previously described(1,2). The gonococcus cells were washed twice with 4 volumes of saline and once with 4 volumes of distilled water. A supply of such cells was kept frozen at –20°C. Washed cells contained 10-12% solids. Three g portions of washed cells were suspended in 47 ml portions of distilled water and subjected to sonic disintegration in Raytheon 10-kc sonic oscillator at 8°C for 60 minutes at maximum intensity. Cell debris, 8.8-9.0%, on dry cell basis, was removed by centrifuging in cold room in Servall angle centrifuge at 3,600 g for 15 minutes. The clear supernatant was placed in cellophane bags and dialyzed for 20 hours with continuous stirring against distilled water. The supernatant, after dialysis, contained 90% of solids originally present in the gonococcus cells (5-6 mg protein/ml). Analysis indicated that this material consisted mainly of nucleo-proteins. Such supernatants were stored at –20°C for several months without loss of toxicity. Precipitates which occasionally formed after thawing contained part of the endotoxin, and were put back in solution by brief sonic treatment. LD_{50} values on the supernatant are listed in Table I. Twenty-five to 50 CFl male mice, weighing 16-18 g, and 5 different endotoxin concentrations were used in the assays(2). Endotoxin solutions were administered intraperitoneally. The animals were observed 5 days. Karber's(3) procedure was employed for calculating LD_{50} values and interpreting their statistical meaning. From sonic treated supernatant a stable endotoxin-containing acetone powder was prepared as follows: a small quantity of sodium chloride was added to the supernatant and

TABLE I. Endotoxin Activities of Fractions Obtained by Sonic Treatment and Ultracentrifuging.

Type of fraction	LD_{50} value, mg
Supernatant at 3,600 g after sonic treatment	1.6
Sediment at 26,500 g in 30 min.	1.1
Idem 60 "	1.3
Supernatant at 26,500 g in 60 min.	1.4
Acetone powder from same supernatant after removal of sediment	1.5
Acetone powder, cells extracted at pH 13	2.3
Protein-free endotoxin	.8

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† The gonococcus strain employed was isolated from male patient by Dr. James D. Thayer of this laboratory.