Protein in Meconium from Meconium Ileus

W. H. SCHUTT* and T. E. ISLES†

From the Department of Child Life and Health, University of Edinburgh

Meconium ileus is the earliest manifestation of cystic fibrosis, and several investigations have been undertaken to determine its cause. In 1952, Buchanan and Rapoport showed that the ash content of abnormal meconium was twice as high as that of normal newborns, and that whereas normal meconium consisted of only 7% protein, this rose to 85% in meconium ileus. We here show that the proteins concerned have similar electrophoretic mobility to normal serum proteins as well as to those found in amniotic fluid, and that their presence can be detected by a simple side-room test.

Material and Methods

Meconium from 9 cases with meconium ileus was collected at operation from a loop of resected small bowel, taking care to avoid contamination with blood from the cut end of the gut. The diagnosis of cystic fibrosis was subsequently confirmed in each case, either by estimation of sweat sodium or at necropsy. Similar specimens were obtained from 2 cases with structural small bowel obstruction, without cystic fibrosis. Meconium passed on the first day of life was also collected from 4 sibs of children with proven cystic fibrosis, as well as from 4 normal newborn infants.

Nitrogen estimation. A known weight (about 2 g.) of each specimen of meconium was homogenized with 0.9% saline and the volume made up to 20 ml. The total nitrogen in each homogenate was estimated by a micro-Kjeldahl method (Varley, 1962) after digestion of the meconium with sulphuric acid (3 parts), phosphoric acid (1 part), and selenium dioxide (1 g.). Non-protein nitrogen was estimated in the supernatant, after precipitating the protein in the meconium homogenate with 10% trichloroacetic acid, by the micro-Kjeldahl method following digestion.

The solid content of the meconium was determined by heating a weighed amount of meconium to constant weight at 100° C.

Zone electrophoresis on cellulose acetate. After passage of the homogenate through a Sephadex G-25

column, the eluate was concentrated tenfold by placing the solution contained in a dialysis sac into solid sucrose.

Approximately 5 ml. of the resulting concentrated solution was placed on a 12 × 2.5 cm. cellulose-acetate strip and electrophoresis in a barbitone-acetate buffer pH 8.6, ionic strength $\mu = 0.1$, was carried out. The protein bands were visualized with 0.2% Ponceau S. stain in 6% sulphosalicylic acid.

The liquor amnii was similarly concentrated and electrophoresed.

Qualitative test for protein (albumin). A small quantity of meconium was mixed well with a few drops of distilled water on a porcelain tile. One side of a test strip (Albustix), containing tetra-bromophenol blue buffered to pH 3 with citrate, was moistened with the diluted meconium. Any colour change in the test strip was noted. Care was taken not to contaminate both sides of the test strip with solid meconium as this stained the strip, making it difficult to observe a colour change.

Faecal excretion of PVP ¹³¹I. After blocking thyroid uptake of iodine by the oral administration of Lugol's iodine, 3 μ Ci ¹³¹I PVP was given intravenously to 4 children with cystic fibrosis. The faecal excretion was measured over a 3-day period.

Results

The average solid content of the meconium from cases with meconium ileus was 32.2% (range 26.0–37.0) compared with 23.7% (range 18.2–32.0) in the normal cases, and the corresponding protein content in each group averaged 69.6% (range 64.4–73.3) and 8.9% (range 3.0–12.1) of the dry weight of the meconium, respectively (Table I). In contrast, the proportion of total nitrogen present as non-protein nitrogen was very low in meconium ileus, averaging 6% (range 1.9–13.8), while in normal meconium it was high at 76.3% (range 64.5–93.5).

Electrophoresis of the concentrated eluate of the normal and abnormal meconium was contrasted with that of serum and the concentrate of liquor amnii (Fig.). In the abnormal meconium, a rather diffuse albumin band was dominant, and further bands were present in the α -, β -, and γ -globulin regions. In the normal meconium, faint bands

Received October 5, 1967.

* Present address: Department of Child Health, University of Bristol.

† Present address: Department of Clinical Chemistry, University of St. Andrews.

TABLE I

Protein and Non-protein-N Content; and Tetra-bromophenol Blue (Albustix) Test on Meconium from Cases of Meconium Ileus, Intestinal Obstruction, Sibs of Cases of Cystic Fibrosis, and Normal Neonates

Diagnosis	Case No.	Meconium		Sweat Sodium (mEq/l.)	Albustix
		Protein (%)	Non-protein Nitrogen (%)		
Meconium ileus*	1	—	—	—	Positive
	2	—	—	130	Positive
	3	73·3	2·8	132	Positive
	4	70·0	1·9	—	Positive
	5	—	—	—	Positive
	6	—	—	158; 130	Positive
	7	64·4	13·8	42; 39	Positive
	8	69·1	8·4	—	Positive
	9	71·0	3·1	158; 132	Positive
	Mean	69·6	6·0		
Intestinal obstruction†	1	22·2	44·8	—	Negative
	2	—	—	—	Negative
Normal sibs of cystic fibrosis cases	1	4·6	87·4	18	Negative
	2	10·7	66·0	24	Negative
	3	—	—	—	Negative
	4	8·6	80·2	20	Negative
	Mean	8·0	77·9		
Normal neonates	1	12·1	64·5	—	Negative
	2	11·6	69·8	—	Negative
	3	11·9	73·0	—	Negative
	4	3·0	93·5	—	Negative
	Mean	8·9	75·2		

* Pancreatic fibrosis was confirmed histologically in Cases 1, 4, 5, 7, and 8 *post mortem*, and in Cases 3 and 4 by surgical biopsy.

† Case 1, duodenal stenosis; Case 2, jejunal atresia.

could be seen in the α - and β -globulin regions. Albumin was absent. Electrophoresis of liquor amnii showed a very similar pattern of proteins.

A specimen of meconium, extracted with water and tested with tetra-bromophenol blue, produced a distinct colour change to a light blue in cases with meconium ileus, but not with the meconium of normal newborns (Table I).

Faecal excretion of PVP ^{131}I was 1% or less over a period of 3 days in all 4 cases of fibrocystic disease tested (Table II). This investigation could not be carried out in control cases, but the excretion is well within the accepted limits for normal adults.

Discussion

Since Buchanan and Rapoport's first report on the high protein content of meconium from these cases, other investigations have confirmed their findings by electrophoretic as well as immunoelectrophoretic techniques (Young, Schwert, and Harris, 1958; Schachter and Dixon, 1965). Albumin has consistently been found to be present in high concentration in the abnormal meconium, and the addition of human serum albumin to samples of

meconium from normal infants resulted in an increase in viscosity approaching that seen in meconium ileus (Young *et al.*, 1958). Mucoproteins are unlikely to contribute significantly to this increased viscosity, as any qualitative or quantitative differences encountered are small and can be explained by the partial proteolysis of the mucoprotein that occurs in the normal gastrointestinal tract (Schachter and Dixon, 1965).

Source of protein. Speculation has centred around the source of the albumin in meconium ileus, and the demonstration of a low PVP ^{131}I excretion

TABLE II
Faecal Excretion of PVP ^{131}I in 4 Cases of Cystic Fibrosis (3-day faecal collection)

Case No.	Age (yr.)	PVP Excretion (%)
1	8/12	1·0
2	2	0·6
3	1 2/12	0·3
4	10	0·8

FIG.—Protein electrophoretic strips of (a) liquor amnii; (b) normal meconium; (c) meconium from meconium ileus; (d) normal serum.

has excluded the possibility of a protein-losing enteropathy similar to that seen in coeliac disease.

Secretions into the gastro-intestinal tract, particularly those from the biliary system, are known to contain some protein, and electrophoresis of duodenal fluid from patients with cystic fibrosis has demonstrated albumin; this is not found under normal circumstances (Chodos, Ely, and Kelley, 1958). The quantities involved are, however, small, and a much more plausible hypothesis for the high protein content of meconium is that the albumin is 'dietary' in origin. There is good circumstantial evidence that amniotic fluid is absorbed by fetal swallowing, and final proof has recently been provided by following the fate of ^{51}Cr -tagged red cells and albumin ^{131}I after injection into the amniotic cavity (Pritchard, 1965, 1966). Chromium is hardly absorbed from the gut. The rate of disappearance from the liquor amnii can be determined and the radioactive chromium subsequently recovered from the meconium after birth. It was shown that a normal mature infant swallowed about 500 ml. of amniotic fluid daily. The albumin ^{131}I disappeared from the amniotic cavity at a similar rate, and most of the ^{131}I was subsequently excreted in the maternal urine. Our finding of a similar electrophoretic pattern in liquor amnii and meconium ileus extracts therefore allows the assumption

that most of the protein in the meconium of babies with meconium ileus is undigested and unabsorbed protein from amniotic fluid, particularly as these cases invariably have pancreatic achylia (Farber, 1944).

Test for protein. Protein detection in meconium by electrophoretic or immunoelectrophoretic techniques or by trichloroacetic acid precipitation of protein in an extract of meconium following ultracentrifugation or filtration (O'Brien and Ibbott, 1962) are not always practicable, require elaborate apparatus, and are time consuming. We have found a simple side-room technique helpful in detecting the presence or absence of albumin in significant quantities in meconium. Tetra-bromophenol blue test strips (Albustix) are available commercially, and, in the 9 cases of meconium ileus tested, provided a good indication of the presence of protein. The dye appears to have a greater affinity for albumin than for the globulins (Rennie and Keen, 1967), and is thus particularly suitable for this test. The high protein levels in the meconium of our cases were subsequently confirmed by Kjeldahl estimation. Tests performed on the meconium of 4 sibs of children with cystic fibrosis were found to be negative on each occasion, and freedom from the disease was subsequently confirmed in each instance.

Tests performed on 2 infants with intestinal obstruction from other causes and in whom the diagnosis of meconium ileus had been briefly considered were also negative on this test.

We have not as yet had the opportunity to detect an affected child amongst newborn sibs of cases with this disease, but Wiser and Beier (1964) have shown that newborn infants with cystic fibrosis have raised protein in the meconium even in the absence of meconium ileus, while unaffected sibs do not. The Albustix test may therefore prove useful for screening this high-risk group and allow early diagnosis as well as effective preventive measures and therapy. It is simpler to carry out than iontophoresis and sweat testing, and could form the basis of simple population screening at birth, though the rare cases of cystic fibrosis without pancreatic achylia would still be missed.

Summary

The protein content of meconium in 9 cases of meconium ileus was abnormally high, averaging 70% against 9% in controls and 22% in a case of neonatal obstruction not due to cystic fibrosis. Albumin is present in meconium from meconium ileus, but not from normals.

The protein is derived from swallowed amniotic fluid, the protein of which accumulates undigested if there is pancreatic achylia.

A simple test using an Albustix strip enables a high protein (albumin) content in meconium to be recognized. This could provide the basis of a screening test at birth for cases of cystic fibrosis.

We are indebted to Miss R. M. Mackay, Mr. F. H. Robarts, and Mr. W. H. Bisset for allowing free access to the cases, and to Miss Marion Dickson for her technical assistance.

REFERENCES

- Buchanan, D. J., and Rapoport, S. (1952). Chemical comparison of normal meconium and meconium from a patient with meconium ileus. *Pediatrics*, **9**, 304.
- Chodos, D. D. J., Ely, R. S., and Kelley, V. C. (1958). Paper electrophoresis of duodenal fluid from patients with cystic fibrosis of the pancreas. *Proc. Soc. exp. Biol. (N.Y.)*, **99**, 775.
- Farber, S. (1944). The relation of pancreatic achylia to meconium ileus. *J. Pediat.*, **24**, 387.
- O'Brien, D., and Ibbott, F. A. (1962). *Laboratory Manual of Pediatric Micro- and Ultramicro-Biochemical Techniques*, 3rd ed., p. 209. Harper & Row, New York.
- Pritchard, J. A. (1965). Deglutition by normal and anencephalic fetuses. *Obstet. and Gynec.*, **25**, 289.
- (1966). Fetal swallowing and amniotic fluid volume. *ibid.*, **28**, 606.
- Rennie, I. D. B., and Keen, H. (1967). Evaluation of clinical methods for detecting proteinuria. *Lancet*, **2**, 489.
- Schachter, H., and Dixon, G. H. (1965). A comparative study of the proteins in normal meconium and in meconium from meconium ileus patients. *Canad. J. Biochem.*, **43**, 381.
- Varley, H. (1962). *Practical Clinical Biochemistry*, 3rd ed. Heinemann, London.
- Wiser, W. C., and Beier, F. R. (1964). Albumin in the meconium of infants with cystic fibrosis: a preliminary report. *Pediatrics*, **33**, 115.
- Young, D. M., Schwert, G. W., and Harris, J. S. (1958). Viscosity and origin of meconium in meconium ileus. *Proc. Soc. exp. Biol. (N.Y.)*, **99**, 673.