References

Avery, G. B., Villavicencio, O., Lilly, J. R., and Randolph, J. G. (1968). Intractable diarrhoea in early infancy. *Pediatrics*, **41**, 712.

Fomon, S. J., Ziegler, E. E., and O'Donnell, A. M. (1974). Infant feeding in health and disease. *Infant Nutrition*, p. 487. Ed. by S. J. Fomon. Saunders, Philadelphia.

Gall, D. G., and Hamilton, J. R. (1974). Chronic diarrhoea in childhood. *Pediatric Clinics of North America*, **21**, 1001.

Criteria for diagnosis of temporary gluten intolerance

Sir,

I agree with McNeish and his colleagues (*Archives*, 1976, **51**, 275) that the criteria they have used would, at the time of challenge, indicate that the patient showed sensitivity to gluten. However, this child almost certainly had lactose intolerance in the early stages of its illness. This is evidenced by (1) the presence of reducing substances in his stools, and (2) the three episodes of diarrhoea with collapse. This problem might be expected in a child with the marked villous atrophy shown. During the time that he was said to be on a lactose, milk, and gluten-free diet he was receiving expressed breast milk and Galactomin 17, and even the latter contains enough lactose to produce symptoms in a highly sensitive child. This view is supported by the beneficial results after 3 weeks of intravenous feeding and a change to Velactin.

In view of these comments, one would anticipate that had this child been challenged earlier, adverse reactions to all of these substances would have been encountered. They go on to say that, '. . . it is safer to delay any gluten challenge until the child is more than one year old, even though it is likely that by that age many states of temporary dietary intolerance will have recovered and formal proof of their existence will be harder to find'. This emphasizes that we are seeing a dynamic situation and they have reverted back to the situation which they described in the text relating to Fig. 1.

I agree with McNeish that IgA deficiency might well have been important and I also agree that the possible mechanisms may be related to the secondary response to gastroenteritis.

The last point which I feel must not be ignored is that according to Fig. 4, this child at the age of 25 weeks was only minimally above his admission weight of 4 kg. Dobbing's work on brain growth would raise anxieties at allowing this situation to persist and while acknowledging that this was a patient who was treated as long ago as 1971 it seems a pity if it were felt that in such a marasmic infant effective therapy with intravenous nutrition or oral elemental diet would not have been a more appropriate way of dealing with this child in the initial stages.

V. MILLER,
Booth Hall Children's Hospital,
Charlestown Road,
Blackley,
Manchester M9 2AA.

Professor McNeish and Dr. Arthur comment:

It is indeed likely that the early illness of this child in Derby was associated with multiple dietary intolerances. The initial lactose intolerance may have been caused by cows' milk protein intolerance (Liu *et al.*, 1968) as we suggested, but we have no formal proof of this. It is experience of the problems of such infants that has been a stimulus to improve the techniques of intravenous feeding. This has allowed us, like our Manchester colleagues, to advocate early intravenous feeding in cases of 'multiple gut dysfunction' in young infants (McNeish, 1976).

A. S. McNEISH
Department of Child Health,
Leicester Royal Infirmary,
Leicester.

L. J. H. ARTHUR
Derbyshire Children's Hospital,
Derby.

References

Liu, H. Y., Tsao, M. U., Moore, B., and Giday, Z. (1968). Bovine milk protein-induced intestinal malabsorption of lactose and fat in infants. *Gastroenterology*, **54**, 27.

McNeish, A. S. (1976). *Acute Diarrhoea in Childhood.* Ciba Symposium. New series no. 42. Ed. by K. Elliott and J. Knight. Elsevier, Excerpta Medica, North Holland, Amsterdam.

Does conjugated bilirubin displace unconjugated bilirubin from albumin?

Sir,

Irrespective of its behaviour in the Van Den Bergh reaction, bilirubin in serum is bound to albumin (Klatskin and Bungards, 1956). Conjugated bilirubin is probably not toxic, though conclusive evidence on this point is still lacking (Zuelzer and Brown, 1961), and its enhancing influence for the genesis of kernicterus in infants with a high level of unconjugated bilirubin cannot be excluded (Poláček, 1966). Spectrometric studies on sera containing conjugated and unconjugated bilirubin showed that the two types of pigment cannot be bound to the same site on the albumin molecule (Fog, 1967). The albumin-binding forces seem to be much less for conjugated than for unconjugated bilirubin and it seems that conjugated bilirubin could compete with unconjugated bilirubin and displace it from albumin (Schmid, 1967).

To check this hypothesis the following *in vitro* experiment was carried out. To a serum containing unconjugated bilirubin various amounts of a serum containing a large fraction of conjugated bilirubin was added, to yield five serum samples with the bilirubin/albumin (Br/Alb) molar ratio in the following ranges: 0·30–0·50, 0·51–0·60, 0·70–0·80, and 0·81–1·22. A serum containing only unconjugated bilirubin with the Br/Alb molar ratio within the above ranges was prepared and fractioned in 5 aliquots for each Br/Alb molar ratio, for statistical analysis.

TABLE

Br/Alb molar ratio	Bilirubin (mg/100 ml)		OD 450 nm free unconjugated Br in $CHCl_3$ trapped by Sephadex G-25	Br/Alb molar ratio	Only unconjugated bilirubin (mg/100 ml)	OD 450 nm free unconjugated Br in $CHCl_3$ trapped by Sephadex G-25 (mean ± SD)
	Unconjugated	Conjugated				
0·48	6·90	8·64	0·0070	0·30 0·41	8·5 11·5	— 0·002 ± 0·001
0·56	8·53	10·19	0·0095	0·60	17·0	0·005 ± 0·001
0·75	11·79	15·29	0·0140	0·80	22·5	0·015 ± 0·003
0·89 1·22	17·21 29·51	15·29 15·29	0·0350 0·0700	1·20	34·0	0·040 ± 0·012

All the sera were run through small columns (h.2 cm; Ø = 1·3 cm) packed with Sephadex G-25 F (Pharmacia, Uppsala, Sweden). After completeness of protein elution with phosphate buffer *p*H 7·4 the bilirubin trapped by the gel was eluted with NaOH 0·1 N. The 'free' nonalbumin-bound unconjugated bilirubin present in this fraction was extracted with chloroform (Kapitulnik, Blondheim, and Kaufman, 1972) and the absorbance at 450 nm was recorded using a Beckman DU Spectro-photometer (Table). For each Br/Alb molar ratio range, as indicated in the Table, the absorbance at 450 nm of the free unconjugated bilirubin extracted with chloroform is higher in sera also containing conjugated bilirubin, in spite of the fact that the concentration of unconjugated bilirubin is lower than that of the serum samples containing only unconjugated bilirubin.

It seems reasonable to assume that, within the limits of the present experiment, conjugated bilirubin displaces unconjugated bilirubin from albumin. If this is true we need to know what happens *in vivo* at a cellular level in the brain, when both pigments are present, in relatively high concentration in the blood stream, during the neonatal period.

ANTONIO PRIOLISI
Istituto di Puericultura
Università, Via Lancia di Brolo, 10,
90145 Palermo, Italy.

REFERENCES

Fog, J. (1967). *Bilirubin Metabolism*, p. 156. Ed. by I. A. Bouchier and B. Billing. Blackwell, Oxford.

Kapitulnik, J., Blondheim, S. H., and Kaufman, N. A. (1972). Sephadex adsorption of bilirubin from neonatal and adult serum. *Clinical Chemistry*, **18**, 43.

Klatskin, G., and Bungards, L. (1956). Bilirubin-protein linkages in serum and their relationship to the Van Den Bergh reaction. *Journal of Clinical Investigation*, **35**, 537.

Poláček, K. (1966). Risk of kernicterus in newborn infants with a high level of conjugated bilirubin. *Acta Paediatrica Scandinavica*, **55**, 401.

Schmid, R. (1967). *Bilirubin Metabolism*, p. 156. Ed. by I. A. Bouchier and B. Billing. Blackwell, Oxford.

Zuelzer, W. W., and Brown, A. K. (1961). Neonatal jaundice. *American Journal of Diseases of Children*, **101**, 87.