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Preliminary Communication

AN ENVELOPED VIRUS IN STOOLS OF CHILDREN AND ADULTS WITH GASTROENTERITIS THAT RESEMBLES THE BRED A VIRUS OF CALVES

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Summary Pleomorphic virus-like particles about 100 nm in diameter with a fringe of closely applied peplomers (7–9 nm in length) were observed by electron microscopy in the stools of 20 children and adults with gastroenteritis. In most of the samples no other viral or bacterial pathogens were detected. In form and under immune electron microscopy these virus-like particles resembled the Breda virus isolated from diarrhoeic calves. These objects may be a viral pathogen of humans.

INTRODUCTION

ALTHOUGH several viral and bacterial causes of acute infectious diarrhoea are known, in many cases no candidate pathogen can be found.¹ In many countries at some times of the year rotaviruses cause up to 85–95% of cases.² But in other studies—eg, in Gabon³—rotaviruses were only found in approximately 17% of cases. A similar low proportion of cases caused by rotavirus was reported from South Africa.⁴ Although adenoviruses were prevalent in Birmingham during the winter of 1981/2 and apparently caused almost as many cases as did rotaviruses, in most studies they seemed to be responsible for less than 10% of cases. Other viruses which have been found in diarrhoeic faeces of children make an even smaller contribution.¹ Bacterial pathogens—eg, *Salmonellae*, *Shigellae*, enterotoxigenic *Escherichia coli*, and campylobacters rarely cause more than 20%⁵ of cases and in developed countries their incidence is usually much lower. It seems likely that at least one other viral pathogen remains to be discovered.

A new virus of calves was reported in 1982 by Woode et al.⁶ This was named the Breda virus after the town in the USA in

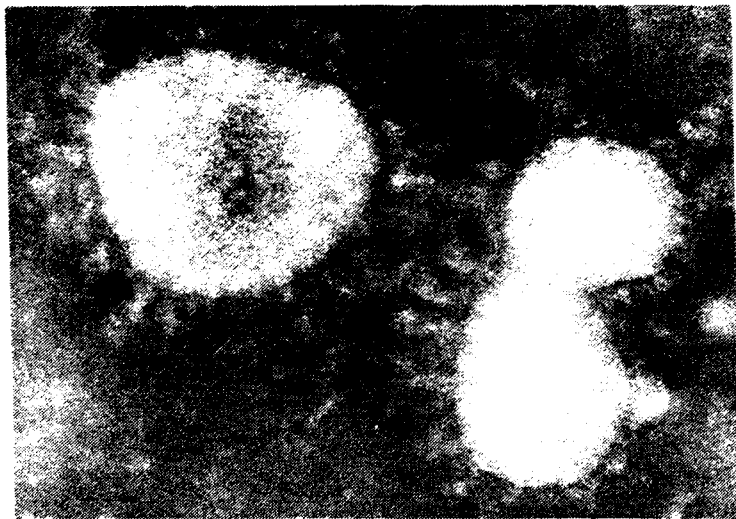


Fig 1—Breda virus particles from gnotobiotic calf faeces.

Viruses are pleomorphic and have peplomers 7 nm to 9 nm long. Larger (20 nm) projections can also be seen. Reduced by 1/4 from $\times 250\,000$ (PTA).

which the calf was found. The virus was shown to be antigenically different from coronaviruses, which it resembles a little in morphology. Faeces from some infected calves strongly agglutinated rat erythrocytes and haemagglutination-inhibiting antibodies developed in convalescent animals. When fed to gnotobiotic calves the virus caused diarrhoea which abated when convalescent serum was given. Viruses of distinctive morphology (fig 1) were detected in the faeces of some infected animals.

Subsequently, another serologically related virus of similar morphology was discovered in calves in Ohio. This showed enough serological differences, however, to be described as a second serotype. Another similar virus was isolated in tissue culture from a horse in Berne in 1972, and is known as the Berne virus.⁷ It was antigenically related to the Breda virus, but apparently did not produce haemagglutinin. This virus is morphologically very similar to the Breda virus, but occasionally large (20 nm) peplomers as well as the shorter ones described by Woode et al, were observed. These viruses are considered as representatives of a hitherto undefined family of viruses.^{6,7}

We have seen morphologically similar particles, occasionally quite numerous, in faeces of 20 patients both in Birmingham and Bordeaux, who had diarrhoea. 2 of the Birmingham patients were among the recent cluster of patients with haemolytic uraemic syndrome in the West Midlands. All the 10 Bordeaux patients had gastroenteritis, and apart from 2 adults all were less than five years old and about half were the children of immigrant Arabs. All stools, both from Bordeaux and Birmingham were negative for other viruses by electron microscopy and enzyme-linked immunosorbent assay.

METHODS

Samples of faeces from children and adults with diarrhoea were diluted to give a final concentration of 10–20% in saline and were centrifuged at 1000 *g* for 10–20 min to deposit bacteria. Preparations for electron microscopy were made from them and phosphotungstate was used as a negative stain.⁸ The stained preparations were examined directly in the electron microscope. Most of the particles were 50 \times 100 nm (if kidney shaped or oval) and 80–90 nm in diameter (if circular), although occasionally particles which appeared to have the same structure could be seen which were no more than 50 nm in diameter; and we found particles which were even smaller than those in samples of faeces from gnotobiotic calves infected with the Breda virus. The particles were surrounded by a ring of peplomers 7–9 nm long. Occasionally what appeared to be a second ring of small peplomers could be seen, partly superimposed upon the first. When the flat surface of a particle faced the electron beam and was well defined by the negative stain, dots similar in size and distribution to the peplomers could be seen on the surface (fig 2). The longer peplomers, believed by Woode et al to be of doubtful importance, and more often seen on the horse virus particles described by Weiss et al⁷ were not seen in the samples we used for immune electron microscopy, although we have occasionally seen such projections on particles in samples of faeces examined by electron microscopy over the last few years. Some supernates, after the initial centrifugation, were centrifuged at high speed (200 000 *g* for 1 h) but perhaps because they were pelleted too violently, it was difficult to distinguish particles in a resuspended pellet. We did not detect an agglutinin for rat erythrocytes by the method described by Woode et al,⁶ when we tested a suspension from the child with the haemolytic uraemic syndrome, which contained these particles in greatest abundance.

Professor Woode kindly sent us samples of faeces and convalescent sera from gnotobiotic calves infected with one or other of the two Breda serotypes. These sera were tested, together with a control serum, to see whether they would agglutinate particles from children. Similarly, sera taken in the acute phase and in convalescence from 4 Bordeaux patients in whose faeces Breda-like

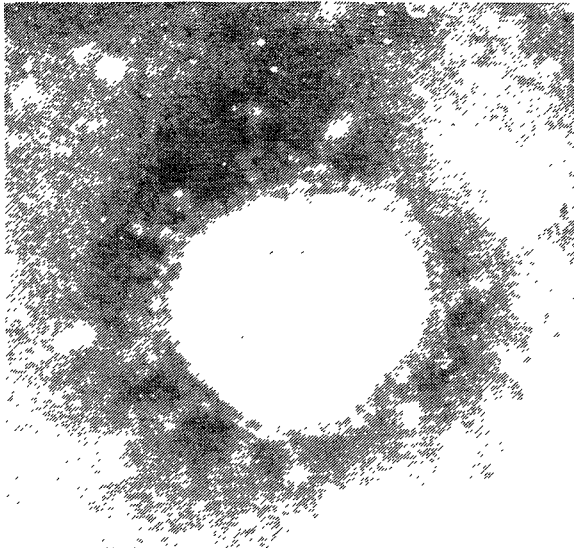


Fig 2—Breda virus-like particle from human faeces.

Some 20 nm projections can be seen on this particle as well as the shorter ones. $\times 250\,000$.

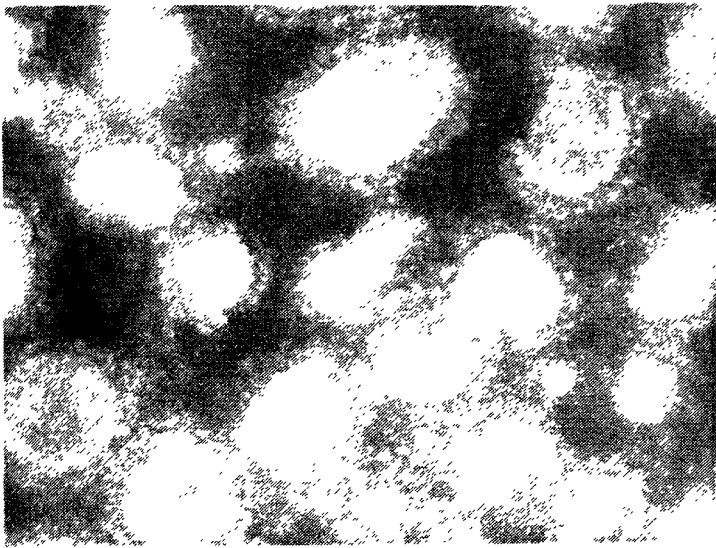


Fig 3—Immune electron microscopy of human Breda virus-like particles and antiserum to Breda virus II.

Reduced by $\frac{1}{2}$ from 200 000.

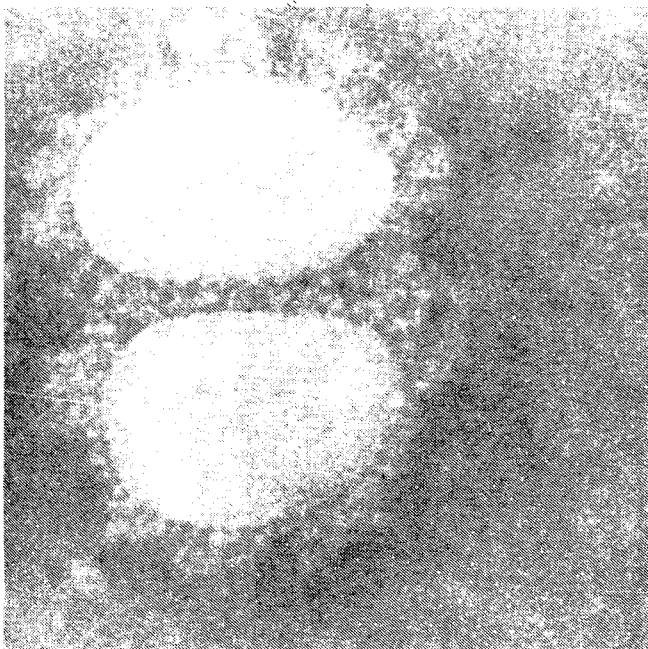


Fig 4—Breda virus particles aggregated by convalescent human serum.

Antibody is clearly visible. $\times 250\,000$.

IMMUNE ELECTRON MICROSCOPY RESULTS

Antiserum	Virus			
	Breda I	Breda II	B'ham 5486	B'ham 5814
Breda I	+++	++	±	±
Breda 2	+++	+++	+++	+++
Case A acute	-	±	NT	NT
Case A convalescent	++	±	NT	NT
Case B acute	-	+	NT	NT
Case B convalescent	±	+++	NT	NT
Case C	+++	+	NT	NT
Case D	++	++	NT	NT
Gnotobiotic calf (rotavirus infected)	-	-	-	-
None	-	-	-	-

NT = Not tested, insufficient material.

particles were found, were tested to determine any serological relation with Breda virus particles and Birmingham particles. Serum was added to a clarified faecal suspension to give a final dilution of 1:10. Samples were incubated overnight at 4°C and centrifuged for 30 min at 10 000 *g*. The deposits were examined for immune complexes and attached antibody by electron microscopy. All samples were prepared by one of us (G. M. B.), given code numbers, and read independently by two of three others (T. H. F., J. G., and C. H. who recorded their findings before the code was broken). Homologous and pre-immune serum controls were included in all experiments.

RESULTS

Birmingham particles were agglutinated by sera from gnotobiotic calves convalescent from infection by the two serotypes of Breda virus. Antiserum to the Breda type II gave a much clearer result than antiserum to type I. The reaction with anti-Breda type II is shown in fig 3. A serum sample from gnotobiotic calves artificially infected with a rotavirus was used as a control and no immune complexes were observed. Similarly, convalescent sera from 2 Bordeaux patients gave an immune complex with Breda serotypes and the Birmingham samples that were tested (fig 4). Results of the immune electron microscopy experiments are summarised in the table.

DISCUSSION

Every electron microscopist who has looked at many samples of faeces in the electron microscope is well acquainted with pleomorphic objects bearing fringes around their periphery, which are often found in both normal and infected faeces, and sometimes also in other body fluids, and are of no importance. They vary considerably in size and shape and are often much larger than the particles we have described. We believe that over the years we have often seen particles morphologically resembling Breda virus, but have simply regarded them as "fringed particles". The particles we describe which we believe are related to the Breda virus, were detected because they were numerous even in the unconcentrated faecal supernate and because they were all in the same size range. They were agglutinated by convalescent sera to the Breda virus types I and II and convalescent human sera. It is not surprising that these viruses have not previously been considered as human pathogens; firstly, because they resemble the non-specific fringed particles, and also because electron microscopy is an inefficient method which does not detect most infections with the Breda calf virus (Woode, personal communication).

The discovery of these particles in faeces of children with haemolytic uraemic syndrome may have been coincidental. They have also been found in faeces of children who had had

only diarrhoea. We have no evidence, other than association with diarrhoea in a few patients, that these particles caused the disease. We report these findings to alert others to the existence of these objects in the hope that evidence to determine their importance may be collected. The serological relations described above are based upon immune electron microscopy. Although we have made the observations as objective as possible, this method like any other single method can never be guaranteed free from error. Confirmation by another serological test is required.

The role of these objects in human infectious disease remains to be determined, but it appears that they probably are previously unrecognised viral agents and, with the Breda and Berne viruses, may belong to a new virus genus.

We thank Professor Gerald Woode for samples of faeces and convalescent sera from gnotobiotic calves infected with the two serotypes of the Breda virus, and for information about their preparation for electron microscopy. This work was in part supported by the Medical Research Council.

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Reviews of Books

Emergency Medicine

Edited by Harold L. May, Harvard Medical School, Boston, Massachusetts. New York and Chichester: John Wiley. 1983. Pp 1062. \$91.95; £65.50 (Part V: Pp 223. \$22.95; £16.35).

THIS book is based on a course offered at Harvard Medical School for fourth-year medical students. Its aim is to suggest the immediate care of the patient admitted to the accident and emergency department. Most of its 79 contributors are specialists in emergency medicine in Boston, Massachusetts. The first part of the book describes the basic principles of life support, discusses major pathophysiological mechanisms, and gives details on management in the emergency admitting department. Part II considers trauma, patient evaluation, and initial management, part III discusses acute disorders relating to various systems, part IV relates to medicolegal matters, and part V describes various emergency procedures. The focus is on evaluation and initial management of the patient in the accident and emergency department, and there is no detailed advice about subsequent definitive care.

The format and style are similar for each chapter; the text is clear, easily readable, and punctuated by excellent diagrams. The section on acute injuries seems to be disproportionately large (260 pages) but includes some excellent contributions on topics rarely found in a text on emergency care; these include the diagnosis and management of facial and eye injuries, and acute disorders of the hand. Certain sections are less detailed, such as those on acute medical disorders and general surgical crises, but sufficient advice is given for any admitting clinician to make a diagnosis and start treatment.

Part V, which can be bought separately, is outstanding, and would be an invaluable manual for all emergency areas, especially where senior assistance for procedures such as relief of upper airway obstruction by needle insertion into the cricothyroid membrane is

some time away. Most of the procedures described may be performed in the resuscitation area of the A & E department. Those generally done in the intensive therapy unit or operating theatre are not included.

The book has to cover such an enormous spectrum of disease that, inevitably, certain aspects are omitted or covered superficially. A section on diagnostic imaging, indicating the most suitable technique for different emergencies, would have been helpful. Many of the first-line drugs used in acute patient care in Great Britain (such as some of the antidysrhythmics and hypotensive agents) are not yet available in the United States of America, but this deficiency is largely made up for by the clear descriptions on diagnosis and other aspects of initial management, which make this book an excellent reference manual for all clinicians who have to initiate patient management in the A & E department.

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Digital Radiography

William R. Brody, Stanford University, California. New York: Raven Press. 1983. Pp 225. \$41.

THE author of this compact and well-presented volume set out to explain the fundamental principles of digital radiographic systems and has succeeded admirably. The subject inevitably demands some knowledge of the mathematics and physics involved and it is to Dr Brody's credit that he manages to keep this requirement to a minimum without detracting from the comprehensive nature of the work.

The first section, which steers the reader through the basic concepts of digital radiography under the general heading of Understanding Digital Imaging Systems, contains excellent chapters on subtraction methods and image processing. Within each chapter small, clearly headed subsections allow the novice to look up topics and come to terms with the new terminology associated with this rapidly evolving imaging modality. The second section of the book discusses digital imaging systems and the topics include digital fluoroscopy, digital subtraction angiography (DSA), and scanned projection radiography. Much of this technology is not yet available in the United Kingdom but the clear and basic knowledge of the subject imparted by this book will allow workers in radiology to make a more critical analysis of the literature and the equipment as it becomes available. The chapter on DSA will prove invaluable to those departments who have just installed or are about to install their own DSA systems. Although the author is not too didactic in his approach he nevertheless tells the reader specifically how examinations are performed in his own department and this provides very useful information on how to obtain optimum image quality.

The quality of the illustrations is uniformly high and the author's liberal use of diagrams helps to make the more complex aspects of the subject more interesting and less confusing. This book is a "must" for any department installing digital equipment, and it should be read for any radiologist intending to stay abreast of modern radiology.

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New Editions

Medicine: Essentials of Clinical Practices.—3rd ed. Edited by R. W. Wilkins and N. G. Levinsky. Boston: Little, Brown. London: Quest. 1983. Pp 1013. \$32.50.

Cardiovascular Pharmacology.—2nd ed. Edited by M. J. Antonaccio. New York: Raven Press. 1984. Pp 592. \$49.

Allergy: Principles & Practice.—Vols 1 & 2. Edited by E. Middleton, C. E. Read, and E. F. Ellis. Oxford: Blackwell. 1983. Pp 1440. £96.

A Practice of Obstetrics & Gynaecology.—2nd ed. By G. Chamberlain and J. Dewhurst. London: Pitman Medical. 1984. Pp 197. £9.95.

Color Atlas of Laparoscopy.—2nd ed. By K. Beck. Philadelphia and Eastbourne: W. B. Saunders. 1984. Pp 508. £129.

The Eye & its Disorders.—2nd ed. By P. D. Trevor-Roper and P. V. Curran. Oxford: Blackwell. 1984. Pp 628. £45.