*J. Hyg. Camb.* (1981), 87, 313
*Printed in Great Britain*



# Virus diarrhoea associated with pale fatty faeces

BY MAIR E. M. THOMAS, P. LUTON* AND JANET Y. MORTIMER

* *Department of Clinical Microbiology, University College Hospital, London, WC1 6AU and Epidemiological Research Laboratory, Central Public Health Laboratory, Colindale, London, NW9 5HT*

(*Received* 21 *January* 1981)

## SUMMARY

Steatorrhoea was a significant feature in an outbreak of rotavirus gastroenteritis which affected adults and infants in hospital. Fat globules or fatty acid crystals were obvious by light microscopy (LM) in faeces from 14 of 25 patients examined. Ten of the fatty stools and two of the remainder were very pale. By electron microscopy (EM) a rotavirus was seen in 11 of the 14 fatty faeces and in only two of 11 specimens without visible fat.

In a further study of pale or fatty faeces 20 such specimens sent for laboratory examination from patients *not* involved in the hospital outbreak were compared microbiologically with a similar number which were neither pale nor fatty. Viruses were found by EM in 11 (55%) of the pale or fatty stools; eight rotaviruses, two astroviruses and an uncultivable adenovirus were seen; one further patient had acute jaundice. In contrast, no viruses were seen by EM in the twenty specimens which were normally pigmented and without evident fat.

Steatorrhoea was significantly associated with rotavirus infection of the alimentary tract which usually presented as a fatty enteritis. We conclude that rotaviruses certainly, and other viruses possibly, can impede both the digestion of fat and the pigmentation of the faeces. Inspection and LM of faeces are easy. In acute enteritis a fatty or pale stool is an indication for virological examination.

## INTRODUCTION

In 1950 an outbreak of non-bacterial gastroenteritis which affected children and adults was characterized by pale stools in many of which undigested globules of neutral fat could be seen microscopically (Thomas, 1952). Similar observations were reported subsequently. In 1952–4 when faeces from 1135 children under 4 years old were examined in a study of juvenile diarrhoea fat globules were the sole abnormality detected in 13% and such 'fatty diarrhoea' cases outnumbered those of shigellosis, colibacillosis and salmonellosis (Thomas & Carter, 1956). During the years 1952–68 fat was seen on microscopy in the faeces of 6·7% of 20273 index cases of diarrhoea of all ages, while *Shigella sonnei* and salmonellas were isolated from 9·2% and 2·3% respectively. Steatorrhoea was more frequent in pre-school children (17·3%) than in older children or adults (3·2% and 2·3%) in that unselected series (Thomas & Tillett, 1975). The diarrhoea commonly associated with fatty stools appeared to be communicable, but searches for a viral agent using culture techniques were unsuccessful and neither the aetiology nor the mechanism of the visible steatorrhoea was elucidated. During the past decade however EM has become more widely available and a number of viruses have been recognized in diarrhoeal faeces by this means. Of these the rotaviruses have been the most common.

In 1980 a hospital outbreak of non-bacterial enteritis presented an opportunity to examine faeces from patients by EM and LM in parallel and to correlate the results with bacteriological findings. The study was extended by including material from the general diagnostic intake.

## PATIENTS AND METHODS

Early in 1980 an outbreak of non-bacterial gastroenteritis with an incubation period of about 2 days affected more than 30 people, mainly adults, in an obstetric unit (Hildreth, Thomas & Ridgway, 1981). Rotaviruses were found by EM in the stools of 26 persons including 7 infants. Faeces from 12 adults and 13 infants were examined in parallel by LM and by EM as well as bacteriologically within a week of the onset of symptoms. The colour and consistency of the faeces was recorded, following which about 1 $mm^3$ of the sample was emulsified in a drop of Gram's iodine. This preparation was then scanned by light microscopy for 1 min at times 400 magnification to determine whether red cells, pus cells or parasites were present. Fat globules and fatty acid crystals were recorded if visible in all fields. EM was performed on a Hitachi H500 machine. Five ml of a 10% watery suspension of 0·5 g faeces was centrifuged at 3000 rev./min for 15 min and the supernatant filtered through a 0·8 micron millipore filter, then centrifuged in a Sorvall OTD 50 ultracentrifuge at 30000 rev./min for an hour at 4 °C. Pellets were resuspended in 0·25 ml distilled water and stained with an equal volume of 3% phosphotungstic acid at pH 6·8. A drop on a 400 mesh carbon-coated grid was examined at a magnification of times 30000 and specimens were not declared negative until they had been examined for 15 min. The method cannot be relied upon to detect virus concentrations under a million per gram of faeces (Madeley, 1979). Specimens were subsequently screened for rotavirus by ELISA (Yolken *et al.* 1977) using the 'Rotazyme' kit supplied by Abbott Laboratories. Salmonella, shigella and campylobacter species, and in infants enteropathogenic *Escherichia coli*, were sought by standard methods. LM, EM, ELISA and bacteriology were undertaken without cross reference.

After the outbreak more fatty or pale faeces were investigated by the same methods. To obtain these, 150 diarrhoeal specimens from the miscellaneous intake of the diagnostic laboratory were inspected directly and by LM. Twenty fatty or pale stools were identified; eight of these were fatty and pale, four were fatty and pigmented, while eight were pale but not fatty (Fig. 1). The next in sequence normally pigmented specimen without evident fat was examined for comparison. No attempt was made to match the age or condition of the series from the outbreak. Seventeen of the fatty or pale faeces, but only two of the 20 pigmented samples came from children.

https://doi.org/10.1017/S0022172400069539 Published online by Cambridge University Press

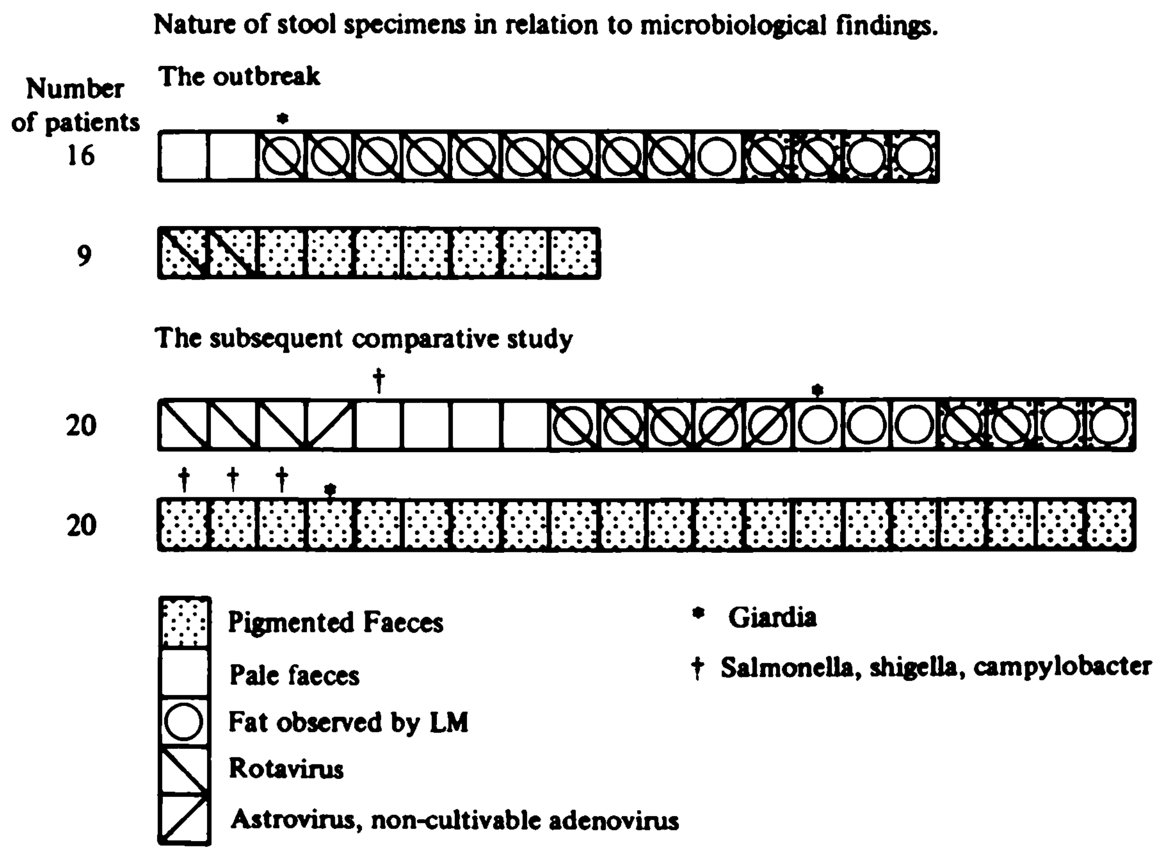


Fig. 1. Nature of stool specimens in relation to microbiological findings.

## RESULTS

In the outbreak study faeces from 25 patients were examined. Nineteen were diarrhoeal and 11 of these were of pale colour as was one formed stool. *Giardia lamblia* was found in one case and pus cells in another but no bacterial pathogens were isolated (Fig. 1). However, globules of neutral fat mostly from 10 to 30 $\mu$m in diameter (Plate 1) were recorded in 12 specimens (two of formed consistency) and fatty acid crystals without globules in a further two. Rotaviruses were visible by EM (Plate 2) and later confirmed by ELISA in 11 of the 14 fatty faeces (79%) and in two of the remaining 11 (18%). Seven of these 13 rotavirus positive patients were adults. The association between rotavirus and undigested fat was statistically significant ($P < 0.01$). The virus was seen in 9 of the 12 pale stools ($P < 0.07$). In specimens from six patients re-examined a few days later pigment had returned but rotaviruses and fat had disappeared.

In the study of 40 diarrhoeal faeces selected from the general laboratory diagnostic intake of faecal specimens viruses were found by EM in 11 of the 20 specimens which were fatty or pale (Fig. 1). Eight contained rotavirus, two astrovirus and one an uncultivable adenovirus. All the virus positive patients were children. One pale fatty specimen came from an adult with non-B infective hepatitis. Pus cells were noted in only one fatty stool and in two pale fat negative specimens, from one of which a salmonella was isolated. In contrast *no* viruses were detected in the 20 pigmented fat-negative stools, but salmonella, shigella, campylobacter and *Giardia lamblia* infections were found, each in one case and pus cells were present in four. Rotaviruses were seen more often in fatty than in fat-negative stools ($P < 0.07$) and in pale than in pigmented specimens ($P < 0.07$;

https://doi.org/10.1017/S0022172400069539 Published online by Cambridge University Press

Table 1. *Characteristics of the faeces from 24 patients (both series) with evidence of a viral infection of the digestive system*

| Virus seen by EM | No. of patients | No. of children | Faeces | | | | |
|---|---|---|---|---|---|---|---|
| | | | Pale | Fatty | Neither | Pus cells | Other pathogen |
| Rotavirus | 21 | 14 | 15 | 16 | 2 | 1 | 1 Giardia |
| Astrovirus | 2 | 2 | 2 | 1 | 0 | 0 | 0 |
| Uncultivable Adenovirus | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| Total | 24 | 17 | 18 | 18 | 2 | 1 | 1 |

Fig. 1). The findings in EM positive faeces from both studies are summarized in Table 1.

## DISCUSSION

During the course of a hospital outbreak of gastroenteritis we observed that rotavirus infection usually presented with a fleeting steatorrhoea detectable by LM for the first few days of illness. This is the stage at which rotaviruses are most easily seen by EM and the concentration of antigen in the faeces is highest (Nagayoshi *et al.* 1980). Among adults and infants we found a statistically significant association between the observation of rotavirus by EM and the passage of fatty faeces ($P < 0{\cdot}01$) and an association with pale stools ($P < 0{\cdot}07$). A subsequent study of diarrhoeal faeces selected from the diagnostic intake of the microbiology laboratory included 20 fatty or pale faeces specimens and 20 pigmented non-fatty specimens. Again an association of rotavirus infection with fatty or pale stools was found ($P < 0{\cdot}07$). EM revealed eight rotavirus and two astrovirus infections and an uncultivable adenovirus among 17 children with fatty or pale faeces, and one of three adults with such stools had non-B hepatitis. No viruses were found in pigmented non-fatty specimens (Fig. 1).

A link between steatorrhoea and virus unfection would explain the infectious fatty diarrhoea recognized in 1950 (Thomas, 1952) and found to be the commonest single diarrhoeal syndrome (13%) among pre-school children (Thomas & Charter, 1956). In a 16-year study (1952–68) of 20273 index cases of diarrhoea in all ages fatty diarrhoea was found in 6·7% of the entire series. The incidence was 17·3% in pre-school children, 3·2% in older children and 2·3% in adults. It occurred as an independent abnormality and was commonly associated with a communicable enteritis of 2–3 days incubation period. Some patients were febrile and a few had a biphasic illness (Thomas & Tillett, 1975, 1981). It was estimated that 15% of the total index cases was probably due to whatever agents had been causing fatty diarrhoea (Tillett, 1981). Analysis of time patterns showed winter peaks as have reports to the Communicable Disease Surveillance Centre of rotavirus infections (PHLS unpublished 1980). At that time EM had not been available and sample virus cultures were negative.

An apparently greater susceptibility of children to fatty diarrhoea and to proven rotavirus infection may be due at least in part to differences in rates of laboratory

https://doi.org/10.1017/S0022172400069539 Published online by Cambridge University Press

investigation as faeces are more likely to be sent for examination from children than from adults. In institutional outbreaks however a more complete investigation is feasible and in two hospital outbreaks of rotavirus infection in London those involved were adults (Cubitt & Holzel, 1980; Holzel *et al.* 1980). In the episode we studied adults were more ill than children (Hildreth *et al.* 1981).

We do not know the mechanism by which a transient steatorrhoea is produced in man by rotaviruses. Fat globules in the faeces are not simply a consequence of intestinal hurry for we have found them in loose and formed stools from patients of all ages involved in outbreaks of non-bacterial enteritis, but rarely in acute bacterial diarrhoeas or giardiasis. In liver disease fatty faeces and defective pigmentation are attributed to lack of bile (British Medical Journal, 1980). The possibility of hepatic or pancreatic disorder as well as of temporary biliary stasis or obstruction merits consideration. The timing of investigation might be critical because of the brief but variable duration of the condition. Fat was seen microscopically in 13% of 1135 cases of juvenile diarrhoea in 1952–54 (Thomas & Charter, 1956) and in samples from a few representative cases examined then the percentage of fat in dried stools ranged from 5 to 60% and more than half was in the split form. A temporary failure of bile emulsification, possibly with a defect in absorption was thought likely. Deficiency of pigment, but not steatorrhoea, has been described in the stools of children with rotavirus infection in Japan where the condition is known as 'Hakuri' (Konno *et al.* 1977; Morishima *et al.* 1980).

Bishop and her colleagues first reported the observation of rotavirus particles (referred to as reovirus, reovirus-like, duovirus and orbivirus by previous workers) in the duodenal mucosa of children with non-bacterial enteritis (Bishop *et al.* 1973). The virus is now recognized as a major cause of diarrhoea in man and animals. During the acute stage rotaviruses are profuse in the duodenum and jejunum but not in the stomach, rectum or mesenteric glands. The viruses multiply in the differentiated villous cells of the small gut causing blunting of the villi. The consequent reduction in digestive surface area has correlated with deficient disaccharide secretion and xylose absorption has been found impaired for about a week (Davison & Barnes, 1979; Hamilton, 1980; Steinhoff, 1980). Replacement of damaged cells can however be slow (Mayr, 1980).

Carbohydrate absorption has been studied, but little has been reported about fat absorption in viral enteritis of man or animals since Sabin described a virus isolated from a domestic outbreak of steatorrhoeic enteritis as a reovirus (Sabin, 1956, 1959) and the later observation by Stanley (1961) that gross steatorrhoea was evident in mice fed a 'reovirus' of human origin. On one occasion such mice developed an islet-sparing pancreatitis. In 1972 Blacklow and colleagues reported transiently raised levels of faecal fat, estimated chemically, in five adults infected experimentally with the Norwalk agent of gastroenteritis (Blacklow *et al.* 1972; Blacklow & Cukor, 1981). Perhaps rotavirus in man like reovirus in animals can interfere with fat digestion in several ways (Stanley, 1961). Interference with the secretion of pancreatic lipases and amylases has been demonstrated in experimental animals (Branski *et al.* 1980). We do not know whether steatorrhoea might be found in viral enteritides in veterinary practice, and it is hoped to investigate this (D. Reynolds, Institute for Research in Animal Disease, Compton, personal communication).

Mathan and colleagues (1975) in South India found pleomorphic virus-like particles by EM in the faeces of 90% of a group of well and ill adults and children but in no neonates. They considered that if these were viruses, their ubiquitous presence might be related to the local high incidence of symptomless intestinal abnormality and to sprue. Malabsorption may complicate chronic giardiasis and it is interesting that Wright & Tomkins (1978) in a quantitative histological study of human infection by *Giardia lamblia* found surface area measurements to be lower in those patients whose absorption of fat and xylose was impaired than in controls.

In conclusion, a transient malabsorption of fat appears to be one common consequence of rotavirus infection in man and a poorly pigmented stool is another. The two often coincide and a pale fatty stool in acute gastroenteritis is suggestive of a viral infection. Pale or fatty faeces are easily selected by inspection and LM and it is among such specimens that a virus is most likely to be detectable by virological techniques.

The World Health Organisation list among priorities the identification of aetiological agents of acute diarrhoea and of means of reducing their transmission (World Health Organization, 1980*a*) and recommends that studies be made of the role, if any, of rotavirus disease in malabsorption and nutritional deficiency (World Health Organization, 1980*b*). Recognition that the acute fatty diarrhoea syndrome is likely to be of viral origin should contribute to this work.

We are very grateful to John Gibson of the Department of Medical Illustration, CPHL, Colindale, for preparing the figures, and to Barrie Mellars of the Clinical Microbiology Department, UCH, London, for performing the ELISA tests.

## REFERENCES

BISHOP, R. F., DAVIDSON, G. P., HOLMES, I. H. & RUCK, B. J. (1973). Virus particles in epithelial cells of duodenal mucosa from children with acute non-bacterial gastroenteritis. *Lancet* ii, 1281.

BLACKLOW, N. R., DOLIN, R., FEDSON, D. S., DUPONT, H., NORTHRUP, R. S. HORNOCK, R. B. & CHANOCK, R. M. (1972). Acute infectious nonbacterial gastroenteritis: etiology and pathogenesis. *Annals of Internal Medicine* **76**, 993.

BLACKLOW, N. R. & CUKOR, G. (1981). Viral gastroenteritis. *New England Journal of Medicine* **304**, 397.

BRANSKI, D., LEBENTHAL, E., FADEN, H., HATCH, T. E. & KRASNER, J. (1980). Reovirus type 3 infection in a suckling mouse: the effects on pancreatic structure and enzyme content. *Pediatric Research* **14**, 8.

BRITISH MEDICAL JOURNAL (1980). **281**, 504 (Correspondent's questions).

CUBITT, W. D. & HOLZEL, H. (1980). An outbreak of rotavirus infection in a long-stay ward of a geriatric hospital. *Journal of Clinical Pathology* **33**, 306.

DAVISON, G. P. & BARNES, G. L. (1979). Structural and functional abnormalities of the small intestine in infants and young children with rotavirus enteritis. *Acta Paediatrica Scandinavica* **68**, 181.

HAMILTON, J. R. (1980). Infectious diarrhoea: implications of recent research. *Canadian Medical Association Journal* **122**, 29.

HILDRETH, C., THOMAS, M. & RIDGWAY, G. (1981). Rotavirus infection in an obstetric unit. *British Medical Journal* **282**, 231.

HOLZELL, H., CUBITT, W. D., MCSWIGGAN, D. A., SANDERSON, P. J. & CHURCH, J. (1980). An outbreak of rotavirus infection among adults in a cardiology ward. *Journal of Infection* **2**, 33.

KONNO, T., SUZUKI, H., IMAI, A. & ISHIDA, N. (1977). Reovirus-like agent in acute epidemic gastroenteritis in Japanese infants: Faecal shedding and serologic response. *Journal of Infectious Diseases* **135**, 259.

https://doi.org/10.1017/S0022172400069539 Published online by Cambridge University Press

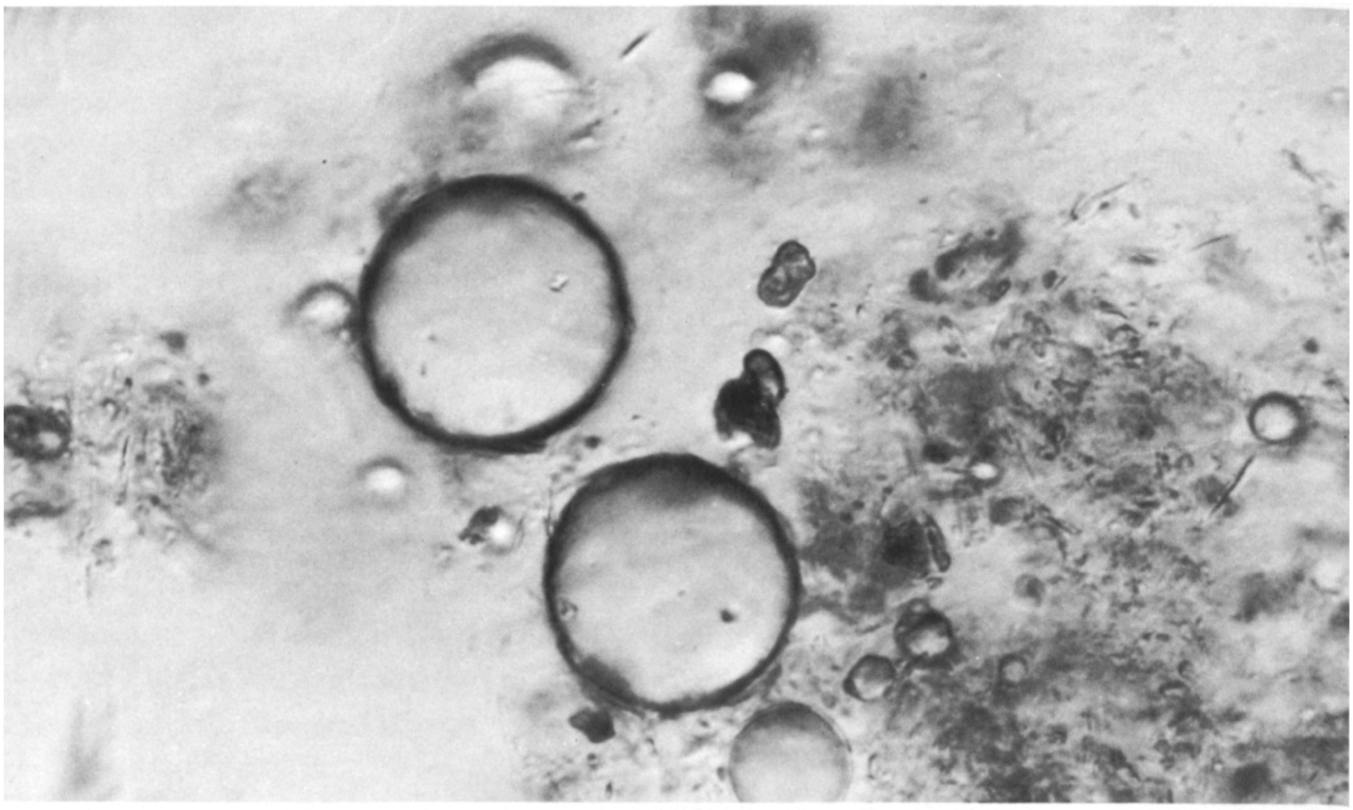

Plate 1

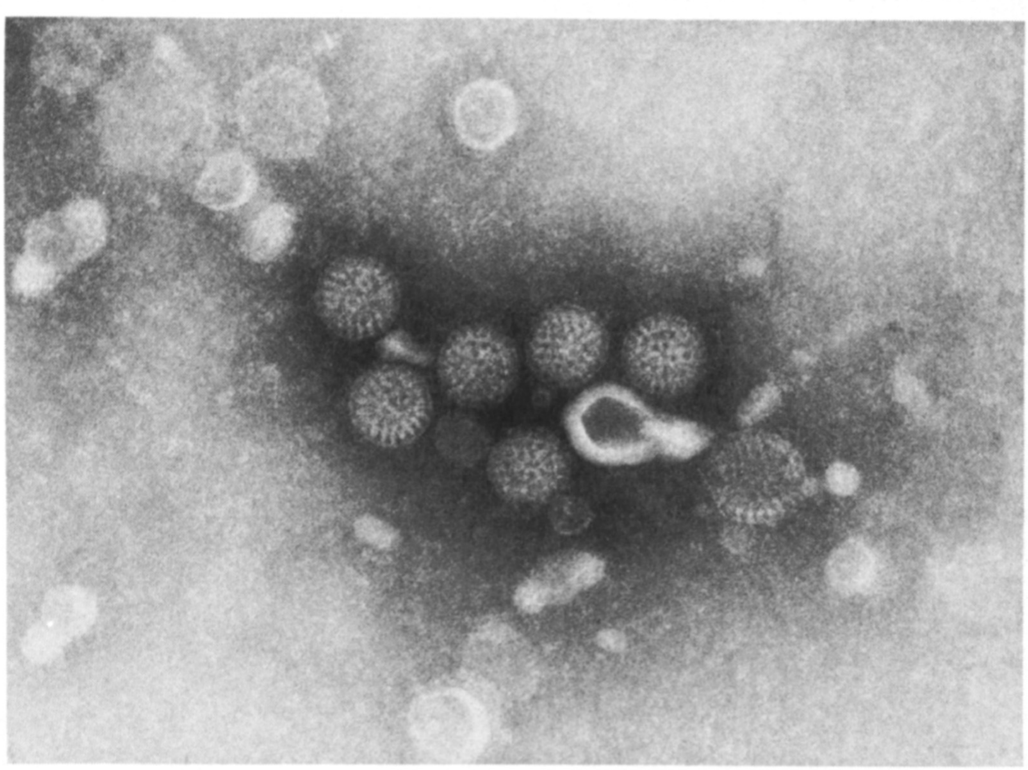

Plate 2

M. E. M. THOMAS, P. LUTON AND JANET Y. MORTIMER

(*Facing p.* 319)

https://doi.org/10.1017/S0022172400069539 Published online by Cambridge University Press

MADELEY, C. R. (1979). Viruses in the stools. *Journal of Clinical Pathology* **32**, 1.

MATHAN, M., MATHAN, V. I., SWAMINATHAN, S. P., YESODOSS, S. & BAKER, S. J. (1975). Pleomorphic virus-like particles in human faeces. *Lancet* i, 1068.

MAYR, A. (1980). New emerging viral zoonoses. *Veterinary Record* **106**, 503.

MORISHIMA, T., YAMAGUCHI, H., NAGAYOSHI, S., OZAKI, T., ISOMURA, S. & SUZUKI, S. (1980). Course of rotavirus gastroenteritis in a closed community. *Archives of Diseases in Childhood* **55**, 147.

NAGAYOSHI, S., YAMAGUCHI, H., ICHIKAWA, T., MIYAZU, M., MORISHIMA, T., OSAKI, T., ISOMURA, S., SUZUKI, S. & HOSHINO, M. (1980). Changes of the rotavirus concentration in faeces during the course of acute gastroenteritis as determined by the immune adherence haemagglutination test. *European Journal of Paediatrics* **134**, 99.

SABIN, A. B. (1956). The significance of viruses recovered from the intestinal tracts of healthy infants and children. *Annals of the New York Academy of Science* **66**, 226.

SABIN, A. B. (1959). Reoviruses. *Science* **130**, 1387.

STANLEY, N. F. (1961). Reovirus – a ubiquitous orphan. *Medical Journal of Australia* **2**, 815.

STEINHOFF, M. C. (1980). Rotavirus: the first five years. *Journal of Pediatrics* **96**, 611.

THOMAS, M. E. M. (1952). 'Epidemic' abdominal colic associated with steatorrhoea. *British Medical Journal* i, 691.

THOMAS, M. E. M. & CHARTER, R. E. (1956). Aetiology of juvenile diarrhoea. *British Medical Journal* ii, 339.

THOMAS, M. E. M. & TILLETT, H. E. (1975). Diarrhoea in general practice. *Journal of Hygiene* **74**, 183.

TILLETT, H. E. & THOMAS, M. E. M. (1981). Monitoring infectious diseases using routine microbiology data. 1. Study of gastroenteritis in an urban area. *Journal of Hygiene* **86**, 49.

TILLETT, H. E. (1981). Monitoring infectious diseases using routine microbiology data. 2. An example of regression analysis used to study infectious gastroenteritis. *Journal of Hygiene* **86**, 59.

WORLD HEALTH ORGANISATION (1980*a*). Diarrhoeal diseases control programme. *WHO Weekly Epidemiological Record* **55**, 209.

WORLD HEALTH ORGANISATION (1980*b*). Scientific working group. Rotavirus and other viral diarrhoeas. *Bulletin of WHO* **58**, 183.

WRIGHT, S. G. & TOMKINS, A. M. (1978). Quantitative histology in giardiasis. *Journal of Clinical Pathology* **31**, 712.

YOLKEN, R. H., KIM, H. W., CLEM, T., WYATT, R. G., KALIKA, A. R., CHANOCK, R. M. & KAPIKIAN, A. Z. (1977). Enzyme-linked immunosorbent assay (ELISA) for detection of human reovirus-like agent of infantile gastroenteritis. *Lancet* ii, 263.

## EXPLANATION OF PLATES

### PLATE 1

Faeces preparation showing fat globules (LM × 1000). The largest globule has a diameter of about 35 $\mu$m.

### PLATE 2

Rotavirus in EM preparation of faeces (magnification × 130000).

https://doi.org/10.1017/S0022172400069539 Published online by Cambridge University Press