association between overt ulcerative colitis and less specific jejunal abnormalities⁶⁻⁸ whose severity reflects the clinical activity of the colitis.

Microscopic colitis is not generally recognised, though Read *et al*⁹ reported minor histological changes in the colon described as microscopic colitis in eight of 27 patients investigated for intractable diarrhoea. No conclusions, however, were drawn from this finding.

The possibility that surreptitious laxative ingestion caused the diarrhoea and microscopic colitis in our patients cannot be totally disproved. Nonetheless, we consider it unlikely as we failed to find laxatives in stool, urine, or personal belongings of the patients; melanosis coli was not found endoscopically and histologically; the histological changes in our cases differed from those described in the cathartic colon^{10 11}; and, most importantly, patients responded favourably to sulphasalazine.

We conclude that microscopic colitis is responsible for a proportion of cases of intractable diarrhoea of obscure origin and may respond to treatment with anti-inflammatory drugs. Rectal and colonic biopsies are mandatory in the investigation of such patients even though findings of colonic radiology and endoscopy are normal.

We thank Miss Helen Trussler for typing the manuscript.

References

- ¹ Lindstrom CG. "Collagenous colitis" with watery diarrhoea—a new entity? *Pathologica Europa* 1976;11:87-9.
- ² Teglbjaerg PS, Thaysen EH. Collagenous colitis: an ultrastructural study of a case. *Gastroenterology* 1982;**82**:561-3.
- ³ Elliot PR, Williams CB, Lennard-Jones JE, et al. Colonoscopic diagnosis of minimal change colitis in patients with a normal sigmoidoscopy and normal air contrast barium enema. Lancet 1982;i:650-1.
- ⁴ Falchuk KR, Falchuk ZM. Selective IgA deficiency, ulcerative colitis and gluten sensitive enteropathy—a unique association. Gastroenterology 1975;69:503-6.
- ⁵ Kumar PJ, O'Donoghue DP, Gibson J, Stansfeld A, Dawson AM. The coexistence of inflammatory bowel lesions in coeliac disease. *Postgrad Med J* 1979;55:753-6.
- ⁶ Salem SN, Truelove SL. Small intestinal and gastric abnormalities in ulcerative colitis. Br Med J 1965;i:827-31.
- ⁷ Jankey N, Price LA. Small intestinal histochemical and histological changes in ulcerative colitis. *Gut* 1969;10:267-9.
- ⁸ Binder V, Soltoft J, Gudmond-Hoyer E. Histological and histochemical changes in the jejunal mucosa in ulcerative colitis. Scand J Gastroenterol 1974;9:293-7.
- ⁹ Read NW, Krejs GJ, Read MG, Santa Ana CA, Morawski SG, Fordtran JS. Chronic diarrhoea of unknown origin. *Gastroenterology* 1980;78: 264-71.
- Morson BC. Histopathology of cathartic colon. Gut 1971;12:867-8.
 Smith B. Pathology of cathartic colon. Proc R Soc Med 1972;65:288.

(Accepted 5 August 1982)

Rate of inactivation of cytomegalovirus in raw banked milk during storage at -20° C and pasteurisation

HENRIK FRIIS, H KERZEL ANDERSEN

Abstract

Samples of milk from 23 mothers attending the department of obstetrics and gynaecology and 36 who donated milk to the department's milk bank were cultured for cytomegalovirus. Virus was isolated from samples from 12 of the milk donors but none of the mothers attending the department; follow-up studies during lactation in seven of these 12 women showed that five continued to excrete the virus. Samples were taken on three occasions from one woman who regularly excreted high titres of the virus. Storage at -20° C for over three days reduced the titre by over 99%; after pasteurisation at 63°C for eight minutes the milk did not contain any viable virus.

It is recommended that raw banked milk used for feeding preterm babies should be kept frozen for at least 72 hours before feeding.

Introduction

Recently it has been questioned whether human milk used to feed infants who cannot be breast-fed should be pasteurised or not because pasteurisation affects antimicrobial substances and nutritional properties of milk.^{1 2} Little attention, however, has been paid to the risk of transmitting cytomegalovirus infection by using raw banked milk. For this reason we studied the amounts of cytomegalovirus in human milk and the rate of

Institute of Medical Microbiology, University of Aarhus, DK-8000 Aarhus C, Denmark inactivation of the virus during storage at -20° C and during pasteurisation at 63° C.

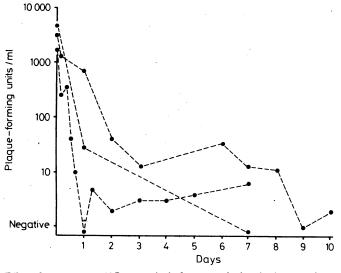
Patients, methods, and results

Milk samples were collected between days 2 and 12 from 23 mothers attending the university department of obstetrics and gynaecology at Aarhus Kommunehospital, and from 36 women who donated milk to the milk department's milk bank. Within four hours after collection 1 ml of skimmed milk was inoculated into a 50 cm² monolayer of human fibroblasts in glass bottles. After one hour of adsorption the cultures were overlaid with Eagle's modified medium and studied weekly for four weeks for a cytopathic effect typical for cytomegalovirus. Titres of cytomegalovirus were estimated by counting viral plaques in the cell layer after staining with methylene blue as described previously.³ Cytomegalovirus was not isolated from any samples from the 23 mothers attending the department but from 45 samples from 12 of the 36 milk donors. The earliest isolation was made in milk collected on day 14 and the latest in milk collected 58 weeks after delivery.

The initial cytomegalovirus titres were about 1000 plaque-forming units (pfu)/ml in two women, 500 and 200 pfu/ml in two others, and about 10 pfu/ml in the rest. Follow-up studies performed during lactation in seven of the milk donors including three of the four women with initial titres of 200 pfu or more/ml showed that all except two continued to excrete cytomegalovirus, in titres of 10 pfu or less/ml. One woman, however, regularly excreted between 1000 and 4600 pfu/ml for 26 weeks of lactation; on three occasions samples obtained from her were divided and cultured after storage at -20° C for up to 10 days. Storage for more than three days reduced the amount of cytomegalovirus in the milk by more than 99% (from 4300 pfu/ml to about 10 pfu/ml) (figure). Pasteurisation at 63°C, by heating aliquots of milk in a water bath for one, two, four, eight, and 16 minutes followed by quick cooling in an ice bath, also reduced the virus titre (from 3600 pfu/ml to 10 pfu/ml after one minute). After eight minutes of pasteurisation the milk did not contain any viable cytomegalovirus at all.

HENRIK FRIIS, medical student H KERZEL ANDERSEN, DMSC, reader in virology

4 DECEMBER 1982



Effect of storage at $-20^\circ C$ on survival of cytomegalovirus in three specimens obtained at weekly intervals from a milk donor.

Discussion

By using a sensitive plaque method to determine excretion of cytomegalovirus in human milk we found that both the number of excretors and the amount of virus in milk may be considerably higher than previously reported.^{4 5} This confirms the importance of breast-milk as a source of transmission of cytomegalovirus infection. The course of infection in infants infected by their mother's milk is usually subclinical, probably owing to antibodies acquired transplacentally. Cytomegalovirus may constitute a hazard, however, when milk is collected and given raw to preterm and mature seronegative babies.

The risk of using raw milk is reduced considerably by keeping the milk at -20° C for at least three days, but high-titred milk may, however, still contain a slight residue of cytomegalovirus of certain infective potential. Pasteurisation at 63°C for eight minutes will kill cytomegalovirus in milk.

This study was supported by grant No 12-3087 from Statens Laegevidenskabelige Forskningsråd.

References

- ¹ Anonymous. Sterilisation of human milk. (Editorial.) Lancet 1979;i:913.
 ² Björkstén B, Burman LG, de Chateau P, Frederikzon B, Gothefors L, Hernell O. Collecting and banking human milk: to heat or not to heat? Br Med J 1980;281:765-9.
- ³ Andersen HK, Brostrøm K, Hansen KB, et al. A prospective study on the incidence and significance of congenital cytomegalovirus infection. Acta Paediatr Scand 1979;68:329-36.
- ⁴ Hayes K, Danks DM, Gibas H, Jack J. Cytomegalovirus in human milk. N Engl J Med 1972;297:177-8.
- ⁵ Stagno S, Reynolds DW, Pass RF, Alford CA. Breast milk and the risk of cytomegalovirus infection. N Engl J Med 1980;**302**:1073-6.

(Accepted 13 August 1982)

Enkephalin inhibits relaxation of the lower oesophageal sphincter

J M HOWARD, M R BELSHEIM, S N SULLIVAN

Abstract

Five healthy volunteers were studied for the effect on oesophageal motility of a single subcutaneous injection of a synthetic analogue of enkephalin as compared with an injection of an equivalent volume (0 5 ml) of saline. The injections were given at random on separate days, and each was followed after 40 minutes by 2 mg naloxone given intravenously. Pressures were measured by manometry after dry and wet (5 ml) swallows at one-minute intervals, and traces were coded and analysed "blind."

Twenty-five minutes after the injection of enkephalin the percentage relaxation of the lower oesophageal sphincter pressure was significantly less (p < 0.005) than at the same time after saline. Within two minutes after intravenous naloxone this effect had disappeared completely. Enkephalin had no noticeable effect on pressure of the sphincter or on amplitude and duration of oesophageal peristalsis.

The mechanism of action of enkephalin in selectively

inhibiting relaxation of the lower oesophageal sphincter remains to be determined. That naloxone rapidly reversed the inhibition may be relevant in achalasia and warrants further study.

Introduction

Opiate receptors and enkephalin-containing neurons occur throughout the gastrointestinal tract. In the oesophagus these neurons lie in the muscularis propria and muscularis mucosae. Other enkephalinergic neurons are found in the vagus nerve. The function of these neurons is unknown. We have studied the effects of a long-acting synthetic enkephalin analogue (Sandoz FK33-824; DAMME) on the motility of the oesophagus.

Methods and results

Five healthy fasting volunteers were studied on two occasions. Manometry was performed with a seven-lumen catheter perfused by a pneumohydraulic capillary infusion pump. Once the lower oesophageal sphincter had been identified the catheter was secured and continuous recordings made in five locations (intragastric, lower oesophageal sphincter, and 5, 10, and 15 cm proximal to the lower oesophageal sphincter). Peristalsis was initiated by alternating dry and wet (5 ml) swallows at one-minute intervals. After a 10-minute basal recording the subjects randomly received on separate days either a subcutaneous

Department of Medicine, Colborne II, Victoria Hospital, London, Ontario, Canada

J M HOWARD, MD, resident in gastroenterology

M R BELSHEIM, MD, FRCP(C), consultant in gastroenterology

S N SULLIVAN, MRCP, FRCP(C), consultant in gastroenterology