

Intestinal bacterial flora and bile salt studies in hypogammaglobulinaemia

D. M. PARKIN, D. B. L. McCLELLAND, R. R. O'MOORE, I. W. PERCY-ROBB, I. W. B. GRANT, AND D. J. C. SHEARMAN

From the Gastrointestinal Section of the University Department of Therapeutics, the University Department of Clinical Chemistry, The Royal Infirmary, and the Respiratory Diseases Unit, Northern General Hospital, Edinburgh

SUMMARY Five patients with adult acquired hypogammaglobulinaemia, four of whom were achlorhydric, were studied. Jejunal bacterial counts were much higher than those in a control group of acid secretors, but were similar to those in a control group of patients with pernicious anaemia; *Giardia lamblia* were isolated from the jejunal content of all patients with hypogammaglobulinaemia. The concentration of conjugated bile acids in the fasting state was lower in hypogammaglobulinaemia than in pernicious anaemia, but in the two hypogammaglobulinaemic patients with steatorrhoea there was a normal bile salt response to a fatty meal.

Gastrointestinal symptoms occur frequently in patients with hypogammaglobulinaemia, and Rosen and Janeway (1966) reported that more than half of such patients have diarrhoea or steatorrhoea. In those patients in whom a flat intestinal biopsy is accompanied by hypogammaglobulinaemia or selective IgA deficiency, a gluten-free diet may result in clinical improvement (Green and Sperber, 1962; Crabbé and Heremans, 1967). In other cases, the administration of fresh plasma which contains IgA has been associated with remission of diarrhoea (Binder and Reynolds, 1967; Gryboski, Self, Clemett, and Herskovic, 1968), and where a response to oral broad-spectrum antibiotics is found (Gryboski *et al*, 1968; Mawas, Sors, and Bernier, 1969; Collins and Ellis, 1965) proliferation of bacteria in the intestine may be the cause of the diarrhoea and steatorrhoea.

Previous studies have included a few observations on the flora of the upper gastrointestinal tract (Collins and Ellis, 1965; McCarthy, Austad, and Read, 1965; Mawas *et al*, 1969; Dubois, Roy, Fulginiti, Merrill, and Murray, 1970) but apart from one study (Hersh, Floch, Binder, Conn, Prizont, and Spiro, 1970) there has been no attempt to quantitate jejunal bacteria in a series of patients with hypogammaglobulinaemia. In the present study, an attempt is made to correlate the gastrointestinal disturbance in patients with hypogammaglobulin-

aemia with the number of bacteria in the jejunum and with possible abnormalities of bile salt metabolism. Deconjugation of bile salts can be the cause of steatorrhoea in patients without immunological deficiency (Gorbach and Tabaqchali, 1969).

Materials and Methods

PATIENTS

The clinical details of five patients with adult acquired hypogammaglobulinaemia are shown in Table I. Three of the patients with hypogammaglobulinaemia were receiving long-term antibiotic therapy for chest infection. This was stopped for a period of three weeks before any of the studies was performed. A further five subjects who had no evidence of gastrointestinal disease were used as normal controls. Eight additional patients with pernicious anaemia were studied because four of the five patients with hypogammaglobulinaemia were achlorhydric.

METHODS

The 24-hour output of faecal fat was estimated from five-day stool collections by the method of van der Kamer, Ten Bokkel Huinink, and Weijers (1949). The normal value is 6 g/24 hr or less while the subject receives a dietary fat load of less than 70 g/24 hr. Vitamin B₁₂ absorption was estimated by the Schilling test and by the whole-body counting method (Finlayson, Simpson, Tothill, Samson, Girdwood,

Patient No.	Age Sex	Serum Immuno-globulins (mg/100 ml)			Acid Output in Post-penta-gastrin Hour	Faecal Fat (g/24 hr)		Vitamin B ₁₂ Absorption ¹	Jejunal Biopsy	Clinical Features
		IgG	IgA	IgM		No Anti-biotic	On Anti-biotic			
1	35 M	10	13	<10	0	18.1	2	6% ²	Normal villi, large lymphoid follicles in lamina propria ³	Recurrent chest infections, intermittent diarrhoea and steatorrhoea, Peripheral neuropathy, Addisonian pernicious anaemia —the vitamin B ₁₂ malabsorption being corrected by intrinsic factor
2	19 F	<10	27	10	0	8.5	6	68%	Normal villi, large lymphoid follicles in lamina propria	Small stature, iron-deficiency anaemia, no gastrointestinal symptoms
3	60 M	<10	<10	<10	0	7	7	47%	Normal mucosa	Recurrent chest infections, and receiving long-term antibiotics; intermittent diarrhoea; iron-deficiency anaemia
4	52 M	140	<10	<10	0	4	—	40%	Normal mucosa	Recurrent chest infections and receiving long-term gammaglobulin im and antibiotics; no gastrointestinal symptoms
5	36 M	<10	<10	<10	18	2.1	—	85%	Normal mucosa	Recurrent chest infections and receiving long-term gammaglobulin im and antibiotics, no gastrointestinal symptoms

Table I Clinical details of patients with hypogammaglobulinaemia

¹Whole body count²The malabsorption in this patient was corrected by oral intrinsic factor (14% absorption as assessed by Schilling test).³Figure 3

and Shearman, 1969) in which the normal patient absorbs not less than 30% of the oral dose vitamin B₁₂.

Jejunal biopsy was performed with the Crosby capsule, the specimen being taken from between 2 and 10 cm beyond the duodeno-jejunal flexure.

SERUM IMMUNOGLOBULINS

Serum levels of immunoglobulins were measured by the Mancini technique as modified by Fahey and McKelvey (1965) using antisera supplied by Hyland Laboratories¹ and standard sera supplied by Hoechst².

INTUBATION AND SAMPLING

All intubations were performed after an overnight fast. Intestinal secretions were aspirated via a sterile radioopaque double-lumen tube, which was passed orally. The distal end of the tube was fitted with a balloon filled with barium sulphate. The tube was guided into the upper jejunum (the first 30 cm of bowel beyond the duodeno-jejunal flexure) under radiological control. The distal end of the tube was unsealed, but the proximal end was firmly clamped during the intubation.

With the tube in position, aspiration of jejunal juice was performed. The first 8 ml of aspirate was discarded. Two 3 ml aliquots were then collected for

bile salt analysis and quantitative bacteriology. Samples for bile salt estimation were immediately stored at -20°C. The first stages of the bacteriological analysis were carried out within three minutes of collection.

The tube was then slowly withdrawn into the stomach and after five to 10 minutes *in situ* aspiration of gastric juice via the second lumen was performed. The first 5 to 10 ml of aspirate was discarded, further samples being analysed for bacterial content and pH.

BACTERIOLOGICAL STUDIES

Serial tenfold dilutions of the intestinal secretions were prepared in broth which had been rendered anaerobic by pre-steaming and storage in an oxygen-free atmosphere for 24 hours. The culture media are shown in Table II. Plates for isolation of anaerobic organisms were stored in an oxygen-free atmosphere until immediately before use.

Media 1 and 2 were inoculated by spreading the surface of the plates with 0.1 ml of undiluted intestinal fluid and of tenfold dilutions up to 10⁻⁶. Media 3 to 10 were inoculated with single drops of each dilution according to the method of Miles and Misra (1938). The plates for anaerobic incubation were immediately sealed in BTL anaerobic jars, incubation being carried out in an atmosphere of hydrogen containing 5% CO₂. In order to limit the loss of oxygen-sensitive organisms the interval between aspiration of intestinal fluid and the completion of the above manoeuvre was less than 15

¹Hyland Division Travend Laboratories, Thetford, Norfolk.²Hoechst Pharmaceuticals, Hoechst House, Kew Bridge, Brentford, Middlesex.

Medium	Incubated	Duration of Incubation (hr)	Organisms Counted
1 10% blood agar	Aerobically	24	Total aerobes
2 Reinforced clostridial agar (Oxoid) + 10% horse blood	Anaerobically	5 days	Total anaerobes
3 McConkey agar	Aerobically	24	Coliforms, enterococci
4 Mannitol—salt agar	Aerobically	24	Staphylococci
5 Sabouraud agar	Aerobically	24	Yeasts
6 Rogosa lactobacillus agar (Rogosa, Mitchell, and Wiseman, 1951)	90% CO ₂	48	Lactobacilli
7 Tomato juice agar	Anaerobically	48	Anaerobic lactobacilli
8 Willis and Hobbs medium (Willis and Hobbs, 1959) with 100 µg/ml of neomycin.	Anaerobically	48	Clostridia
9 Rogosa Veillonella agar (Rogosa, Fitzgerald, MacKintosh, and Beaman, 1958)	Anaerobically	48	Veillonellae
10 Reinforced clostridial agar with 10% blood, 100 µg/ml neomycin, 7.5 µg/ml vancomycin (Finegold, Miller, and Posnick, 1964)	Anaerobically	48	Bacteroides

Table II Details of bacteriological analyses

minutes. Incubation was carried out at 37°C for the times specified in Table II.

Bacteria were identified using the tables of Cowan and Steel (1970). Plates were set up in triplicate. The colonies on each plate were counted and the mean value was used to calculate the number of organisms present in the original sample.

Samples of jejunal juice were also examined microscopically for the presence of *Giardia lamblia*.

BILE SALT STUDIES

Analysis of samples of jejunal juice was performed by a thin-layer chromatography technique. Samples were extracted and thin-layer chromatograms developed as described previously (Percy-Robb, Jalan, McManus, and Sircus, 1971). The developed chromatograms were heated at 100°C for five minutes in a hot-air oven and sprayed with acetic acid, sulphuric acid, anisaldehyde: 30, 1, 0.5 v/v/v (Kritchevsky, Martak, and Rothblatt, 1963). The sprayed plates were returned to the hot-air oven for six and a half minutes and after 20 minutes at room temperature they were scanned on a flying-spot densitometer (Vitatron TLD 100). Quantitation of the individual spots was performed by analysing standard samples containing known concentrations of bile acid conjugates with glycine and taurine as well as unconjugated acids. Comparison of the areas under the peaks produced by the unknown samples was carried out with a calibration curve prepared from the standard samples. Each analysis was performed in duplicate. The concentration of total conjugated bile acids was estimated by summing the concentrations of the individual conjugates. The coefficient of variation of these quantitative analyses is between 1.5 and 5.5%. The recoveries of conjugated bile acids added to jejunal juice were 96 to 103% (O'Moore and Percy-Robb, in preparation).

In patients 1 and 2, a formal assessment of the concentration of bile salts in the upper jejunum in the 90 minutes following ingestion of a fat-containing

meal (sucrose 50 g, corn oil 20 g, egg albumen 20 g, water 200 ml) was carried out. Samples were collected immediately before ingesting the meal and thereafter at 30, 60, and 90 minutes.

Organisms isolated from the jejunal juice were incubated in the presence of taurocholate³ (4.0 m moles/l) for a period of 48 hours and the appearance of unconjugated cholic acid assessed by thin-layer chromatography.

Results

The results of the faecal fat output in the patients with hypogammaglobulinaemia are shown in Tables I and IV, and in the patients with pernicious anaemia in Table IV. The findings on jejunal biopsy, and the vitamin B₁₂ absorption of the patients with hypogammaglobulinaemia are shown in Table I.

SERUM IMMUNOGLOBULIN LEVELS

The serum immunoglobulin levels in the control patients, both normal and with pernicious anaemia, were all within the normal range for the laboratory (IgG: 700 to 1,675 mg/100 ml. IgA: 89 to 315 mg/100 ml. IgM: 45 to 250 mg/100 ml). The results in the patients with hypogammaglobulinaemia are shown in Table I.

BACTERIOLOGICAL STUDIES

The pH of the gastric contents of the normal control subjects was between 1.1 and 4.0. In these subjects, bacteriological analysis of jejunal juice showed only a scanty growth of organisms, mainly streptococci, lactobacilli, and veillonellae, the mean logarithmic counts being 3.6, 2.4 and 2.5 organisms per ml, respectively.

The results of bacteriological studies in the patients with hypogammaglobulinaemia are shown in Table III, and compared with those in pernicious anaemia

³Maybridge Chemical Co., Tintagel, Cornwall.

Patient	Enteric Organisms (Enterobacteriaceae and Enterococci)	Streptococci (Viridans/Salivarius)	Neisseriae	Staphylococci	Yeasts	Aerobic Lactobacilli	Anaerobic Lactobacilli (Bifidobacteria)	Veillonellae	Clostridia	Bacteroides and Fusiforms
1	3.2 ^E	7.1	6.1	3.0	—	3.8	6.5	6.3	—	4.4
2	4.6 ^S	3.1	4.4	3.7	—	3.0	3.6	3.5	—	—
3	7.1 ^E	7.3	7.1	2.5	—	2.3	6.7	7.8	—	7.0
4	6.9 ^E	7.5	6.2	4.8	—	—	7.2	5.7	—	6.9
5	—	3.4	3.5	—	—	—	5.0	3.6	—	—

Table III Jejunal organisms

E = Enterobacteriaceae

S = Streptococci (enterococci)

Counts = log no. of organisms per ml — = < 1.7

in Figures 1 and 2. Although the patients with hypogammaglobulinaemia have relatively high bacterial counts in the jejunum when compared with normal subjects, they are unremarkable in comparison with counts obtained from the group of patients with pernicious anaemia.

When the anaerobic flora of the jejunum in the patients with hypogammaglobulinaemia and patients with pernicious anaemia are compared (Fig. 2), it is seen that three of the hypogammaglobulinaemics had bacteroides species (when these are regarded separately from the 'fusiforms'), whereas the patients with pernicious anaemia did not.

Microscopic examination of jejunal juice showed that all the patients with hypogammaglobulinaemia harboured the protozoon *Giardia lamblia* in the

jejunum. This organism was also found in one of the control patients with pernicious anaemia, but was not present in the normal subjects.

BILE SALT STUDIES

The results of the bile salt analyses on the jejunal juice of fasting patients are shown in Table IV. Unconjugated cholic acid was found in four of the patients with hypogammaglobulinaemia and in six of the patients with pernicious anaemia. The concentration of total conjugated bile acids was between 3.2 and 15.5 m moles/l in the patients with pernicious anaemia and 0.5 to 2.5 m moles/l in the patients with hypogammaglobulinaemia.

In response to the fat-containing meal, the concentrations of bile salts in the upper jejunum of

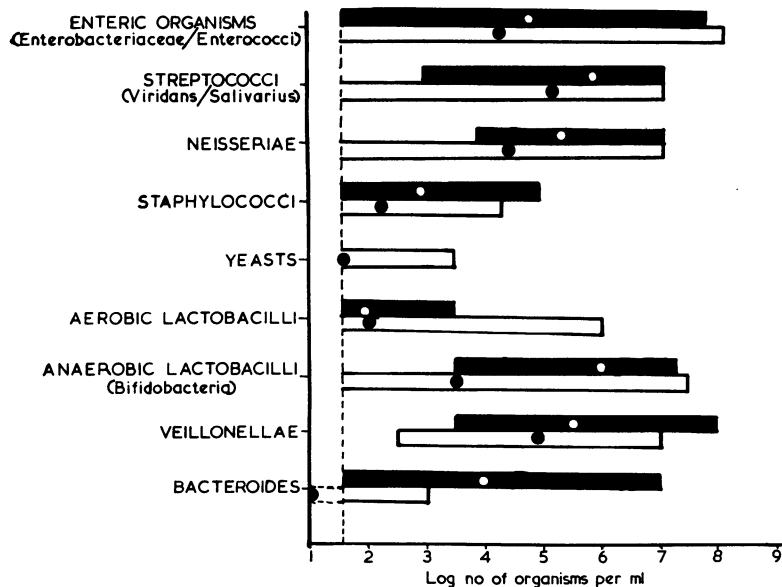


Fig. 1 Black bars—patients with hypogammaglobulinaemia (5) White bars—patients with pernicious anaemia (8)

The bars represent the range of counts. The central point is the logarithmic mean. The vertical dotted line at 1.5 represents the lower limit of sensitivity of the counting method employed.

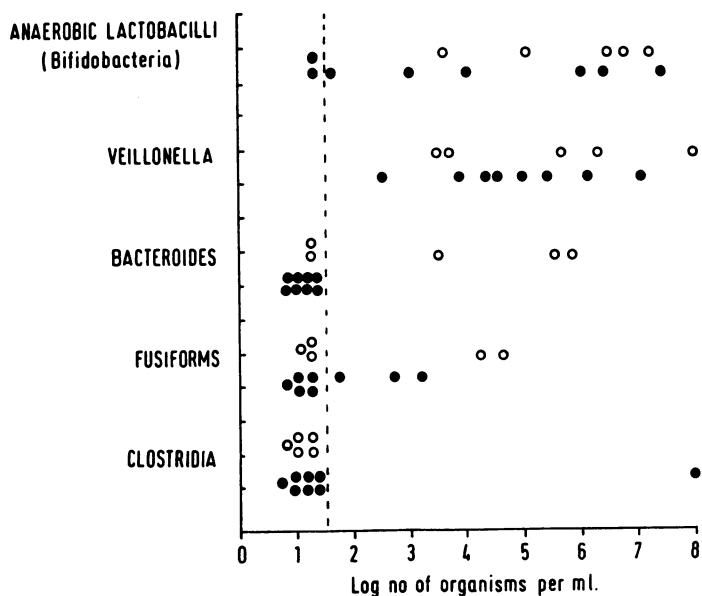


Fig. 2 White points—patients with hypogammaglobulinaemia
Black points—patients with pernicious anaemia
Each point represents the count of a particular organism. The dotted line running vertically at 1.5 is the lower limit of sensitivity of the counting method used. Points to the left of this line indicate that the organism was not detected.

patient 1 at 30, 60, and 90 minutes were 10, 7, and 5 m moles/l, and in patient 2 the concentrations were 17, 10, and 13 m moles/l.

Organisms which were isolated from the jejunum of the patients and found to be capable of deconjugating taurocholate *in vitro* were *Streptococcus faecalis*, a strain of *Bacteroides sp.*, a strain of *Veillonella sp.*, and two types of anaerobic lactobacilli.

Discussion

The role of the immunoglobulins in external secretions is not fully understood. IgA is the pre-

dominant immunoglobulin in the gastrointestinal tract, where it is secreted as a complex with a glycoprotein, the 'secretory piece' as 11S, secretory IgA. It has been suggested (Tomasi, 1969) that immunoglobulins play an important part in the regulation of the normal flora, and the resistance of mucous surfaces to colonization by potentially pathogenic microorganisms and viruses. If this is correct, then in hypogammaglobulinaemia, the intestinal conditions might exist for the proliferation of bacteria. In the patients with hypogammaglobulinaemia, the results of jejunal culture which showed large numbers of organisms compared to normal controls were similar to those described by Hersh *et*

Patient	Concentrations of Bile Acids in Jejunum (Fasting) (mmol/l)				Faecal Fat Output (g/24 hr)
	Total Conjugated	Cholic	Chenodeoxycholic	Deoxycholic	
1	2.5	0.17	0.10	0	18.1
2	1.5	0.04	0	0	8.5
3	1.5	0.08	0	0	7
4	1.5	0.06	0	0	4
5	0.5	0	0	0	2.1
6	15.5	0	0	0	7.0
7	3.2	0	0	0	3.2
8	5.3	0.10	0	0	4.7
9	5.4	0.16	0.06	0	4.9
10	10.6	0.20	0.05	0	7.9
11	4.0	0.12	0.07	0.04	3.6
12	5.6	0.22	0.10	0.05	5.5
13	6.5	0.55	0.15	0.08	5.3

Table IV Bile salt concentrations in jejunal juice from patients with hypogammaglobulinaemia (patients 1 to 5) and pernicious anaemia (patients 6 to 13)

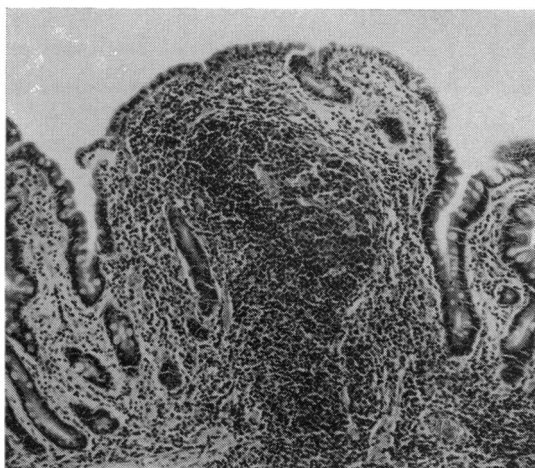


Fig. 3 Jejunal biopsy from patient no. 1, showing nodular lymphoid hyperplasia.

al (1970) except that we found relatively large numbers of *Bacteroides* in three out of five patients. Gastric achlorhydria is a common finding in patients with immunoglobulin deficiency diseases (Twomey, Jordan, Laughter, Meuwissen, and Good, 1970), and the absence of gastric acid is associated with increased numbers of bacteria in the jejunum (Sherwood, Goldstein, Haurani, and Wirts, 1964). For this reason, it is essential that the gastric acidity of all patients studied should be known before counts of organisms obtained from the upper small bowel can be considered abnormal. In the study by Hersh *et al* (1970) only one of the patients with significant numbers of organisms in the upper jejunum was shown to have 'normal' gastric acidity. Because four of our patients were achlorhydric, we have compared them with patients with pernicious anaemia. The differences between the two groups were unremarkable, apart from the presence of *Bacteroides* in three of the patients with hypogammaglobulinaemia (Fig. 2). In spite of the presence of bacterial colonization in the upper small intestine, none of the patients with pernicious anaemia had gastrointestinal symptoms. Two of the five patients with hypogammaglobulinaemia had gastrointestinal symptoms but we did not demonstrate any correlation between the jejunal flora and the clinical and laboratory features of the bowel disorder. It was noted that *Giardia lamblia* was present in the jejunal aspirate of all patients with hypogammaglobulinaemia compared with only one of the patients with pernicious anaemia. Others have found the diarrhoea to be relieved in the occasional

patient by eradicating the *Giardia* but in the present study this treatment could not be carried out separately because of the limited time that three of the five patients could remain untreated with antibiotics.

The presence of an abnormal flora in the upper small intestine can be associated with malabsorption of fat due to the production of unconjugated bile acids (being the products of bacterial deconjugation of bile acid conjugates). This causes a reduction in the concentration of these conjugates to a level below that necessary for the production of micelles, and fat malabsorption results. The concentration of total conjugated bile acids in the jejunum of fasting patients was lower in the hypogammaglobulinaemic group than in the eight patients with pernicious anaemia.

However, it was demonstrated that in response to a fatty meal, patients 1 and 2 (faecal fat excretion 18.1 and 8.5 g respectively) achieved adequate concentrations (Badley, Murphy, and Bouchier, 1969) of conjugated bile acid for normal fat absorption to occur. It seems unlikely that these low fasting bile acid concentrations in the hypogammaglobulinaemic group were due to malabsorption of bile salts since it has been demonstrated that a subnormal response to a fatty meal accompanies bile salt malabsorption, eg, in ileal resection (Van Deest *et al*, 1968; Percy-Robb *et al*, 1970). Decreased bile salt synthesis is unlikely in view of the normal bile salt response to a fat load. A dilutional effect has not been excluded in this study. Thus, although the fasting levels of conjugates in hypogammaglobulinaemia and pernicious anaemia appeared different, there was no evidence that the low level in hypogammaglobulinaemia reflected differences in bile salt concentration in response to food.

In the present study, some of the patients with hypogammaglobulinaemia and some of those with pernicious anaemia had low concentrations of unconjugated bile acids in the jejunal aspirate, and studies *in vitro* defined some of the bacteria which were capable of deconjugation. Both the morphology (Dawson and Isselbacher, 1960; Low-Beer, Schneider, and Dobbins, 1970) and function (Dawson and Isselbacher, 1960; Dietschy, 1967) of the small intestine of the rat, guinea pig, and hamster may be altered by the presence of unconjugated bile acids in the intestinal lumen. However, the concentrations of unconjugated acids required to produce these effects have been shown to be, in general, greater than 1mM although a concentration of 5×10^{-4} M of deoxycholate has been shown to inhibit fatty acid esterification (Dawson and Isselbacher, 1960). It is unlikely that the presence of unconjugated bile acids which we have found in our patients is related to the

mechanism of malabsorption since there was no relation between their concentration and the presence of a raised faecal fat excretion.

Whilst it is appreciated that the present study did not proceed to assess the bacteriological flora at different levels in the small intestine (Gorbach and Tabaqchali, 1969) and did not assess whether the bile salt response to a fatty meal could be sustained on subsequent occasions (van Deest, Fordtran, Morawski, and Wilson, 1968; Percy-Robb *et al*, 1971) we could demonstrate no specific factors causing gastrointestinal disturbance and steatorrhoea. It may be that disturbance is due to a combination of minor mucosal abnormalities and repeated viral and bacterial infections, together with the presence of *Giardia lamblia*.

Dr D. M. Parkin and Dr D. B. L. McClelland were in receipt of MRC junior research fellowships. We thank our technical staff Mr R. R. Samson and Mr K. Marwick, for their assistance.

References

- Badley, B. W. D., Murphy, G. M., and Bouchier, I. A. D. (1969). Intraluminal bile salt deficiency in the pathogenesis of steatorrhoea. *Lancet*, 2, 400-402.
- Binder, H. J., and Reynolds, R. D. (1967). Control of diarrhoea in secondary hypogammaglobulinemia by fresh plasma infusions. *New Engl. J. Med.*, 277, 802-803.
- Collins, J. R., and Ellis, D. S. (1965). Agammaglobulinemia, malabsorption and rheumatoid-like arthritis. *Amer. J. Med.*, 39, 476-482.
- Cowan, S. T., and Steel, K. J. (1970). *Manual for the Identification of Medical Bacteria*. Cambridge University Press, London.
- Crabbé, P. A., and Heremans, J. F. (1967). Selective IgA deficiency with steatorrhoea. *Amer. J. Med.*, 42, 319-326.
- Dawson, A. M., and Isselbacher, K. J. (1960). Studies on lipid metabolism in the small intestine with observations on the role of bile salts. *J. clin. Invest.*, 39, 730-740.
- Dietschy, J. M. (1967). Effects of bile salts on intermediate metabolism of the intestinal mucosa. *Fed. Proc.*, 26, 1589-1598.
- Dubois, R. S., Roy, C. C., Fulginiti, V. A., Merrill, D. A., and Murray, R. L. (1970). Disaccharidase deficiency in children with immunologic deficits. *J. Pediatr.*, 76, 377-385.
- Fahey, J. L., and McKelvey, E. M. (1965). Quantitative determination of serum immunoglobulins in antibody-agar plates. *J. Immunol.*, 94, 84-90.
- Finegold, S. M., Miller, A. B., and Posnick, D. J. (1964). Further studies on selective media for bacteroides and other anaerobes. *Ernährungsforschung*, 10, 517-528.
- Finlayson, N. D. C., Simpson, J. D., Tothill, P., Samson, R. R., Girdwood, R. H., and Shearman, D. J. C. (1969). Application of whole-body counting to the measurement of vitamin B₁₂ absorption with reference to achlorhydria. *Scand. J. Gastroent.*, 4, 397-405.
- Gorbach, S. L., and Tabaqchali, S. (1969). Bacteria, bile and the small bowel. *Gut*, 10, 963-972.
- Green, I., and Sperber, R. J. (1962). Hypogammaglobulinemia, arthritis, sprue, and megaloblastic anemia. *N.Y. St. J. Med.*, 62, 1679-1686.
- Gryboski, J. D., Self, T. W., Clemett, A., and Herskovic, T. (1968). Selective immunoglobulin A deficiency and intestinal nodular lymphoid hyperplasia: correction of diarrhoea with antibiotics and plasma. *Pediatrics*, 42, 833-837.
- Hermans, P. E., Huizenga, K. A., Hoffman, H. N., II, Brown, A. L., Jr., and Markowitz, H. (1966). Dysgammaglobulinemia associated with nodular lymphoid hyperplasia of the small intestine. *Amer. J. Med.*, 40, 78-89.
- Hersh, T., Floch, M. H., Binder, H. J., Conn, H. O., Prizont, R., and Spiro, H. M. (1970). Disturbance of the jejunal and colonic bacterial flora in immunoglobulin deficiencies. *Amer. J. clin. Nutr.*, 23, 1595-1601.
- Van de Kamer, J. H., ten Bokkel Huinink, H., and Weyers, H. A. (1949). Rapid method for the determination of fat in feces. *J. biol. Chem.*, 177, 347-355.
- Kritchevsky, D., Martak, D. S., and Rothblat, G. H. (1963). Detection of bile acids in thin-layer chromatography. *Analyt. Biochem.*, 5, 388-392.
- Low-Beer, T. S., Schneider, R. E., and Dobbins, W. O. (1970). Morphological changes of the small-intestinal mucosa of guinea pig and hamster following incubation *in vitro* and perfusion *in vivo* with unconjugated bile salts. *Gut*, 11, 486-492.
- McCarthy, C. F., Austad, W. I., and Read, A. E. A. (1965). Hypogammaglobulinemia and steatorrhoea. *Amer. J. dig. Dis.*, 10, 945-957.
- Mawas, C., Sors, C., and Bernier, J. J. (1969). Amyloidosis associated with primary agammaglobulinemia, severe diarrhoea and familial hypogammaglobulinemia. *Amer. J. Med.*, 46, 624-634.
- Miles, A. A., and Misra, S. S. (1938). The estimation of the bactericidal power of the blood. *J. Hyg. (Lond.)*, 38, 732-749.
- Percy-Robb, I. W., Jalan, K. N., McManus, J. P. A., and Sircus, W. S. (1971). Bile salt metabolism in ileostomy patients with and without ileal resection. *Clin. Sci.*, in press.
- Rogosa, M., Fitzgerald, R. J., MacKintosh, M. E., and Beaman, A. J. (1958). Improved medium for selective isolation of veillonella. *J. Bact.*, 76, 455-456.
- Rogosa, M., Mitchell, J. A., and Wiseman, R. F. (1951). A selective medium for the isolation and enumeration of oral and fecal lactobacilli. *J. Bact.*, 62, 132-133.
- Rosen, F. S., and Janeway, C. A. (1966). The gamma globulins. III. The antibody deficiency syndromes. *New Engl. J. Med.*, 275, 709-715.
- Sherwood, W. C., Goldstein, F., Haurani, F. I., and Wirts, C. W. (1964). Studies of the small-intestinal bacterial flora and of intestinal absorption in pernicious anemia. *Amer. J. dig. Dis.*, 9, 416-425.
- Tomasi, T. B., Jr. (1969). On the mechanisms of transport and biological significance of antibodies in external secretions. *Arthr. and Rheum.*, 12, 45-50.
- Twomey, J. J., Jordan, P. H., Jr., Laughter, A. H., Meuwissen, H. J., and Good, R. A. (1970). The gastric disorder in immunoglobulin-deficient patients. *Ann. intern. Med.*, 72, 499-504.
- Van Deest, B. W., Fordtran, J. S., Morawski, S. G., and Wilson, J. D. (1968). Bile salt and micellar fat concentration in proximal small bowel contents of ileostomy patients. *J. clin. Invest.*, 47, 1314-1324.
- Willis, A. T., and Hobbs, G. (1959). Some new media for the isolation and identification of clostridia. *J. Path. Bact.*, 77, 511-521.