THE ABILITY OF CHOLESTYRAMINE RESIN AND OTHER ADSORBENTS TO BIND *ESCHERICHIA COLI* ENTEROTOXINS

N. A. MULLAN, M. N. BURGESS, R. J. BYWATER AND P. M. NEWSOME

*Beecham Pharmaceuticals Research Division, Animal Health
Research Centre, Walton Oaks, Tadworth, Surrey KT20 7NT*

NEONATAL diarrhoea in piglets and calves is commonly caused by colonisation of the small intestine by enteropathogenic strains of *Escherichia coli*. The *E. coli* strains concerned secrete a heat-labile enterotoxin (LT) and two heat-stable enterotoxins (STa and STb) (Smith and Gyles, 1970; Burgess *et al.*, 1978). STa is active in neonatal piglets and infant mice, but inactive in weaned pigs; STb is active in weaned pigs and in ligated intestinal loops of rabbits, but inactive in neonatal piglets and infant mice (Burgess *et al.*, 1978). These toxins presumably attach to receptors on the epithelial surface and lead to excessive intestinal secretion, causing diarrhoea and dehydration.

A substance able to compete with the epithelial receptors for attachment of these enterotoxins might be expected to reduce their effect in the gut. Several pharmaceutical products containing adsorbent materials are intended to control diarrhoea by adsorbing by-products of bacterial metabolism (Martin, 1955). Kaolin has long been employed as an adsorbent in diarrhoea remedies although activated attapulgite has superior adsorptive properties (Barr and Arnista, 1957).

There is little firm evidence that these adsorbents are useful in the treatment of diarrhoea of infective origin. The present study was undertaken to evaluate some materials for their effectiveness in reducing the fluid secretion caused by STa, STb or LT.

One compound, cholestyramine, a quaternary ammonium anion-exchange resin with a high molecular weight, was highly effective in binding STa *in vitro*, and STa, STb and LT *in vivo*. This compound was further studied in piglets given either enterotoxin or enteropathogenic *E. coli*.

MATERIALS AND METHODS

Experimental animals were all bred on the premises. Suckling mice were produced by a colony originally stocked with outbred MS1 mice (OLAC, Oxford). Neonatal piglets and weaned pigs were mainly from pure Landrace stock. The neonatal piglets were 2 days old at the time of use.

Organisms. *E. coli* strains P16 (09:K103), P307 (08:K87:K88a,b) and P155 (0149:K91:K88a,c:H10) were obtained from Dr H. Williams Smith, Houghton Poultry

Received 12 Mar. 1979; accepted 29 Mar. 1979.

Research Station, Houghton, Huntingdonshire. These strains secrete STa + STb, STb + LT, and STa + STb + LT respectively.

Preparation of infective inocula. For injection into pig ligated intestinal loops, *E. coli* strain P16 from a Dorset Egg Slope (Oxoid) was inoculated into 10 ml of peptone water and incubated at 37°C for 18 h. For infection of neonatal piglets, 10 ml of peptone water was seeded with 0.1 ml of an 18-h culture of *E. coli* strain P155 and incubated at 37°C for 6 h. The 6-h cultures were diluted to contain 6×10^3 viable organisms/ml before administration.

Preparation of enterotoxin inocula. Culture filtrates containing STa and STb from *E. coli* strain P16 were produced as previously reported (Mullan, Burgess and Newsome, 1978); the defined medium of Mitchell, Tame and Kenworthy (1974) containing 0.5% glucose was used. LT from *E. coli* strain P307 consisted of a cell-suspension lysate (5% w/v) prepared as described by Burgess *et al.* (1979).

Suckling-mouse assay for STa was performed as described by Dean *et al.* (1972), with modifications previously reported (Mullan *et al.*, 1978). The response of suckling mice to culture filtrates containing STa was determined by dosing groups of six animals orally with 0.1 ml of the sample and measuring the ratio of gut weight to remaining body weight (GW:BW) 2 h later. A measure of the fluid secretion (FS) caused by STa was obtained by subtracting from the value obtained the GW:BW ratio of animals inoculated with physiological saline.

The amount of STa given was expressed in terms of units obtained from a dose-response curve previously reported (Mullan *et al.*, 1978). One unit of STa was equivalent to a GW:BW ratio of 0.105. The reduction in FS caused by various adsorbents was calculated from the following formula.

$$\text{Adsorbent effect (\%)} = \left(\frac{\text{FS toxin} - \text{FS adsorbent}}{\text{FS toxin}} \right) \times 100$$

"FS toxin" was a measure of the fluid secretion caused by STa alone, and "FS adsorbent" was a measure of the fluid secretion caused by STa after reaction with adsorbent. A 100% effect indicated no FS.

Assay for STb, LT and viable organisms in ligated intestinal loops of pigs. The technique used was basically that of Smith and Halls (1967). Loops 10 cm in length were prepared in pigs aged 7–9 weeks, and no more than 18 loops were used in each pig. Only one type of enterotoxin material was tested in each pig. Ligated loops were inoculated in pairs. In each pair one loop received either 5 ml of STb + 5 ml of physiological saline (PS), 2 ml of LT + 2 ml of PS, or 0.2 ml of an 18-h culture of strain P16 + 5 ml of PS; this loop served as a positive control for the adjacent loop in which PS was replaced by PS containing 500 mg of cholestyramine. Pigs were killed 18 h later and the inoculated loops were removed. The fluid content (ml) and length (cm) of each loop were measured and the volume-to-length ratio was calculated. The response of each treated loop was compared with the response of the positive control loop immediately proximal to it.

Piglet oral-dosing assay for STa. The method of Kohler (1968) was employed. Animals were dosed orally with 25 ml of a filtrate containing approximately 400 units of STa and observed for diarrhoea on a 0–3 scale every hour for 7 h; they were recorded as diarrhoeic if they produced fluid faeces for at least 1 h. When cholestyramine was used it was mixed with the STa filtrate before administration.

Infection assay in neonatal piglets. Colostrum-deprived piglets were inoculated orally with 5 ml of a suspension of *E. coli* strain P155 containing 6×10^3 organisms/ml. Animals were then returned to the sow and 24–36 h later, when they were scouring, treatment was begun with either cholestyramine resin, amoxycillin, or both. The consistency of the faeces was scored twice daily on a 0–3 scale for 5 days. Rectal swabs were collected at the time of treatment and from survivors at the end of the experiment. By means of antiserum to *E. coli* strain P155, the strain administered was identified in rectal swabs from a representative number of animals. Pigs were weighed at the time of treatment and survivors were weighed at the end of the experiment.

Reaction of adsorbents with STa in vitro. Equal volumes of culture filtrate containing STa and aqueous adsorbent suspensions were shaken at ambient temperature (22°C) for 30 min. The supernates were removed and tested for enterotoxin activity in suckling mice. When the effect of

sow's milk on the efficacy of the adsorbent was studied, the adsorbent was suspended in an equal volume of milk.

Recovery of cholestyramine from piglets. In one experiment, after scouring piglets had been treated for 3 days, the animals were killed and the contents of the stomach, small intestine and large intestine removed and washed. Portions were then either assayed for adsorbent activity, or treated with 1N sodium hydroxide and boiled and dried to constant weight to determine the amount of sodium hydroxide-insoluble material (cholestyramine).

Antibiotic therapy. Amoxycillin in an oral dose formulation (Clamoxyl, Beecham Animal Health, Brentford) was used in experiments concerned with antibiotic therapy of scouring piglets.

Adsorbents. Pharmasorb Regular and Pharmasorb Colloidal (attapulgis clay products) were obtained from Lawrence Industries, Mitcham, Surrey. The Bentone gellants were obtained from Steeley Mineral Products, Worksop, Nottinghamshire; these gellants were formed by replacing the inorganic cations of a clay mineral lattice with organic cations.

Kaolin, light B.P. was obtained from Evans Medical Ltd, Liverpool. Cholestyramine, a commercial preparation of the anion-exchange resin AG-1-X2, was marketed by Merck, Sharpe and Dohme Ltd under the trade name of Cuemid. Other ion-exchange resins were obtained from Bio-Rad Labs., Watford, Herts.

Statistics. Analysis of variance, Student's *t* test (paired and unpaired) and linear correlation were used.

RESULTS

STa adsorption in vitro

Table I shows the adsorbent effect of various substances. After allowing each substance to react with STa *in vitro*, any residual enterotoxigenic effect was assessed in suckling mice. Neither kaolin nor attapulgit removed any of the STa activity from sterile culture filtrates of *E. coli* strain P16. Three of four modified bentone materials bound significant amounts of STa. The most effective adsorbents were the strong anion-exchange resin, AG-1-X2, and bentone 27. Both removed virtually all of the STa activity. The strong cation-exchange resin, AG-50W-X4, and the non-ionic macroreticular XAD-2 resin also bound significant amounts of STa.

TABLE I
Binding of STa to a selection of adsorbents in vitro

Adsorbent	Amount (μ g) of adsorbent mixed with each STa unit before dosing mice	Adsorbent effect (%) (mean $\pm$ standard error of the mean [SEM])
Kaolin	2500*	0 $\pm$ 26
Non-colloidal attapulgit	2500*	0 $\pm$ 19
Colloidal attapulgit	2500*	0 $\pm$ 14
Bentone 18	625†	34 $\pm$ 13‡
Bentone 27	625†	98 $\pm$ 6‡
Bentone 34	625†	25 $\pm$ 4
Bentone 38	625†	33 $\pm$ 2‡
AG-50W-X4	625†	39 $\pm$ 2‡
DEAE-cellulose	625†	18 $\pm$ 4
XAD-2	625†	37 $\pm$ 8‡
AG-1-X2	625†	98 $\pm$ 5‡

* Each suckling mouse received 2 units of STa (equivalent to a GW:BW ratio of 0.117 $\pm$ 0.005).

† Each suckling mouse received 4 units of STa (equivalent to a GW:BW ratio of 0.135 $\pm$ 0.007).

‡ Significant reduction from control response as determined by Student's *t* test ($p = < 0.05 - < 0.001$).

STa adsorption in the alimentary tract of suckling mice

The two most effective adsorbents, bentone 27 and cholestyramine resin (AG-1-X2), were evaluated *in vivo*. Each was administered to suckling mice at different times in relation to STa challenge. Table II shows that only cholestyramine resin bound significant amounts of STa. It was effective in reducing fluid secretion when given 40 min. before, and up to 30 min. after, STa challenge.

TABLE II

Administration of selected adsorbents to suckling mice at various times in relation to a dose of STa

Adsorbent	Concentration of adsorbent ($\mu\text{g}/\text{STa unit}$)	Time (minutes before or after dosing with STa) at which mice treated with adsorbent	Adsorbent effect (%) (mean $\pm$ SEM)
Cholestyramine	720*	-40	95 $\pm$ 3†
	720*	0	89 $\pm$ 4†
	720*	+10	62 $\pm$ 7†
	720*	+20	48 $\pm$ 11†
	720*	+30	37 $\pm$ 15
Bentone 27	1100†	-40	19 $\pm$ 8
	1100†	0	0 $\pm$ 5
	1100†	+10	0 $\pm$ 2

* Each animal received 3.5 units of STa (equivalent to a GW:BW ratio of 0.132 ± 0.005).

† Each animal received 2.5 units of STa (equivalent to a GW:BW ratio of 0.124 ± 0.003).

‡ Significant reduction from control response as determined by Student's *t* test ($p < 0.05-0.001$).

STa adsorption by graded concentrations of cholestyramine resin in vitro

Twofold dilutions of cholestyramine suspension were allowed to react with equal volumes of culture filtrate containing STa. The supernates were then tested in suckling mice. The figure shows that the resin produced a reduction in fluid secretion that showed a significant linear correlation with the log concentration of cholestyramine ($r = 0.999$, $p < 0.001$).

Adsorptive effect of cholestyramine resin in pig ligated intestinal loops treated with STb, LT or viable E. coli

The results are shown in table III. The cholestyramine resin significantly reduced the secretion caused by STb, LT or viable *E. coli*.

STa adsorption in the alimentary tract of piglets

Cholestyramine resin was mixed with STa and administered to piglets aged 2 days. The piglets were observed for diarrhoea and compared with litter mates dosed with STa alone. In five of seven animals dosed with the mixture of resin and STa, no diarrhoea was noted and the mean diarrhoea score was significantly reduced; each of seven control piglets had diarrhoea (table IV).

FIGURE.—Capacity of cholestyramine resin to adsorb STa. ●—●=Cholestyramine suspended in water; ○—○=cholestyramine suspended in sow's milk. Each point represents the mean $\pm$ S.E.M.

TABLE III

Fluid secretion in pig ligated intestinal loops treated with either STb, LT, or *E. coli* strain P16, with and without cholestyramine resin

Loop treated with	Volume: length ratio (mean $\pm$ SEM) of		Significance* (p)
	control loops	resin-treated loops	
STb	1.65 $\pm$ 0.36 (11)	0.44 $\pm$ 0.16 (11)	<0.005
LT	4.30 $\pm$ 1.02 (12)	1.54 $\pm$ 0.55 (12)	<0.001
<i>E. coli</i>	2.24 $\pm$ 0.31 (17)	1.29 $\pm$ 0.13 (17)	<0.005

* Paired *t* test.

Figures in parenthesis indicate number of loops inoculated.

TABLE IV
Effect of cholestyramine given orally with STa to piglets

Treatment	Number of animals with diarrhoea/total number	Diarrhoea score* (mean $\pm$ SEM)
STa (400 units)	7/7	2.0 $\pm$ 0.4
STa (400 units) + 2 g cholestyramine	2/7	0.5 $\pm$ 0.2†

* The diarrhoea of each animal was assessed on a 0-3 scale every hour for 7 h and a mean scour score for the group determined from the ratio of summed diarrhoeal scores to total number of observations.

† Significant reduction as determined by Student's *t* test ($p < 0.01$).

Effect of cholestyramine alone on E. coli-induced diarrhoea in piglets

After the commencement of diarrhoea, pigs were given either 1 g or 0.5 g of resin twice daily as shown in table V. Comparison with control piglets showed that neither treatment significantly affected the duration of diarrhoea. Moreover, treated animals gained weight more slowly than untreated controls ($p < 0.01$).

TABLE V
Effect of cholestyramine resin on piglets infected with E. coli strain P155

Treatment	Number of piglets	Duration of diarrhoea (days) in survivors (mean $\pm$ SEM)	Weight gain (%) (mean $\pm$ SEM)	Mortality (%)
Controls	9	2.8 $\pm$ 0.6	88 $\pm$ 13	11
Cholestyramine 1 g, twice daily	11	3.2 $\pm$ 0.3	37 $\pm$ 9*	9
Cholestyramine 0.5 g, twice daily	9	3.7 $\pm$ 0.6	45 $\pm$ 6	22

* Significantly less than controls ($p < 0.01$) by analysis of variance.

TABLE VI
Effect of cholestyramine resin and antibiotic therapy in piglets infected with E. coli strain P155

Treatment*	Number of piglets	Duration of diarrhoea (days) in survivors (mean $\pm$ SEM)	Diarrhoea score† in survivors (mean $\pm$ SEM)	Weight gain (%) (mean $\pm$ SEM)	Mortality (%)
Controls	16	5.0 $\pm$ 0.5	1.0 $\pm$ 0.2	93 $\pm$ 7	13
Amoxycillin	16	3.8 $\pm$ 0.6	0.6 $\pm$ 0.1	101 $\pm$ 11	0
Amoxycillin + cholestyramine	16	3.9 $\pm$ 0.7	0.8 $\pm$ 0.2	56 $\pm$ 10‡	13

* The resin was given in a dose of 1 g in 15 ml water twice daily; amoxycillin was given in a dose of 40 mg twice daily.

† See footnote to table IV.

‡ Significantly less than controls ($p < 0.01$) by analysis of variance.

Effect of cholestyramine and amoxycillin on E. coli-induced diarrhoea in piglets

After the commencement of diarrhoea, pigs were given either cholestyramine resin (1 g, twice daily) and amoxycillin (40 mg, twice daily), or amoxycillin alone (40 mg, twice daily). Table VI shows that amoxycillin reduced the amount and duration of diarrhoea. Animals receiving cholestyramine resin as an adjunct to antibiotic therapy showed no additional advantage. Piglets that received both cholestyramine and amoxycillin gained weight more slowly than amoxycillin-treated piglets or untreated controls ($p < 0.01$).

Intestinal distribution and adsorptive capacity of cholestyramine in piglets with E. coli-induced diarrhoea

The failure of cholestyramine resin to produce a curative effect might have been caused by (1) retention in the stomach as a result of stasis, or (2) insufficient adsorptive capacity of the resin in the intestine.

Scouring piglets were given amoxycillin (40 mg, twice daily) and cholestyramine (1 g, twice daily) for 3 days; the amount of resin in the intestines was then determined. Table VII shows that sodium hydroxide-insoluble material (cholestyramine) was not present in the small intestine, but significant amounts were present in the stomach and large intestine.

TABLE VII

Sodium hydroxide-insoluble material (cholestyramine resin) present in intestines of cholestyramine-treated diarrhoeic piglets, and its capacity to bind STa

Samples removed from	Amount of resin recovered* (mean $\pm$ SEM)	Residual adsorbent effect (%)†
stomach	1.23 $\pm$ 0.16‡	0
small intestine	0.00 $\pm$ 0.03	...
large intestine	0.64 $\pm$ 0.09‡	1

* Adjusted to take account of the values obtained in animals that did not receive resin.

† Compared with that of a similar quantity of fresh resin mixed with an equal quantity of STa (1 unit).

‡ Significant amounts of resin present by Student's *t* test ($p = 0.001$).

Portions of the gastric and intestinal samples were mixed with STa and centrifuged. The supernates were then administered to infant mice to determine the percentage adsorbent activity remaining. Table VII shows that the samples from the resin-treated animals had no residual adsorbent effect.

Interference by milk with the adsorption of STa by cholestyramine

The figure shows that sow's milk significantly interfered with the capacity of cholestyramine to bind STa *in vitro* ($p < 0.001$, paired *t* test).

DISCUSSION

The use of colloidal clays in the treatment of gastrointestinal disturbances has been based on the belief that adsorption of bacteria or bacterial toxins or both occurs (Martin, 1955); activated attapulgite was reported to be superior to kaolin (Barr and Arnista, 1957). This belief is supported by little evidence, apart from in-vitro observations.

Nalin and Cash (1970), in a study made with Thiry-Vella loops in the intestine of dogs, found that kaolin, put into the loops before or with cholera toxin, protected the loops against the toxin; kaolin had no effect on the duration or volume of diarrhoea in cholera patients.

Gyles and Zigler (1978) reported that 10% colloidal attapulgite and 25% Pepto-Bismol (a commercial preparation containing bismuth subsalicylate) reduced the accumulation of fluid in pig ligated intestinal segments infected with enteropathogenic *E. coli*. Drucker *et al.* (1977) found that colloidal attapulgite reduced the fluid accumulation caused by LT and cholera toxin in rabbit ligated intestinal segments, but only when the toxin and attapulgite (10%) were incubated before injection. Unlike Gyles and Zigler (1978) they were unable to show that attapulgite had any effect in loops inoculated with viable *E. coli* producing LT.

Ericsson *et al.* (1977) showed that pretreatment of rabbit ligated intestinal loops with Pepto-Bismol significantly reduced the fluid accumulation caused by crude *E. coli* and cholera toxins; the preparation had no effect when given after toxin administration. The active ingredient, bismuth subsalicylate, was further evaluated by DuPont *et al.* (1977), who showed it to be effective in the treatment of diarrhoea.

In the present study neither kaolin nor activated attapulgite adsorbed STa secreted by strains of *E. coli* enteropathogenic for piglets. Kohler (1971) noted that neither ST—as assayed in neonatal piglets, hence STa—nor LT were adsorbed to kaolin; he also found that enterotoxin activity (STa) was adsorbed by a cation- and an anion-exchange resin. These observations have been confirmed and extended in this report. In addition we have noted that STa was adsorbed by the macroreticular resin XAD-2, a non-ionic resin designed for the adsorption of water-soluble organic substances. This suggests that STa is lipophilic and amphoteric. We found that the most effective adsorbent for STa was the strong anion-exchange resin, cholestyramine; it also showed significant activity against STb, LT and *E. coli* in pig ligated intestinal loops.

Berant, Wagner and Cohen (1976) have reported cholestyramine to be effective in infantile diarrhoea of infectious origin, the diarrhoea ceasing within 1–3 days of treatment. Cholestyramine appeared to alleviate the symptoms in antibiotic-associated colitis associated with lincomycin and clindamycin therapy (Burbige and Milligan, 1975). This disease is now believed to be caused by toxin-producing strains of *Clostridium difficile* (Bartlett *et al.*, 1978). Chang, Onderdonk and Bartlett (1978) showed that cholestyramine adsorbed the toxin produced by such strains; this might explain the remission of symptoms noted by Burbige and Milligan (1975). Chang *et al.* (1978) mentioned that

E. coli enterotoxins were not adsorbed by cholestyramine, an observation contrary to our own.

In this study cholestyramine was found to bind STa *in vitro* and STa, STb and LT *in vivo*. In infant mice the fluid secretion that rapidly followed challenge with STa was significantly reduced. Cholestyramine also significantly reduced the fluid secretion in pig ligated intestinal loops after exposure to either STb, or LT, or viable organisms. When cholestyramine was mixed with STa and administered orally to piglets aged 2 days, diarrhoea was reduced.

However, the crucial test was to examine the effectiveness of cholestyramine in the treatment of diarrhoeic piglets after infection with viable enteropathogenic *E. coli*. The results were disappointing. No beneficial effect was noted when doses of 1 g or 0.5 g of resin twice daily, either alone or in conjunction with an antibiotic, were administered to diarrhoeic piglets. Indeed, resin-treated animals gained weight significantly more slowly than controls. Resin could still be recovered from the stomach and large intestine at necropsy, but resin could not be recovered from the small intestine where the toxins are believed to exert their biological effect. The capacity of the recovered material to react with STa after passage through the stomach was significantly reduced, probably due to saturation with some constituents of the sow's milk. The reduced ability of cholestyramine to adsorb STa in the presence of sow's milk was demonstrated *in vitro*. The binding of essential nutrients or bile salts to the resin might explain the effect on weight gain in these animals.

These results do not encourage the use of cholestyramine in the treatment of diarrhoea in unweaned piglets. Its possible value in weaned piglets or in other species remains to be determined.

SUMMARY

Several adsorbent materials were evaluated for their ability to bind *Escherichia coli* enterotoxins. Cholestyramine, a strong anion-exchange resin, bound the heat-labile and the heat-stable types of enterotoxin and reduced significantly their effects in some animal models. However, its efficacy in the treatment of diarrhoeic piglets appeared to be adversely affected by the presence of milk in the alimentary tract.

We thank C. Cowley, P. Diprose and P. Whittington for excellent technical assistance, and Mrs E. T. Gaskin for excellent secretarial services.

REFERENCES

- BARR, M. AND ARNIST, E. J. 1957. Adsorption studies on clays III. The adsorption of diphtheria toxin by activated attapulgit, halloysite and kaolin. *J. Am. pharm. Ass.*, **46**, 493.
- BARTLETT, J. G., CHANG, T. W., GURWITH, M., GORBACH, S. L. AND ONDERDONK, A. B. 1978. Antibiotic-associated pseudomembranous colitis due to toxin producing clostridia. *New Eng. J. Med.*, **298**, 531.

- BERANT, M., WAGNER Y. AND COHEN, N. 1976. Cholestyramine in the management of infantile diarrhoea. *J. Pediat.*, **88**, 153.
- BURBIGE, E. J. AND MILLIGAN, F. D. 1975. Pseudomembranous colitis. Association with antibiotics and therapy with cholestyramine. *J. Am. med. Ass.*, **231**, 1157.
- BURGESS, M. N., BYWATER, R. J., COWLEY, C. M., MULLAN, N. A. AND NEWSOME, P. M. 1978. Biological evaluation of a methanol-soluble, heat-stable *E. coli* enterotoxin in infant mice, pigs, rabbits and calves. *Infect. Immun.*, **21**, 526.
- BURGESS, M. N., COWLEY, C. M., MELLING, J., MULLAN, N. A. AND NEWSOME, P. M. 1979. Assay of the heat-labile enterotoxin of *Escherichia coli* in infant rabbits. *J. med. Microbiol.*, **12**, 291.
- CHANG, T. W., ONDERDONK, A. B. AND BARTLETT, J. G. 1978. Anion exchange resins in antibiotic-associated colitis. *Lancet*, **2**, 258.
- DEAN, A. G., CHING, Y. C., WILLIAMS, R. G. AND HARDEN, L. B. 1972. Test for *Escherichia coli* enterotoxin using infant mice: application in a study of diarrhea in children in Honolulu. *J. infect. Dis.*, **125**, 407.
- DRUCKER, M. M., OGRA, P. L., GOLDHAR, J. AND NETER, E. 1977. The effect of attapulgit and charcoal on enterotoxicity of *Vibrio cholerae* and *Escherichia coli* enterotoxins in rabbits. *Infection*, **5**, 211.
- DUPONT, H. L., SULLIVAN, P., PICKERING, L. K., HAYNES, G. AND ACKERMAN, M. S. 1977. Symptomatic treatment of diarrhoea with bismuth subsalicylate among students attending a Mexican University. *Gastroenterology*, **73**, 715.
- ERICSSON, C. D., EVANS, D. G., DUPONT, H. L., EVANS, D. J. AND PICKERING, L. K. 1977. Bismuth subsalicylate inhibits activity of crude toxins of *Escherichia coli* and *Vibrio cholerae*. *J. infect. Dis.*, **136**, 693.
- GYLES, C. L. AND ZIGLER, M. 1978. The effect of adsorbent and anti-inflammatory drugs on secretion in ligated segments of pig intestine infected with *Escherichia coli*. *Can. J. comp. Med.*, **42**, 260.
- KOHLER, E. M. 1968. Enterotoxic activity of filtrates of *Escherichia coli* in young pigs. *Am. J. vet. Res.*, **29**, 2263.
- KOHLER, E. M. 1971. Enterotoxic activity of whole cell lysates of *E. coli* in young pigs. *Am. J. vet. Res.*, **32**, 731.
- MARTIN, G. J. 1955. Ion-exchange and adsorption agents in medicine: the concept of intestinal bionomics, *London*.
- MITCHELL, I. DE G., TAME, M. J. AND KENWORTHY, R. 1974. Conditions for the production of *Escherichia coli* enterotoxin in a defined medium. *J. med. Microbiol.*, **7**, 395.
- MULLAN, N. A., BURGESS, M. N. AND NEWSOME, P. M. 1978. Characterisation of a partially purified, methanol-soluble, heat-stable *Escherichia coli* enterotoxin in infant mice. *Infect. Immun.*, **19**, 779.
- NALIN, D. R. AND CASH, R. A. 1970. Kaolin and cholera. *J. Pakistan med. Ass.*, **20**, 177.
- SMITH, H. W. AND GYLES, C. L. 1970. The relationship between two apparently different enterotoxins produced by enteropathogenic strains of *E. coli* of porcine origin. *J. med. Microbiol.*, **3**, 403.
- SMITH, H. W. AND HALLS, S. 1967. Observations by the ligated intestinal segment and oral inoculation methods on *E. coli* infections in pigs, calves, lambs and rabbits. *J. Path. Bact.*, **93**, 499.