

## ENTEROPATHOGENICITY OF *PLESIOMONAS SHIGELLOIDES*

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*PLESIOMONAS SHIGELLOIDES* has been implicated as an aetiological agent in sporadic cases and outbreaks of diarrhoea, and in food poisoning, in various parts of the world. (Schmid, Velaudapillai and Niles, 1954; Osada and Shibata, 1956; Vandepitte *et al.*, 1957; Ueda, Yamasaki and Hori, 1963; Aldova, Rakovsky and Chovanova, 1966; Geizer, Kopecky and Aldova, 1966; Hori *et al.*, 1966; Cooper and Brown, 1968; Paučova and Fukalova, 1968; von Graevenitz and Mensch, 1968; Winton, 1968; Sakazaki *et al.*, 1971; Chatterjee and Neogy, 1972; Sanyal *et al.*, 1972*a* and *b*; Zajc-Satler, Dragas and Kumelj, 1972; Bhat, Shantha Kumari and Rajan, 1974; Vandepitte, Makulu and Gatti, 1974; Sanyal, Singh and Sen, 1975; Jandl and Linke, 1976; Tsukamoto *et al.*, 1978). *P. shigelloides* was isolated in pure culture from stools of patients with diarrhoea of otherwise unexplained origin, and more often from patients than from symptomless persons. Experimental evidence has not, however, been reported in support of its enteropathogenicity, except our preliminary communication on the activity of a few strains in the rabbit ileal-loop model (Saraswathi, Sharma and Sanyal, 1978). The present investigation was undertaken to examine its enterotoxicity and invasiveness.

### MATERIALS AND METHODS

*Strains of Plesiomonas.* Thirteen strains of *P. shigelloides* were included in the study; seven were our laboratory isolates, three each from healthy individuals and patients with diarrhoea, and one from water. Three strains each were obtained from the Institut Pasteur, Paris (strain nos. 10.77, 16.70 and 53.76), and from the National Collection of Type Cultures, London (NCTC nos. 10360, 10363 and 10364). The strains were maintained in peptone-agar stab cultures at room temperature.

*Ileal-loop test.* This was done with live cells and culture filtrates in ileal loops of adult rabbits as described by Sanyal *et al.* (1975). The inoculum was 1 ml of a 1 in 10<sup>3</sup> dilution in isotonic saline of a live 3–4-h culture of the organism in peptone water, or 1 ml of a cell-free filtrate. Live cells or culture filtrate of the toxigenic *Vibrio cholerae* strain 569B served as a positive control, and isotonic saline or Trypticase Soy Broth (TSB) (Baltimore Biological Laboratories) with 1% (w/v) inositol was the negative control. Each strain was tested in three rabbits. Up to eight loops were prepared in each animal.

*Multiplication of bacteria in the ileal loops* was measured as described by Sanyal *et al.* (1975) with two strains, one from a patient with diarrhoea and one from a healthy individual. A 3–4-h culture in peptone water was diluted 1 in 10<sup>3</sup>–10<sup>4</sup> in isotonic saline and 1 ml was inoculated into

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Received 20 Aug. 1979; accepted 29 Nov. 1979.

each loop. For determination of the minimal ileal-loop reacting dose of live cells, different dilutions of the culture were made in isotonic saline and 1-ml samples were inoculated into separate loops. Each experiment was done in triplicate, and the rabbits were killed after 6 h.

*Passage of bacteria in ileal loops.* The method described by Annapurna and Sanyal (1977) was used, with three of the strains that had caused negligible amounts of fluid accumulation in three tests. A 1-ml sample of a 3-4-h peptone-water culture, diluted 1 in 10 in isotonic saline, was injected into a loop. After 6 h, the contents of the loop were removed aseptically and cultured on nutrient agar. Six colonies were picked, cultured in peptone water and inoculated into another loop, and the process was repeated until the strain gave a positive reaction.

*Preparation of culture filtrates* was by the method of Annapurna and Sanyal (1977), but with the substitution of a different culture medium; a 2-ml sample of a 3-4-h peptone-water culture was inoculated into 50 ml of TSB containing 1% inositol in place of brain-heart infusion broth in a 250-ml conical flask. This was incubated at 37°C in a shaking water bath (120 oscillations/min.). The culture was centrifuged at 4°C for 30 min. at 22 000 g and the supernate was filtered through a Millipore filter of 0.45 µm average pore diameter and stored at 4°C.

*Determination of minimum ileal-loop reacting dose of culture filtrate.* Filtrates (0.1-2.0 ml) were inoculated into different loops in each rabbit. The animals were killed after 6 h and the accumulation of fluid per cm of gut was measured. The smallest amount of filtrate that caused maximal accumulation of fluid was regarded as the minimal reacting dose. All experiments were done in triplicate.

*Time course of fluid accumulation in ileal loops.* The minimal reacting dose (0.25 ml of filtrate as described in *Results*) of five strains of *P. shigelloides* was inoculated into loops of different rabbits. Culture filtrate of *V. cholerae* strain 569B was also inoculated into each rabbit. The animals were killed at 1, 2, 4, 6, 8 and 18 h and the accumulation of fluid was noted. Each experiment was done in triplicate.

*Effect of temperature on culture filtrates.* Filtrates were heated at different temperatures for various lengths of time in a water bath or an autoclave and 1-ml samples were inoculated into separate loops. The animals were killed after 6 h and the reactions were noted. Each culture filtrate was tested in three rabbits.

*Suckling-mouse assay* was by the method of Dean *et al.* (1972). Filtrates (0.1-ml volumes) prepared from three strains, two from patients with diarrhoea and one from a healthy individual, were inoculated into the stomach of each of the 2-day-old suckling mice which were arranged into groups of four each, after separation from the mothers. The mice in the control groups each received TSB containing 1% inositol. The animals were killed after 4 h and the ratio of gut weight to body weight was determined. Each experiment was done three times.

*Tissue-culture methods* were those of Donta, Moon and Whip (1974) and Guerrant *et al.* (1974). Monolayer cultures of Chinese hamster ovarian cells (CHO) and mouse adrenal cells (Y<sub>1</sub>) in disposable tissue-culture plates were inoculated with 0.2-ml samples of cell-free culture filtrates of *P. shigelloides* and incubated at 37°C in a humidified atmosphere of 95% air and 5% CO<sub>2</sub>. The cultures were examined for morphological changes after 18-24 h. The filtrates were prepared by growing *P. shigelloides* in TSB for 24 h in a shaking water bath (120 oscillations/min.) at 37°C, centrifuging the culture and filtering the supernate through Millipore membranes (0.2 µm average pore diameter). The positive control was Craig's cholera toxin (Schwartz/Mann), and TSB served as the negative control.

*Sereny test.* This was performed according to Sereny (1957) whereby 0.1-ml samples of a suspension of the test strain, grown on nutrient agar containing 10<sup>5</sup>-10<sup>7</sup> colony-forming units (c.f.u.) were instilled into the conjunctival sacs of rabbits or guinea-pigs. The animals were examined during 72 h for evidence of keratoconjunctivitis. A known positive strain of *Shigella flexneri* (strain 543) isolated in our laboratory was included for comparison. Each strain was tested thrice.

*HeLa-cell culture* was as described by LaBrec *et al.* (1964). Monolayers of HeLa cells were infected by simple addition of bacteria, which had been washed in Hanks's balanced salt solution to give a final concentration of *c.* 3 × 10<sup>7</sup> c.f.u./ml and incubated. The cover-slip samples were taken out at 1, 3, 5, 7 and 24 h after inoculation, rinsed thrice in phosphate-buffered saline, fixed with methanol:acetic acid mixture (in the ratio of 3:1) and stained with Giemsa's stain. The positive control was *S. flexneri* strain 543.

## RESULTS

## Ileal-loop experiments

*Live cultures*

*Activity of strains.* All 13 strains of *P. shigelloides* caused accumulation of fluid (0.8–2.0 ml/cm of gut in ileal loops, comparable with that caused by the toxigenic *V. cholerae* strain 569B (table I). The amount of fluid that accumulated, however, varied from strain to strain and from loop to loop. Several of the strains required one or two passages through ileal loops before they gave a positive result.

*Multiplication in ileal loops and the effect of inoculum size.* Two strains, one from a patient with diarrhoea and one from a healthy individual, multiplied in

TABLE I

*Accumulation of fluid in ileal loops, caused by live cultures of Plesiomonas shigelloides*

| Strain no.  | Source             | Volume of fluid in loop (range, ml/cm of gut) |
|---|--------------------|---|
| 292   | Diarrhoeal patient | 1.0–2.0                                       |
| 3237  | Diarrhoeal patient | 1.0–1.8                                       |
| 3186  | Diarrhoeal patient | 1.1–1.8                                       |
| 10.77   | Diarrhoeal patient | 0.8–1.6                                       |
| 53.76   | Diarrhoeal patient | 1.0–1.8                                       |
| 16.70   | Diarrhoeal patient | 1.2–2.0                                       |
| 10360   | Diarrhoeal patient | 0.9–2.0                                       |
| 10363   | Diarrhoeal patient | 1.0–1.7                                       |
| 10364   | Diarrhoeal patient | 1.0–1.8                                       |
| 5879  | Healthy individual | 0.8–1.6                                       |
| 7123  | Healthy individual | 1.0–1.8                                       |
| V-19-4  | Healthy individual | 0.9–2.0                                       |
| 36.79   | Well water         | 1.0–1.8                                       |
| Positive control<br>( <i>V. cholerae</i> strain 569B) |                    | 0.8–1.8                                       |
| Negative control<br>(isotonic saline)                 |                    | 0   |

loops by nearly  $10^4$ -fold within 6 h (table II). Only slight, or no, accumulation of fluid was observed when  $10^1$ – $10^3$  c.f.u. were used as the inoculum; maximum accumulation was obtained with *c.*  $10^4$ – $10^5$  c.f.u. There was no increase of fluid accumulation when the inoculum was increased to  $10^6$  c.f.u. (table II).

*Culture filtrates*

*Activity of strains.* The filtrates prepared from all the 13 strains gave a positive reaction in the rabbit ileal loop. Fluid accumulation varied from strain to strain; the amounts of fluid were similar to those obtained in tests with live cultures. There were no significant differences between strains from cases

TABLE II

Relationship between multiplication and production of fluid by strains of *Plesiomonas shigelloides* in ileal loops

| Strain no.<br>(and source)       | Viable count<br>(c.f.u./ml) of |                          | Volume of fluid<br>in loop (range,<br>c.f.u./cm of gut) |
|----------------------------------|--------------------------------|--------------------------|---|
|                                  | inoculum                       | loop fluid<br>after 6 h* |   |
| 10.77<br>(diarrhoeal<br>patient) | $6.1 \times 10^1$              | $3.0 \times 10^4$        | 0   |
|                                  | $6.2 \times 10^2$              | $2.8 \times 10^6$        | 0   |
|                                  | $5.8 \times 10^3$              | $3.8 \times 10^7$        | 0.5-0.7   |
|                                  | $6.4 \times 10^4$              | $3.6 \times 10^8$        | 0.8-1.2   |
|                                  | $1.3 \times 10^5$              | $2.2 \times 10^9$        | 1.2-2.0   |
|                                  | $2.4 \times 10^6$              | $8.2 \times 10^9$        | 1.0-2.0   |
| 5879<br>(healthy<br>individual)  | $6.5 \times 10^1$              | $2.0 \times 10^4$        | 0   |
|                                  | $6.0 \times 10^2$              | $2.5 \times 10^5$        | 0   |
|                                  | $6.4 \times 10^3$              | $2.8 \times 10^7$        | 0.6-0.9   |
|                                  | $6.8 \times 10^4$              | $1.4 \times 10^8$        | 0.8-1.1   |
|                                  | $5.6 \times 10^5$              | $2.3 \times 10^9$        | 1.2-1.8   |
|                                  | $7.0 \times 10^6$              | $5.2 \times 10^9$        | 1.2-1.8   |

\* Mean from three loops.

of diarrhoea and those from healthy individuals; all the strains caused fluid accumulation comparable to that of culture filtrates from *V. cholerae* strain 569B.

*Minimal reacting dose.* Inocula of 0.25-ml samples of filtrates prepared from two strains, one from a patient with diarrhoea and one from a healthy individual, produced accumulation of fluid similar to that produced by large inocula (up to 2.0 ml) of these filtrates, and by 1.0 ml of filtrate from *V. cholerae* strain 569B. There was no accumulation of fluid with inocula of 0.1 ml.

*Effect of temperature.* Culture filtrates prepared from five strains did not cause accumulation of fluid in ligated loops after heating at 100°C or 121°C for 30 min. Inhibition of fluid outpouring was not, however, noted when the filtrates had been treated at 65°C for 30 min., or at 100°C or 121°C for 10 min.

*Time course of fluid accumulation.* Fluid accumulation in the range 0.6-1.0 ml/cm of gut was observed with culture filtrates of *P. shigelloides* during the first 1-2 h whereas there was very little, or no, fluid accumulation during that period with filtrates from *V. cholerae* strain 569B. There was a sharp increase in the amount of fluid accumulated (1.2-1.8 ml/cm) at 4 h with no further appreciable change up to 8 h. An increase of 0.2-0.5 ml/cm of gut was noted at 18 h. In the case of *V. cholerae* strain 569B, a maximum outpouring of fluid was noted at 6-8 h (1.5 ml/cm) which increased to 1.7-2.0 ml/cm at 18 h.

## Other experiments

### *CHO- and Y<sub>1</sub>-cell cultures*

All the 13 culture filtrates caused dislodgement of the cells from the plate surfaces, or lysis. Steroidogenic activity was not noted.

*Suckling-mouse assay*

The activities of filtrates from three strains are shown in table III. All the strains tested were positive.

*Sereny's test*

Keratoconjunctivitis was not observed with any of the 13 strains tested, while the control *S. flexneri* strain 543 always gave a positive reaction.

*HeLa-cell cultures*

With most of the strains no change was apparent at 1, 3, 5, 7 and 24 h in cell cultures, and bacteria were not seen inside the epithelial cells. In some cases, however, degenerative changes were noted at 24 h. Bacteria were always seen inside epithelial cells in the control plate containing *S. flexneri* strain 543.

TABLE III

*Suckling-mouse assay of culture filtrates of Plesiomonas shigelloides*

| Strain no.       | Gut weight/body weight (mean* $\pm$ SD) |
|------------------|---|
| V-19-4           | 0.101 $\pm$ 0.005                       |
| 10.77            | 0.102 $\pm$ 0.010                       |
| 292              | 0.102 $\pm$ 0.007                       |
| Negative control | 0.059 $\pm$ 0.007                       |

\* From three groups, each of four mice.

## DISCUSSION

Fluid outpouring into ileal loops is considered to be due to liberation of endotoxic substances by the bacteria during multiplication in the intestine. This has been shown with *V. cholerae* (De and Chatterje, 1953), enterotoxic *Escherichia coli* (De, Battacharyya and Sarkar, 1956), *Clostridium perfringens* (Duncan and Strong, 1969), *V. parahaemolyticus* (Sakazaki *et al.*, personal communication) and *Aeromonas hydrophila* (Annapurna and Sanyal, 1977). Results of the present study with 13 strains of *P. shigelloides* clearly indicate the enterotoxigenicity of the organisms irrespective of their source and country of origin. All the strains caused accumulation of fluid comparable to that from the toxigenic *V. cholerae* strain 569B. Differences in accumulation of fluid between strains and between individual ileal loops inoculated with a single strain were probably due to quantitative variation in the release of toxin. Fluid accumulation was usually greater in New Zealand and Belgian rabbits than in those available to us. Three strains gave negative reactions on repeated tests and later caused accumulation of fluid after only one or two passages in ileal loops—an indication of potential enterotoxigenicity. The initial cultures

were probably mixed populations containing a small proportion of toxigenic members and these have probably increased in proportion during passage in the loops (Annapurna and Sanyal, 1977); or the toxin gene in the initial culture was in a repressed form that became expressed during the passages (Singh and Sanyal, 1978). These phenomena might explain why we could not demonstrate enterotoxicity in *P. shigelloides* in earlier experiments (Sanyal *et al.*, 1975).

We observed that although small amounts of fluid accumulated in the loops after inocula of about  $10^3$  c.f.u.,  $10^4$ – $10^5$  c.f.u. were necessary to cause a maximal reaction. Accumulation of fluid was not noted when smaller inocula of  $10^1$ – $10^2$  were used, even though the bacteria multiplied by *c.*  $10^4$ -fold. These negative results from smaller inocula may have been due to the formation of less toxin during the 6-h period of the experiment. Larger inocula gave a proportional increase in fluid accumulation until the maximal response was reached.

Cell-free culture filtrates of all the 13 strains of *P. shigelloides* gave a positive reaction, similar to that of toxigenic *V. cholerae* strain 569B, indicating that an enterotoxic substance or substances were produced during multiplication in the medium. Although heating at  $100^\circ\text{C}$  or  $121^\circ\text{C}$  for 30 min. destroyed the biological activity, treatment at these temperatures for 10 min. did not, and this indicates that the strains may elaborate a heat-stable enterotoxin. Evans, Evans and Pierce (1973) demonstrated that heat-stable enterotoxin of *E. coli* induces early fluid outpouring in the rabbit gut loop. A similar phenomenon was observed with culture filtrates of *P. shigelloides*, which initiated the outpouring of fluid at 1–2 h. The positive results obtained in the suckling-mouse test for heat-stable enterotoxin with culture filtrates from *P. shigelloides* confirmed this. That a heat-labile enterotoxin may also be formed is indicated by the slight increase in fluid accumulation at 18 h (Sack, 1975).

The CHO- and  $Y_1$ -cell cultures were lysed by the filtrates of all the strains tested. This was perhaps due to the liberation of a separate cytotoxic factor or factors by the bacteria. Similar observations have also been made for *A. hydrophila* (Donta and Haddow, 1978; Ljungh, Wretling and Wadström, 1978) and for the so-called non-agglutinating (NAG) vibrios (Sanyal, unpublished data). The enterotoxin of *A. hydrophila*, when purified so that it was homogeneous on electrophoresis and free from haemolytic and cytotoxic activity, while giving a positive reaction in the ileal-loop model, causes rounding of  $Y_1$  cells and elongation of CHO cells in culture (Sanyal, Dubey and Annapurna, 1978). These data indicate that morphological changes in  $Y_1$ - and CHO-cell cultures are useful indicators for the detection, in culture filtrates, of the enterotoxins of *V. cholerae* and *E. coli* only, and not for other enterotoxins in similar crude preparations.

None of the 13 strains tested could produce keratoconjunctivitis in guinea-pig or rabbit eyes, nor could they be found inside the cells of HeLa monolayers; these findings indicate that *P. shigelloides* lacks the capacity for invasion.

Further studies on characterisation of the enterotoxin and its mode of action are in progress.

## SUMMARY

Live cultures of 13 strains of *Plesiomonas shigelloides* isolated from different sources caused accumulation of fluid in rabbit ileal loops in the range 1–2 ml/cm of gut. Some strains required one or two passages in ileal loops before they gave a positive reaction. Inocula multiplied by *c.*  $10^4$ -fold in the loops within the 6 h of the experiment. Maximal fluid accumulation was obtained with an inoculum of  $10^4$ – $10^5$  colony-forming units. Culture filtrates, in volumes of 0.25 ml, also caused fluid accumulation comparable with that caused by live cells. The fluid accumulation started within 1–2 h, and showed a marked increase up to 4 h, with no appreciable change by 6–8 h, and a slight increase at 18 h. Heat treatment at 121°C for 10 min. did not affect the ability of filtrates to cause fluid accumulation.

Suckling-mouse assay was positive for all the strains tested. The culture filtrates caused dislodgement of Chinese hamster ovarian and mouse adrenal tissue-culture cells from plate surfaces, or lysis of the cells. None of the strains caused keratoconjunctivitis in rabbit or guinea-pig eyes, and bacteria were not seen inside the epithelial cells of HeLa tissue cultures.

The results of the investigation indicate the production of a heat-stable enterotoxin by *P. shigelloides*, while the elaboration of a heat-labile enterotoxin cannot be excluded. They do not suggest invasiveness. They give experimental support for an aetiological role for *P. shigelloides* in the production of diarrhoea, as has been previously suggested by epidemiological evidence.

We are grateful to Dr R. K. Agarwal for his constant help during the work, and to Professor P. C. Sen for stimulating criticism; and to Dr S. Richardson of the Institut Pasteur, Paris, for the supply of three strains of *P. shigelloides*. The work was partially supported by a grant from the Indian Council for Medical Research.

## REFERENCES

- ALDOVA, E., RAKOVSKY, J. AND CHOVANCOVA, A. 1966. The microbiological diagnostics of strains of *Aeromonas shigelloides* isolated in Cuba. *J. Hyg. Epidem. Microbiol. Immun.*, **10**, 470.
- ANNAPURNA, E. AND SANYAL, S.C. 1977. Enterotoxicity of *Aeromonas hydrophila*. *J. med. Microbiol.*, **10**, 317.
- BHAT, P., SHANTHA KUMARI, S. AND RAJAN, D. 1974. The characterization and significance of *Plesiomonas shigelloides* and *Aeromonas hydrophila* isolated from an epidemic of diarrhoea. *Ind. J. med. Res.*, **62**, 1051.
- CHATTERJEE, B. D. AND NEOGY, K.N. 1972. On the aetiology of choleraic diarrhoea. *Ind. J. med. Res.*, **60**, 531.
- COOPER, R. G. AND BROWN, G. W. 1968. *Plesiomonas shigelloides* in South Australia. *J. clin. Path.*, **21**, 715.
- DE, S. N., BHATTACHARYYA, K. AND SARKAR, J. K. 1956. A study of the pathogenicity of strains of *Bacterium coli* from acute and chronic enteritis. *J. Path. Bact.*, **71**, 201.
- DE, S. N. AND CHATTERJE, D. N. 1953. An experimental study of the mechanism of action of *Vibrio cholerae* on the intestinal mucous membrane. *J. Path. Bact.*, **66**, 559.
- 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.

- DONTA, S. T. AND HADDOW, A. D. 1978. Cytotoxic activity of *Aeromonas hydrophila*. *Infect. Immun.* **21**, 989.
- DONTA, S. T., MOON, H. W. AND WHIPP, S. C. 1974. Detection of heat-labile *Escherichia coli* enterotoxin with the use of adrenal cells in tissue culture. *Science*, **183**, 334.
- DUNCAN, C. L. AND STRONG, D. H. 1969. Ileal loop fluid accumulation and production of diarrhea in rabbits by cell free products of *Clostridium perfringens*. *J. Bact.*, **100**, 86.
- EVANS, D. G., EVANS, D. J. AND PIERCE, N. F. 1973. Differences in the response of rabbit small intestine to heat-labile and heat-stable enterotoxins of *Escherichia coli*. *Infect. Immun.*, **7**, 873.
- GEIZER, E., KOPECKY, K. AND ALDOVA, E. 1966. Isolation of *Aeromonas shigelloides* in a child. *J. Hyg. Epidem. Microbiol. Immun.*, **10**, 190.
- GUERRANT, R. L., BRUNTON, L. L., SCHNAITMAN, T. C., REBHUN, L. I. AND GILMAN, A. G. 1974. Cyclic adenosine monophosphate and alteration of chinese hamster ovary cell morphology: a rapid sensitive *in vitro* assay for the enterotoxins of *Vibrio cholerae* and *Escherichia coli*. *Infect. Immun.*, **10**, 320.
- HORI, M., HAYASHI, K., MAESHIMA, K., KIGAWA, M., MIYASATO, T., YONEDA, Y. AND HAGIHARA, Y. 1966. Food poisoning caused by *Aeromonas shigelloides* with an antigen common to *Shigella dysenteriae* 7. *J. Jap. Ass. infect. Dis.*, **39**, 441.
- JANDL, G. AND LINKE, K. 1976. Report on two cases of gastroenteritis caused by *Plesiomonas shigelloides*. *Zentbl. Bakt. ParasitKde*, I. Abt. Orig., **236**, 136 (in German).
- LA BREC, E. H., SCHNEIDER, H., MAGNANI, T. J. AND FORMAL, S. B. 1964. Epithelial cell penetration as an essential step in the pathogenesis of bacillary dysentery. *J. Bact.*, **88**, 1503.
- LJUNGH, A., WRETLIND, B. AND WADSTROM, T. 1978. Evidence for enterotoxin and two cytolytic toxins in human isolates of *Aeromonas hydrophila*. *Toxicon*, suppl. I.
- OSADA, A. AND SHIBATA, I. 1956. On the fraternal diarrhoea supposedly caused by the paracolon organisms with the major somatic antigens of *Shigella sonnei*, with the addendum on the identification of *Shigella* group organisms. *Acta ped. Jap.*, **60**, 739 (in Japanese).
- PAUČOVA, V., AND FUKALOVÁ, A. 1968. Occurrence of *Aeromonas hydrophila* and *Aeromonas shigelloides* in feces. *Zentbl. Bakt. ParasitKde*, I. Abt. Orig., **206**, 212.
- SACK, R. B. 1975. Human diarrheal disease caused by enterotoxigenic *Escherichia coli*. *Ann. Rev. Microbiol.*, **29**, 333.
- SAKAZAKI, R., TAMURA, K., PRESCOTT, L. M., BENCIC, Z., SANYAL, S. C. AND SINHA, R. 1971. Bacteriological examination of diarrhoeal stools in Calcutta. *Ind. J. med. Res.*, **59**, 1025.
- SANYAL, S. C., GAUR, S. D., SHRIVASTAVA, D. L., SEN, P. C., MARWAH, S. M. AND SINGH, H. 1972a. Enteric infections in Sunderpur slum area. *Ind. J. med. Res.*, **60**, 979.
- SANYAL, S. C., DUBEY, R. S. AND ANNAPURNA, E. 1978. Enteropathogenicity and pathogenesis of *Aeromonas hydrophila* in experimental models. Proceedings of the 8th National Conference on Communicable Diseases, Calcutta, Dec. 1978.
- SANYAL, S. C., SEN, P. C., CHAKRABARTY, D. P. AND HARDAS SINGH. 1972b. Bacteriological investigations of gastroenteritis amongst Bangladesh refugees. *Ind. J. prev. soc. Med.*, **3**, 183.
- SANYAL, S. C., SINGH, S. J. AND SEN, P. C. 1975. Enteropathogenicity of *Aeromonas hydrophila* and *Plesiomonas shigelloides*. *J. med. Microbiol.*, **8**, 195.
- SARASWATHI, B., SHARMA, P. AND SANYAL, S. C. 1978. Enteropathogenicity of *Plesiomonas shigelloides*. Proceedings of the 2nd All India Congress, Indian Association of Medical Microbiologists, Hyderabad. Dec. 1978, p. 22.
- SCHMID, E. E., VELAUDAPILLAI, T. AND NILES, G. R. 1954. Study of paracolon organisms with the major antigen of *Shigella sonnei*, form 1. *J. Bact.*, **68**, 50.
- SERENY, B. 1957. Experimental keratoconjunctivitis shigellosa. *Acta Microbiol. hung.*, **4**, 367.
- SINGH, S. J. AND SANYAL, S. C. 1978. Enterotoxicity of the so-called NAG vibrios. *Annls Soc. belge Méd. trop.*, **58**, 155.
- TSUKAMOTO, T., KINOSHITA, Y., SHIMADA, T. AND SAKAZAKI, R. 1978. Two epidemics of diarrhoeal disease possibly caused by *Plesiomonas shigelloides*. *J. Hyg., Camb.*, **80**, 275.



- UEDA, S., YAMASAKI, S., AND HORI, M. 1963. The isolation of paracolon C27 and halophilic organisms from an outbreak of food poisoning. *Jap. J. publ. Hlth.*, **10**, 67.
- VANDEPITTE, J., GHYSELS, G., VAN GOETHEM, H. AND MARRECAU, N. 1957. Sur les colibacteries aberrantes ayant l'antigène somatique de *Shigella sonnei* en phase I. *Anals Soc. belge Méd. trop.*, **37**, 737.
- VANDEPITTE, J., MAKULU, A., AND GATTI, F. 1974. *Plesiomonas shigelloides* survey and possible association with diarrhoea in Zaire. *Anals Soc. belge Méd. trop.*, **54**, 503.
- VON GRAEVENITZ, A. AND MENSCH, A. H. 1968. The genus *Aeromonas* in human bacteriology. Report of 30 cases and review of the literature. *N. Engl. J. Med.*, **278**, 245.
- WINTON, F. W. 1968. *Plesiomonas shigelloides*: An unusual isolate from faeces. *J. Path. Bact.*, **95**, 562.
- ZAJC-SATLER, J., DRAGAS, A. Z. AND KUMELJ, M. 1972. Morphological and biochemical studies of 6 strains of *Plesiomonas shigelloides* isolated from clinical sources. *Zentbl. Bakt. ParasitKde*, I. Abt. Orig., **219**, 514.