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Gastric acidity in cholera and noncholera diarrhoea*

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Gastric acid production, unstimulated and following stimulation with betazole hydrochloride, was measured in Indian men with cholera or acute vibrio-negative diarrhoea. Measurements were made during acute illness and after different periods of convalescence. Men from the same socioeconomic group and from a higher one served as controls. Stimulated acid production was severely reduced during diarrhoea caused by V. cholerae *and related vibrios but not during acute vibrio-negative diarrhoea. Acid production returned to stable convalescent values 1–3 days after cessation of diarrhoea. Stimulated acid production was significantly lower in controls from the lower socioeconomic group than in those from the higher socioeconomic group. Achlorhydria that did not respond to betazole administration occurred in 32% of the convalescent cholera patients but in none of the controls or convalescent vibrio-negative diarrhoea patients. It is concluded from these results that diarrhoea produced by* V. cholerae *and related vibrios is accompanied by transient inhibition of gastric acid secretion, that cholera occurs largely in a population with impaired acid secretion, and that preexisting achlorhydria may predispose to infection with* V. cholerae.

Studies of cholera in an experimental canine model indicate that the stomach and colon do not contribute to faecal losses of salt and water (Sack et al., 1966). It is nevertheless possible that other functions of these organs are impaired during cholera. Recent studies have shown that cholera enterotoxin alters the function of a variety of tissues (Graybill et al., 1970; Vaughan et al., 1970) and indicate that its effects on water and electrolyte transport by the small bowel are mediated by an alteration in the activity of adenyl cyclase in the mucosal membrane with a resultant change in tissue levels of cyclic 3′5′-adenosine monophosphate (cyclic AMP (Kimberg et al., 1971; Schafer et al., 1970; Sharp & Hynie, 1971). Since secretion of gastric acid appears to be mediated by intracellular cyclic AMP (Harris et al., 1969), we have investigated the possibility that secretion of gastric acid might be altered during cholera.

Studies of gastric acidity and cholera are of interest for a second reason. *Vibrio cholerae* is a very acid-labile organism (Read et al., 1939; Napier & Gupta, 1942) and it has been proposed that normal gastric acidity presents a natural barrier to the establishment of intestinal infection (Napier & Gupta, 1942). Earlier studies of cholera patients during early convalescence demonstrated achlorhydria to alcohol stimulation in 36% and suggested that preexisting impairment of gastric acid secretion predisposed these individuals to infection with *V. cholerae* (Pasricha et al., 1940). The late convalescent and control studies necessary to support this conclusion have not been reported, however.

This report describes the effects of acute cholera and noncholera diarrhoea upon gastric acid secretion in man. The results support the hypothesis that persons with impaired gastric acid production may be predisposed to infection with *V. cholerae*.

METHODS

Studies were conducted at the Infectious Diseases Hospital, Calcutta, on men between 14 and 60 years of age with severe cholera or cholera-like diarrhoea. In all of them diarrhoea had begun within 24 hours

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before admission and none had received antibiotics: all had evidence of acute volume depletion, including tachycardia, decreased skin turgor, and hypotension. Salt, water, and base deficits were rapidly corrected intravenously, subsequent stool losses were measured and replaced with equal volumes of appropriate intravenous fluids, and the adequacy of base and saline replacement was frequently monitored, as previously described (Pierce et al., 1968). Some patients were given oral tetracycline to shorten the duration of diarrhoea. All the acute studies were performed when the patients were normally hydrated, free from base deficit, free from nausea or vomiting, and had normal vital signs. Convalescent studies were performed at three different intervals after the cessation of diarrhoea: 1–3 days, 2–7 weeks, and 1 year, the latter group being patients who had previously been treated in this unit in 1969. All patients studied during early (1–3 days) or late (2–7 weeks) convalescence were also studied during the acute illness. However, only some of those studied in late convalescence were also studied in early convalescence. In the patients studied after 1 year gastric acidity had not been studied previously. Some patients were studied at daily intervals during the acute illness.

Two groups of controls, representing different socioeconomic levels of the local population, were studied. The first included 20 men from the same socioeconomic group as those selected for study with diarrhoea. These men were hospitalized at the Infectious Diseases Hospital for tetanus. They were studied immediately prior to discharge, which was 3–4 weeks after admission. At this time there was no residual evidence of tetanus, the men were receiving a normal diet, there had been no diarrhoea during the period of observation, and they were apparently healthy. They, as well as the diarrhoea patients, were mainly labourers, usually uneducated, frequently migrants from rural areas outside Calcutta. The second control group was composed of 12 men from established middle-class families in Calcutta, frequently with some college education, also apparently healthy. Control groups were matched for age and weight as closely as possible to the acute diarrhoea patients.

During the acute episode of diarrhoea studies were carried out on 39 men, 27 of whom had cholera, 6 NAG-vibrio diarrhoea, and 6 vibrio-negative diarrhoea. Control groups consisting of 20 persons from the same socioeconomic group and 12 from the higher socioeconomic group were also studied. The initial gastric acid studies were performed on acutely ill patients on the day of admission, 2–8 hours after rehydration had been completed. A No. 16 French polyethylene nasogastric tube was passed to 50 cm and patients were placed in a recumbent position on their left side. Frequent intermittent syringe suction and occasional repositioning of the tube between 40 and 60 cm ensured the collection of the maximum amount of gastric fluid. The gastric contents were aspirated and discarded for 30 min prior to the recorded measurements. The unstimulated gastric contents were then collected during four 15-min periods before the administration of 50 mg of betazole hydrochloride intramuscularly in the biceps: the injection was followed by four more 15-min collection periods.

The acid content of the gastric juice was determined by titration with 0.1N sodium hydroxide using Töpfers reagent (dimethylaminoazobenzene, 0.5% in ethanol—pK = 2.5) as the indicator. The basal measurements were based on gastric juice collected during the entire 60-min collection period while the stimulated response was taken as the highest rate of acid secretion during any 15-min period following administration of betazole hydrochloride. The arterial pH, plasma specific gravity, and plasma-CO_2 combining power were determined as previously described (Pierce et al., 1968). Liquid stool was obtained at admission by a sterile rectal catheter and cultured for enteric pathogens as previously described (Peirce et al., 1968). The patients were designated as having cholera if the stool samples taken on admission yielded large numbers of *V. cholerae*, as having nonagglutinable vibrio (NAG) diarrhoea if direct inoculation of the admission stool sample on to solid media yielded many colonies of vibrios that did not agglutinate with group-specific *V. cholerae* antiserum, and as having vibrio-negative diarrhoea if no vibrios or other recognized enteric pathogens were isolated.

Statistical evaluation was by Student's "*t*" test applied to unpaired values except where a comparison of paired data was specifically indicated.

RESULTS

The means of age and weight for the three study groups with acute diarrhoea and for the controls from the same socioeconomic group were 32–36 years and 42–45 kg, respectively. The members of the

Table 1. Gastric acid output [a]

Stage	No. studied	Total volume (ml/h)		Rate of acid output (mEq/h)	
		Basal	Stimulated	Basal	Stimulated
Cholera patients					
acute	27	41±6	87±12	0.04±0.03	3.8±0.9
early convalescence (1–3 days)	18	54±7	126±18	0.5 ±0.3	9.1±2.0
P [b]		NS	NS	<0.10 [c]	<0.025
late convalescence (2–7 weeks)	11	65±9	121±16	1.0 ±0.6	7.3±2.0
very late convalescence (1 year)	8	97±16 [d]	194±23 [d]	2.4 ±0.9	11.0±3.5
Patients with NAG-vibrio diarrhoea					
acute	6	28±8	79±10	0.2 ±0.2	4.7±1.4
early convalescence (1–3 days)	4	44±11	121±23	1.5±1.2	10.9±2.9
late convalescence (2–7 weeks)	2	56	118	1.2	11.8
Patients with vibrio-negative diarrhoea					
acute	6	42±9	210±66	0.1 ±0.1	14.3±3.8
late convalescence (2–7 weeks)	2	128	163	2.1	12.5
very late convalescence (1 year)	5	127±28	247±53	2.9 ±1.0	14.0±4.5
P [e]		<0.01	NS	<0.01	NS
Controls					
same socioeconomic group	20	89±11	196±22	1.8 ±0.6	12.9±2.4
higher socioeconomic group	12	110±12	228±8	4.9 ±0.8	21.0±1.8
P [f]		NS	NS	<0.025	<0.025

[a] Data are given as means±SEM.

[b] Comparison of paired acute and early convalescent values by the '*t*' test.

[c] The distribution of observations was not a normal distribution. The proportions of persons in each group producing no free acid after betazole hydrochloride were as follows: acute 25/28, early convalescence 11/18, P<0.05.

[d] Significantly different from the corresponding acute value (P<0.005).

[e] Comparison of acute and very late convalescent values by the '*t*' test.

[f] Comparison of lower and higher socioeconomic groups by the '*t*' test.

higher socioeconomic control group tended to be younger (mean, 26 years) and heavier (mean, 49 kg) but these differences were not statistically significant. The results of the acute and convalescent studies of the three groups of patients with diarrhoea are summarized in Table 1.

In cholera patients studied during the acute stage of the illness, basal acid output was markedly reduced and stimulated acid output was about 40% of the convalescent values. These changes were due to reductions in both the volume and the acid content of the gastric secretions. The basal and stimulated rates of gastric acid output were significantly lower during acute illness than during early convalescence. Basal and stimulated secretory volume increased during early convalescence but the changes were not statistically significant. However, when measured after 1 year of convalescence both these values differed significantly from acute values (P <0.005). Although the gastric acid output tended to rise

from early to very late convalescence, especially the basal acid output, none of these differences are statistically significant. It is possible that the apparent rise in basal acid output from early to very late convalescence of patients with cholera was a result of using different groups of patients for the two examinations. Alternatively, this could represent a real rise that is not demonstrable statistically with the number of observations made in this study. In 12 cholera patients gastric acid output was measured daily until diarrhoea ceased and in no instance did basal or stimulated acid output increase appreciably until the day diarrhoea ended. In several patients the increase appeared 1–3 days after diarrhoea ended.

Patients with NAG-vibrio diarrhoea also showed a marked reduction in gastric acid output during the acute illness, mean values being slightly, but not significantly, higher than in cholera patients. The number of these patients studied in convalescence was too small to permit valid statistical comparison with measurements during acute illness but the course of recovery of acid secretion was similar to that observed in convalescent cholera patients.

Patients with vibrio-negative diarrhoea showed marked reduction of basal acid output during the acute illness, the values being significantly lower than those obtained after 1 year of convalescence. This group clearly differed from the others, however, in that measurements of stimulated acid output during acute illness did not differ significantly from the convalescent values.

The gastric acid output values in the control patients who were members of the same socioeconomic group as the diarrhoea study patients were virtually identical with those obtained for the cholera patients after 1 year of convalescence and were similar to the convalescent values obtained in the other two study groups. The control group with the higher socioeconomic level clearly differed from the lower socioeconomic controls, showing significantly higher values for rate of acid output both before and after stimulation.

The incidence of achlorhydria following administration of betazole hydrochloride was significantly higher ($P < 0.02$) in patients after 2 weeks to 1 year of convalescence from cholera (6/19) than in controls from the same population (0/20). Achlorhydria following betazole hydrochloride was not observed in patients convalescent from vibrio-negative diarrhoea for a similar period of time (0/7) or in the higher socioeconomic level controls (0/12).

DISCUSSION

The mechanism by which acid secretion is altered during cholera and NAG-vibrio diarrhoea is not clear. The study design excludes the possibility that hypotension, hypovolemia, or base-deficit acidosis contributed to altered gastric secretory function since these were corrected before measuring gastric acid outputs. A decrease in the acid content of gastric fluid and in the total amount of acid aspirated from the stomach could be the result of its neutralization by fluid reflux from the duodenum. Two observations suggest, however, that small bowel reflux had little, if any, effect on the gastric secretory measurements made during acute diarrhoea. First, if reflux had occurred the volume of gastric fluid available for aspiration should have been increased above control or convalescent values. Assuming a maximum duodenal HCO_3^- concentration of 30 mEq/litre and a stimulated gastric H^+ concentration of 120 mEq/litre, a 50% reduction of apparent acid secretion would require a reflux volume equal to twice the gastric secretory volume. However, the volume of gastric aspirate was actually significantly decreased during acute diarrhoea. Secondly, acute vibrio-negative diarrhoea, which is also associated with increased flow of HCO_3^- containing fluid in the upper small bowel (Banwell et al., 1971), was not associated with a decrease in stimulated acid output, suggesting that the inhibition of gastric acid secretion was related to diarrhoea of specific etiology and not to small bowel diarrhoea *per se*.

Inhibition of gastric acid secretion might be due to either a direct or an indirect effect of cholera enterotoxin on gastric mucosal function. *V. cholerae* are present in large numbers in gastric fluid during the acute illness (Gorbach et al., 1970) and it is likely that the gastric mucosa is bathed with cholera enterotoxin as well. Since acid secretion is altered by agents that alter the cyclic AMP levels in parietal cells (Harris et al., 1969), and since cholera enterotoxin is capable of altering the cyclic AMP levels in the jejunal mucosa (Kimberg et al., 1971; Schafer et al., 1970) and in several other tissues (Baker et al., 1971; Hewlett & Greenough, 1971), it is possible that the enterotoxin is capable of directly altering the cyclic AMP-mediated acid secretory process of the stomach. Another possibility is that cholera enterotoxin, acting on the small bowel mucosa, might stimulate the production and release of a blood-borne agent that inhibits acid secretion. Several products of the small bowel mucosa, including secretin and

cholecystokinin, are known to inhibit gastric acid secretion (Johnson & Grossman, 1969, 1970).

The similarity in the effects of cholera and NAG-vibrio diarrhoea on acid secretion suggests that their mechanism of action is similar. There is recent evidence that NAG vibrios associated with acute cholera-like diarrhoea produce an enterotoxin similar to, or identical with, cholera enterotoxin (Y. Zinnaka & C. C. J. Carpenter, Jr, unpublished observations).

This study demonstrates that hypochlorhydria is more common in persons from the lower socio-economic levels in Calcutta, in which cholera and other acute diarrhoeal diseases commonly occur, than in persons from higher socioeconomic levels. Although the dose of betazole employed was slightly below the maximum (Desai et al., 1969) this could not account for the significant difference in acid output by controls from the two socioeconomic levels. The etiology of gastric hypochlorhydria in these persons has not been determined.

Pasricha et al. (1940) reported from Calcutta that 36% of adult male cholera patients 7–10 days convalescent had achlorhydria following alcohol stimulation. An additional 52% had low acid output when compared with normal Indians. Read et al. (1939) had already shown that *V. cholerae* failed to grow at a pH of less than 6 in media that would otherwise support vigorous growth, and Napier & Gupta (1942) subsequently demonstrated, that *V. cholerae* survived well in normal gastric juice when the pH was adjusted to neutrality but were rapidly killed at pH values less than 4.8. These observations led to speculation that normal gastric acidity was a natural barrier to cholera infection and that preexisting achlorhydria or hypochlorhydria predisposed individuals to infection.

In this study the mean values for acid output in convalescent cholera patients did not differ significantly from controls from the same population. There was, however, a significantly greater occurrence of achlorhydria following betazole administration in cholera patients after 2 weeks to 1 year of convalescence (32%) than in the appropriate controls (0%. $P<0.02$), strongly suggesting that preexisting achlorhydria predisposes individuals to cholera infection. The alternative possibility, that cholera induces long-lasting achlorhydria in some patients, is not ruled out by this study, but seems unlikely in view of the rapid and complete recovery of other abnormalities in intestinal function associated with cholera (Banwell et al., 1970). The high rate of occurrence of cholera in a socioeconomic group with widespread impairment of gastric acid secretion also suggests that the two could be related.

The role of normal gastric acidity as a barrier to cholera infection is supported by the observation that neutralization of gastric acid with HCO_3^- prior to the introduction of viable *V. cholerae* markedly reduces the vibrio innoculum required to induce cholera in human volunteers (Music et al., 1970). Recent reports also suggest that normal gastric acid is a barrier to intestinal infection with salmonella (Giannella et al., 1970) and *Escherichia coli* (DuPont et al., 1971).

In a study performed in East Pakistan, Cash et al. (1970) were unable to demonstrate any abnormality in stimulated gastric acid secretion by adult patients during early convalescence from cholera. The reasons for the difference in the results of their study and ours are not clear and an explanation may have to await definition of the mechanism responsible for hypochlorhydria in our patients.

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RÉSUMÉ

ACIDITÉ GASTRIQUE DANS LA DIARRHÉE CHOLÉRIQUE ET NON-CHOLÉRIQUE

A Calcutta (Inde), on a mesuré la sécrétion gastrique d'acide, avant et après stimulation par le chlorhydrate de bétazole, chez des sujets atteints de diarrhée aiguë due à une infection par *Vibrio cholerae* ou des vibrions non agglutinables (NAG) ou d'origine non cholérique. Les résultats obtenus en cours de maladie ont été comparés avec ceux fournis par les examens pratiqués 1-3 jours, 2-7 semaines et 1 an après l'épisode diarrhéique. Des examens de contrôle ont été effectués chez des sujets appartenant au même groupe socio-économique que les malades ou à un groupe de niveau plus élevé.

Chez les patients atteints de diarrhée provoquée par *V. cholerae* ou des vibrions NAG, le volume et l'acidité des sécrétions gastriques étaient fortement diminués, le phénomène étant particulièrement net après stimulation par le chlorhydrate de bétazole. Dans les 1 à 3 jours suivant l'arrêt de la diarrhée, la sécrétion a repris progressivement des valeurs d'acidité quasi normales. Chez les malades souffrant de diarrhée non cholérique, on a également noté une réduction du volume des sécrétions et une hypoacidité, mais aucune diminution de la sécrétion d'acide après stimulation. Il semble que par un mécanisme non élucidé la production d'acide soit inhibée en cas de diarrhée due à *V. cholerae* ou à des vibrions NAG, alors qu'elle ne l'est pas durant les épisodes diarrhéiques d'origine non cholérique.

Chez 6 convalescents de choléra sur 19 (32%) examinés après 2 semaines à 1 an, on a constaté une achlorhydrie après stimulation par le chlorhydrate de bétazole, alors qu'aucune achlorhydrie n'a été décelée, dans des conditions identiques, chez les témoins appartenant au même groupe de population ($P<0,02$). En outre, la production d'acide, stimulée ou non, était significativement plus faible chez les témoins appartenant au même groupe que les malades que chez les témoins de niveau socio-économique plus élevé.

Ces observations semblent indiquer que la réduction de la production gastrique d'acide est fréquente dans les couches les moins favorisées de la population qui sont aussi celles qui sont le plus souvent atteintes du choléra. Etant donné l'incidence élevée de l'achlorhydrie chez les convalescents de choléra, ce trouble pourrait être antérieur à la maladie et accroître la réceptivité à l'infection par *V. cholerae*, micro-organisme très labile en présence d'acide.

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