INVESTIGATIONS INTO THE PATHOGENESIS OF DIARRHEAL DISEASES

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Diarrheal diseases of an infectious nature continue to be prevalent. At this particular moment at least two million persons around the world are having diarrhea. An epidemic of Salmonellosis (gastroenteritis) in Baltimore during July, 1970 instigated federal and local inquests into nursing home practices. That epidemic was unfortunate because it was associated with 25 deaths in the debilitated aged population involved. Such occurrences will probably not cease to happen but can be expected to increase in frequency. With our changing food technology, pre-packaged foods ready to eat after heating are excellent sources of this particular pathogen, and no ready way to sterilize these products has yet been devised.

A recurrence of an old species of shigella has appeared in Central America. The Shiga bacillus or *Shigella dysenteriae* has been isolated from severe, sometimes fatal, cases of dysentery in these countries. This pathogen had been absent from that region for many years. It is conceivable it will now spread into the United States.

Traveler's diarrhea is familiar to all, frequently on a very personal basis as an annoying malady. Hopefully, recent discoveries have enabled us to gain some insight into the pathogenesis of this problem. A similar type of illness occurs in military personnel serving in Vietnam. The troops who make the four or five day scouting sorties are especially compromised by diarrhea. Several strains of *E. coli* have been isolated from these cases and have been proven to cause diarrhea in volunteers.

As a final part of this introduction to the current status of diarrheal diseases it is appropriate to mention viral causes. This group of agents has always been suspected of causing diarrhea but extensive efforts by several groups have failed to establish any significant etiological role for viruses in diarrhea. However, with improvement in isolation techniques, some evidence has been acquired indicating potential importance.

The intestinal tract has only a limited number of ways of reacting to an

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infectious agent. These reactions include motility, tonus, absorption, secretion, cell turnover and removal, defense and endocrine mechanisms.[1] Whenever several or all of these functions are activated by an infectious agent, the end result is the passage of a liquid stool. The clinical manifestations of enteric infections therefore are similar and overlapping. Sprinz[1] has emphasized the interaction of the various types of diarrheal diseases. The specific clinical illnesses initiated by shigella, salmonella, and vibrio cholera are but a small proportion of the large mass of undifferentiated diarrheal illnesses. The majority of bacteriologically proven cases of diarrhea are indistinguishable from nonspecific enteritis. A few cases with classic symptoms and signs allow for clinical differentiation of shigellosis from salmonellosis for instance. On the other hand cases of diarrhea which appear to be cholera or shigellosis for example may be initiated by salmonella or *E. coli* rather than the expected agents. This overlapping of classic clinical symptoms is an unusual occurrence. This type of similarity however does point up the limited forms of reaction inherent in the intestinal tract. It is understanding of the pathogenesis and etiology of the large background of undifferentiated illness that concerns part of this presentation.

The anatomic relationships of enteropathogenic agents to the intestinal epithelium have been tabulated by Sprinz.[1] Much of this information has been gained from studies in animals but confirmatory evidence has been accumulating in man. Table I lists various relationships that can exist between the infecting agent and the intestinal epithelium. The agents in Group A have the capability of releasing an enterotoxin which alters the integrity of the living cells. This alteration in most instances is physiological as no definite anatomical lesions have been identified. As a result of the toxin activity there is an outpouring of fluid and diarrhea ensues. Group B contains organisms not known to be pathogenic for man. Shigella

TABLE I

Enteric Infections

Localization of Infectious Agent to Host

A.	Organisms which do not invade host tissues Examples: *Vibrio cholerae;* staph food poisoning, Giardia, *E. coli*
B.	Partial penetration of the epithelial lining Examples: intestinal spirochetes—no clinical significance
C.	Superficial epithelial layers penetrated Examples: Shigella species, *E. coli*
D.	Transportation through epithelial cells—little or no surface cell damage Example: Salmonella

Modified after Sprinz

TABLE II

Infective Dose of Enteric Pathogens for Man

Shigella	200
Typhoid	100,000
Escherichia	100,000,000–10,000,000,000
Cholera	10,000,000,000

species (group C) characteristically penetrate superficial epithelial layers and as a result microabscesses develop as the early phase of bacillary dysentery (the diarrhea phase) is completed. Healing occurs, rapidly with an antibiotic, and the dysentery subsides. Strains of *E. coli* have been isolated from patients with diarrhea that have this same pathogenic virulence. Salmonellae appear to pass through the superficial layers of the epithelium without causing any damage. Those strains causing gastroenteritis do not progress any further than the lamina propria region whereas *S. typhosa* gain entrance to the lymphatic drainage and disseminate into the lymph nodes and liver. The reasons for the differences in the form of disease caused by the strains are unknown.

The previous statements outlining bacteria-host cell interaction presupposes that bacteria reach the susceptible areas of the gut where they can cause diarrhea. Cholera is mainly a disease of the small bowel, shigellosis causes dysentery by producing mucosal injury in the large bowel, salmonella gastroenteritis may be an infection of small bowel and colon. *E. coli* diarrhea in adult man may be either small bowel or colon depending on the characteristics of the strain. If the strain is the penetrating type—i.e. resembles shigella—disease is produced in the colon. On the other hand the enterotoxin producing species appear to initiate disease in the upper small bowel and are incapable of producing disease when present in the large intestine. The number of organisms needed to produce disease in man varies. Table II tabulates the numbers required to cause typhoid, cholera, shigellosis and *E. coli* diarrhea. The attack rates following the listed doses are approximately 20–30% for man except for the *E. coli* strains and insufficient evidence does not allow for an accurate estimation as yet with these organisms. Note only 200 shigella cells can cause diarrhea whereas it takes over 10 billion to cause cholera. Differences in virulence accounts for these variations. One simple virulence factor pertains to the organisms' ability to resist acid degradation in the stomach.

This barrier has varying effects on enteric pathogens. Typhoid bacilli were recovered from our volunteers for as long as 45 minutes in gastric juice with a pH of 2.0 but the recovery rate was low. Shigella was surprisingly resistant to acid digestion and indeed can cause gastritis in mon-

keys.[2] As noted, only 200 organisms cause disease in man—the attack rate was about 20%. This remarkable ability of a few bacteria to cause disease accounts for the frequent laboratory acquired infections with shigella, and also suggests that shigella are readily adaptable organisms for waterborne disease and would not need food to act as a buffer to promote survival in the stomach. A very striking example of the effect of gastric acidity on susceptibility to infection has been documented in man infected with cholera vibrios. These organisms can be shown to be very sensitive to degradation by acid. In vitro studies using aspirated gastric juice have shown that vibrios do not survive over six minutes. This information suggested that the stomach may be an effective defense against cholera. Hence the need to ingest as many as 10 billion organisms to initiate disease, a highly unlikely naturally acquired inoculum. During the course of studies to develop a model of cholera in order to evaluate vaccines, the effect of gastric acidity on subsequent infection rates was quantitated. Volunteers were pretreated with $NaHCO_3$ and subsequently analyses of the dynamic buffering response was measured at 15 minute intervals. It was found that all men (54) had markedly acid pH of gastric juice prior to $NaHCO_3$. In the subsequent 15 minute intervals the expected loss of buffer action occurred but with varying speeds in each volunteer. By 30 minutes the group was evenly divided—those who had returned to a low pH and those with residual buffering effect. (Table III). There is no overlap of these pH values. Since the vibrio would be expected to survive longest in those men with the more prolonged buffering effect, attack rates of disease were correlated with the 30 minute pH value of gastric juice. (Table IV) There was a higher attack rate in the group with the alkaline pH values. An additional indication of the importance of buffering can be estimated from comparing attack rates of disease with and without bicarbonate. Volunteers have been given doses of virulent vibrios as high as 10^8 to 10^{10} without predictable induction of diarrhea. In contrast 10^4 of the same vibrios with a pretreatment of $NaHCO_3$ caused diarrhea in 69% of 13 volunteers. Thus in human cholera buffering of stomach contents has enhanced virulence equivalent to approximately four logs of organisms. Of timely interest has been the report from the CDC that in the present epidemic of cholera in Israel, 3 of 150 patients with cholera had had a preceding gastrectomy, a high percentage in this select group of patients. It is intriguing to speculate that cholera may selectively occur in those individuals with hypo or achlorhydria, either absolute or relative.

Diarrhea caused by so-called enteropathogenic strains of *E. coli* is mainly a disease of children. It has not been definitely established as to which antigens or functions of these bacteria characterize them as diarrheogenic. Usually the major types responsible for infant diarrhea are

TABLE III

Gastric pH, 30 minutes after $NaHCO_3$ (2 Grams)

Low pH Group	High pH Group
1.59	6.51
1.59	5.68*
1.70*	5.83
1.71	5.85
1.75*	6.10*
1.75	6.37
1.76	6.42*
1.78	6.49
1.80*	6.70
1.82	6.79
1.88	6.81
1.88	6.92
1.89	7.00
1.92	7.26
2.12	7.27
2.79	7.28*
3.72	7.51
	7.75*
	8.05
Av. pH = 1.97 ± 0.53	Av. pH = 6.72 ± 0.72

* pH data from rechallenged volunteer.

TABLE IV

Induced Cholera in Volunteers

Dose Specific Attack Rates in Low pH and High pH Groups

	30 Minutes after $NaHCO_3$	
	Low pH	High pH
10^4	1/5 = 20%	4/4 = 100%
10^6	5/7 = 71%	9/10 = 90%
Totals	6/12 = 50%	13/14 = 93%

found among about 10 serotypes. Adults presumably are immune to these species because of prior exposures. However, new isolates have been found which cause human disease. These have been found to have one of two characteristics. Either they have the ability to penetrate the epithelial surface similar to virulent shigella species or else the *E. coli* releases an enterotoxin which presumably damages the integrity of the cell walls resulting in diarrhea, an explanation which has been associated with

cholera toxin activity. In Table V these strains are listed according to their virulence characteristics for animal systems. The clinical manifestations of the illnesses induced by 100 million organisms of each type are listed in Table VI. Note the enterotoxin producing types (B2C, B7A, 334A) cause an illness not unlike salmonella or mild cholera diarrhea. These organisms appear to colonize in the small bowel. When this section of the intestine is cleansed of the *E. coli*, the diarrhea ceases despite the persistence of large numbers in the colon. This truly is a small bowel form of diarrhea. The capability of *E. coli* strains to penetrate gut epithelium results in a different form of enteritis. The volunteers ingesting these strains became acutely ill within 24 hours with abrupt fever, severe mucoid bloody diarrhea and marked toxicity requiring antibiotic therapy. These were cases of severe "shigella" dysentery, in fact more intense than any induced shigellosis in our experience. Both classes of *E. coli* can cause diarrheal disease in man and may account for a large segment of undiffer-

TABLE V

Serologic and Biologic Properties of E. coli *Strains*

Strain	O-Group	Enterotoxin Production	Epithelial Penetration
B2C	6	+	−
B7A	148	+	−
334A	15	+	−
C1272	124	−	+
1624	144	−	+
4608	143	−	+
HS	Nontyped	−	−

TABLE VI

Reactions Following Ingestion of 10^8 E. coli *Cells*

E. coli strain	Number Volunteers	Diarrhea			Mucoid Stools	Positive Stool Isolation	4xAb. Rise
		Fever	Mild	Severe*			
B2C	5	0	2		1	5	4
B7A	5	0	1		0	4	1
334A	5	0	2		1	5	1
C1272	5	0	1		0	4	0
1624	5	3	0	3	3†	3	4
4608	5	3	1	2	1	5	5
HS	5	0	0		0	4	1

* 5 or more watery stools/24 hrs. for 2 consecutive days.

† one volunteer had bloody mucoid dysentery.

entiated diarrhea. A large proportion of such diarrheal cases may be due to similar types of ubiquitous *E. coli* species. Much of the information regarding toxigenic *E. coli* and the colonization of small bowel has come as a result of earlier studies with cholera. The uncovering of the role of cholera enterotoxin in causing this infection has led to the results noted with the toxin producing *E. coli*. In recent months these observations have been extended to *Sh. dysenteriae*. This organism has been known to produce a neurotoxin and this or a similar toxin will induce diarrhea in rabbit ileal loops.[3] Yet another enteric pathogen has been found which may produce illness by means of a toxin rather than direct tissue penetration alone. The search for enterotoxins in the other shigella and salmonella species has begun.

Finally, cell-free filtrates of stools of patients ill with "winter-vomiting" disease in Ohio have been administered to volunteers. The men were pretreated with $NaHCO_3$. A febrile, diarrheal disease resulted. In order to confirm the infectious nature of this infection, a pooled cell-free filtrate from one to the ill volunteers has been fed to additional men. Diarrhea has appeared in them. All that is needed to fulfill Koch's postulates is to grow the suspected virus in tissue or intestinal cell or organ cultures and reinfect volunteers with the harvested virus. This work is in progress. The results to date are exciting and suggest that perhaps another block of undifferentiated enteritis can be assigned to a specific etiology.

Summary

The etiological basis of several forms of undifferentiated diarrhea have been uncovered. Strains of *E. coli* produce an enterotoxin that produces diarrhea, a mechanism similar to cholera infection. Other strains of *E. coli* can cause bacillary dysentery by the same means as shigella. Viral forms of diarrheal disease have been identified. It remains to be determined whether a simple method can be found to counteract the limited number of intestinal dysfunctions initiated by infectious agents and hence prevent the end result of these reactions—diarrhea.

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REFERENCES

1. Sprinz, H.: Pathogenesis of intestinal infections. *Arch. Path.* **87:** 556–562, 1969.
2. Kent, T. H., Formal, S. B., LaBrec, E. H., Sprinz, H. and Maenza, R. M.: Gastric shigellosis in rhesus monkeys. *Am. J. Path.* **51:** 259–267, 1967.
3. Keusch, G. T., Mata, L. J. and Grady, G. F.: Shigella enterotoxin: Isolation and characterization. *Clin. Res.* (Abt.) **18:** 442, 1970.