Studies on Natural Gastric Flora:

I. Bacterial Flora of Fasting Human Subjects

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The presence and survival of ingested micro-organisms, both pathogenic and commensal, is of practical interest because the stomach is one of the principal portals of entry of bacteria in humans. A study of the environmental factors governing the survival of indigenous flora in the gastric lumen will assist in determining whether potentially pathogenic organisms will survive in the stomach and pass on. The authors set out to determine the significance of gastric mucin in such survival, with the ultimate objective of learning what factors provide optimal bactericidal conditions.

Samples of gastric juice were aspirated from 149 fasting individuals; samples of saliva, nose and throat swabs were also obtained. Positive gastric cultures were found in 82%. The study of the effects of gastric pH demonstrated an increase in the number of samples showing growth above pH 2.0 and a concomitant increase in total bacterial growth. At a pH lower than 2.0, certain organisms are selected out. It appears that even minor changes in gastrointestinal physiology disrupt the normal clearing of the flora.

Comme chez l'être humain l'estomac constitue une des bouches principales d'admission de bactéries la présence et la survie de microorganismes ingérés, tant pathogéniques que commensaux, constitue un fait d'intérêt pratique. Une étude de facteurs d'environnement qui gouvernent la survie de la flore indigène du lumen gastrique, s'avère intéressante à l'estimation du passage d'organismes puissamment pathogéniques. Dans notre projet nous nous proposons de déterminer l'importance de la mucine gastrique, notre objet primordial étant de trouver les facteurs apportant les conditions bactéricides maximales.

Nous avons aspiré des échantillons de jus gastrique chez 149 personnes à jeun. Nous avons également obtenu des échantillons de salive, de mucus nasal et de la gorge. Conséquemment nous avons trouvé des cultures gastrique positives dans 82% des cas.

L'étude de l'effet du pH gastrique peut démontrer l'augmentation du nombre d'échantillons faisant preuve de culture au-dessus de pH 2.0 et une augmentation concomitante de la culture bactérienne totale. A des pH inférieurs à 2.0, nous avons observé une certaine sélection d'organismes.

Il semble que des changements mineurs dans la physiologie gastrointestinale peuvent déranger le dégagement normal de la flore.

THE passage of micro-organisms through the stomach has interested bacteriologists and clinicians ever since the role of bacteria as etiological agents of disease was first elucidated. It is of practical interest that the stomach is one of the principal portals of entry for micro-organisms that are either pathogenic or potentially pathogenic. The fate of ingested micro-organisms, both pathogens and commensals, is one of the problems that require clarification. Our knowledge of natural gastric flora in humans is not only incomplete but is frequently founded on inadequate bacteriological studies.

The ultimate objective of the project described in these communications is to determine the significance of gastric mucin and other factors that affect the passage of micro-organisms through the stomach, within the framework of the *gastric let* concept.[1] The *gastric let* is the sum of mechanisms which protect the stomach against mechanical and chemical injuries, and the whole body against toxic and bacterial agents entering by this route. A review of the literature yielded very little recent data on this subject, although it has been shown that many gastric aspirates in diseased individuals show bacterial growth.[2] The present communication describes a study undertaken in our laboratory to identify the normal gastric flora in a large number of individuals of various ages. It corrects some current misconceptions that have been perpetuated by the use of old data.

The nasopharyngeal and salivary secretions constantly supply viable organisms to the gastric lumen: a fact which must be considered, not only in chemical analysis of gastric secretions, but also in bacteriological studies of gastric juice. The volume of saliva deposited in the stomach each day varies between one and one and one-half litres. Although scrupulous aseptic techniques should be employed to avoid extraneous contamination in studies involving gastric intubation, exclusion of the nasopharyngeal and salivary flora, which drain constantly into the stomach, appear to be unnecessary.

It has been reported many times in the literature that the gastric juice is sterile. Hewetson,[3] in 1904, was the first to show experimentally that bacteria are killed in the stomach. By intubation, he implanted cultures of *Staphylococcus aureus* and *Pseudomonas aeruginosa* into his own stomach and aspirated samples of gastric juice every 15 minutes; the staphylococci were killed in 30 to 45 minutes and the Pseudomonas in 90 minutes. However, when Hewetson removed gastric secretion samples from patients directly from the stomach during operations, 50% of the cultures were positive. He disregarded these organisms, considering them to be non-pathogens and therefore insignificant.

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The stomach was called a germicidal barrier by Knott[4] and, under conditions of prolonged high acidity, it is. Unquestionably the acidity and proteolytic activity of gastric juice has a definite effect on the viability of micro-organisms. This oversimplification of the bactericidal mechanisms has, no doubt, deterred further exploration of the problem. Historically, the most dramatic demonstration of the barrier effect was given by Pettenkoffer in 1892,[5] when he swallowed a tube full of *Vibrio comma* to disprove the epidemic pathogenicity of the vibrio.

It is well known that many micro-organisms induce disease through gastric entry. Therefore, a study of the environmental factors governing the survival of indigenous flora in the gastric lumen could be of value in determining whether potentially pathogenic organisms survive in and pass through the stomach, and could elucidate the relationship between these indigenous organisms and gastric secretion.

MATERIALS AND METHODS

We studied 154 samples from 149 persons, ranging in age from 8 to 79 years; three had had a partial gastrectomy. In the morning, after a 12-hour fast, a nasogastric tube was passed into the stomach and approximately 5 ml. of resting secretion was aspirated. Before intubation, nose and throat swabs and a sample of saliva were obtained. A second aspiration was done on six subjects to compare the growth in the two samples. Sterile gastric tubes, syringes, glycerol, lubricants and collecting jars were employed. Samples of gastric juice were taken to the bacteriology laboratory immediately and processed within minutes of collection. The pH was determined in order to estimate the range of dilution for quantitative analysis; this was not an infallible technique, as will be seen subsequently. Qualitative and quantitative analyses of bacterial growth were done on aliquots obtained from a thoroughly mixed specimen. Two methods were used for counting the number of organisms: (1) a strictly quantitative method, the standard serial dilution pour-plate technique, and (2) a quantitative, qualitative surface-spread-plate technique.[6] In the latter method, the specimen or serial dilution of the specimen was placed on the surface of a blood-agar plate (sheep erythrocytes) with a calibrated (0.01 ml.) platinum-wire loop. Each sample of gastric mucin was also placed on two blood-agar plates, Bacto-Mitis-Salivarius agar (Difco), tomato juice agar and Endo agar. Brewer's thioglycollate broth (Difco) and Brewer's cooked meat broth (Difco) were also inoculated. The blood-agar and the tomato-juice–agar plates were incubated in a Brewer anaerobic jar in the presence of H_2. It was found that H_2 was essential in isolating obligate anaerobes.

The anaerobic cultures were examined after 48 hours; the aerobic cultures were examined at 24 and 48 hours. Negative cultures were discarded after one week. Isolated colonies were identified as far as was practicable. At times, the exact definition of some species of the Corynebacteriaceae and Micrococcaceae was quite difficult, with the result that the identification was carried out only to the level of the genus. The source of definition for identification was Bergey's Manual of Determinative Bacteriology.[7]

A complete taxonomic differentiation was not attempted for any of the species, and for the identification of both genus and species, important characters were weighted.

The nose and throat swabs were cultured qualitatively in the same manner as the gastric specimens. The saliva was cultured quantitatively by the serial-dilution pour-plate method. Direct smears of gastric juice were prepared and stained by Gram's method.

RESULTS AND DISCUSSION

One hundred and fifty-four samples, obtained from 149 subjects, were studied; in 127 samples (82%), bacterial growth was demonstrated, which varied from sparse (10^2 organisms/ml.) to abundant (10^8 organisms/ml.). Cultures showing growth of less than 100 organisms per ml. were classified as negative to compensate for possible contamination due to introduction of the nasogastric tube.

Upon direct microscopic examination, all of the gastric specimens contained many Gram-positive and Gram-negative bacilli and cocci of varying shapes. Spirochetes and fusiforms were usually found, in addition to many intracellular bacteria of several morphological types. The tissue cells encountered were those normally sloughed from the gastric mucosa but were usually intact. There were generally only a few organisms per cell but these were well entrenched in the matrix, because careful focusing often demonstrated the bacterial cell at the level of the nucleus.

The positive cultures were studied as follows: (1) frequency of occurrence of positive gastric cultures with (2) relative numbers of each strain appearing in each specimen; (3) effects of gastric pH on bacterial growth; and (4) age distribution in subjects with positive gastric cultures.

Frequency of Positive Gastric Cultures

It soon became apparent that micro-organisms are found in gastric juice frequently, sporadically or rarely. The 127 positive specimens were considered as 100% in evaluating the frequency of occurrence of strains. Accordingly, the strains have been classified as: GROUP 1—frequent; those bacteria or yeasts found in 15-60% of positive cultures; GROUP 2—sporadic; strains found in 2-15% of positive cultures; and GROUP 3—rare; strains occurring in less than 2%.

TABLE I.—FREQUENCY OF OCCURRENCE OF BACTERIAL SPECIES IN FASTING HUMAN SUBJECTS

Organisms found frequently in gastric specimens (15-60%)	%	*Organisms found sporadically in gastric specimens (2-15%)*	%	*Organisms found rarely in gastric specimens (less than 2%)*	*No.**
Streptococcus mitis	55	*Staphylococcus aureus*	8	*Vibrio percolans*	1
Staphylococcus epidermidis	50	*Streptococcus pyogenes*	8	Proteus spp.	2
Streptococcus salivarius	47	Fusobacterium	7	*Alkaligenes fecalis*	1
Lactobacillus spp.	37	Bacteroides	5	*Alkaligenes recti*	2
Yeasts	35	Bacillus spp. (*B. subtilis, B. cereus*)		*Klebsiella pneumoniae*	2
Micrococcus spp.	30				
Neisseria spp. (*N. catarrhalis, sicca, subflava*)	25	Enterococci	3	Achromobacter sp.	1
Corynebacterium spp. (*C. xerosis, bovis, pyogenes pseudodiphtheriticum, parvum*)	19	*Diplococcus pneumoniae*	4	*Aerobacter aerogenes*	1
		Gaffkya tetragena	3	Peptostreptococcus spp.	2
		Escherichia coli	3		
		Veillonella spp.	3		

*These figures are given in actual numbers because the percentage was always less than 2% (out of 127).

The quantitative and qualitative relationships of the bacterial flora are shown in Table I.

Group 1 contains the bacteria found most commonly. They are classified in some cases as species or genus, in others as general groups (as in the case of yeasts). Many of the latter were classified as *Candida albicans,* Candida spp., *Geotrichum* or *Saccharomyces.* Examination of the Corynebacterium species yielded *C. xerosis, C. bovis, C. pyogenes, C. pseudo-diphtheriticum* and certain anaerobic species such as *C. parvum.* However, many of the Corynebacteria found could not be classified accurately. Organisms found in 2% or less of positive cultures (Group 3) included Proteus spp., *Vibrio percolans, Alkaligenes fecalis, Alkaligenes recti, Klebsiella pneumoniae, Aerobacter aerogenes,* Achromobacter and Peptostreptococcus species. Many of the Group 3 are ubiquitous intestinal organisms, and it was surprising to find them so infrequently.

Relative Frequency of Viable Strains in Each Specimen

In order to evaluate the relative occurrence of the various strains within each specimen, we had to obtain very good distributions on the viable-count spread-plates. Of the 127 samples which showed growth of 10^2 or greater, 72 met the minimum standards for a reasonably correct analysis. The order of predominance is recorded in Table II. Three categories were chosen: (1) *predominant,* these organisms comprised two-thirds to one-half of the total viable organisms within the specimen being examined (column 1); (2) *predominant, but sharing* the predominance with one or two other organisms (column 2), and (3) *second in predominance* (column 3). The viridans group, *Streptococcus mitis* and *salivarius,* were in the greatest numbers, shared this position, or were second in predominance on 82 separate occasions. These organisms outnumber all others, both in frequency of occurrence and in total count within the individual specimen. However, it has been definitely established that these organisms are present in the saliva of 100% of normal subjects.[8] *Staphylococcus epidermidis* and Corynebacterium spp. of the diphtheroid type are found in moderate numbers. The other organisms of Group 1 rarely predominate and are usually found in relatively smaller numbers. *Vibrio percolans* and *E. coli,* indigenous to the intestine, predominated each on one occasion. *Gaffkya tetragena* and *Staphylococcus aureus* predominated on one occasion each.

TABLE II.—RELATIVE FREQUENCY OF VIABLE STRAINS IN EACH SPECIMEN—NUMBER OF SAMPLES IN WHICH ORGANISMS PREDOMINATE

Organism	*Number of samples*		
	Predominant	*Equally distributed*	*Second in predominance*
Streptococcus mitis	17	15	12
Streptococcus salivarius	14	13	11
Staphylococcus epidermidis	9	6	5
Diphtheroids	5	4	6
Micrococci	3	—	6
Yeast	3*	—	—
Lactobacilli	0	3	1
Neisseria spp.	1	2	1†
A. aerogenes	2	0	0

*In one case only yeast was present.
†Few.

In the evaluation of the relative numbers of the organisms, the total count and pH must also be examined. *S. mitis* and *S. salivarius* predominated within a wide range of pH that permitted growth of 10^2 or greater. Eleven of the 72 cultures showed a pH of 7.8 or greater. *S. mitis* was predominant in two, *S. salivarius* in three, and the two species were the most numerous in four specimens.

Analysis of occurrence of predominantly *S. mitis* or *S. salivarius,* or equal distributions of these organisms, gave the following results:

Predominant organism	*Average pH*
Streptococcus mitis	5.9 (range 2.0 - 8.4)
Streptococcus salivarius	6.5 (range 3.8 - 8.4)
Equal distribution of *S. mitis* and *S. salivarius*	6.8 (range 2.4 - 8.5)
Other species	4.6 (range 1.8 - 8.4)

This is perhaps too small a sample to predict that *S. salivarius* survives longer at very high pH values than does *S. mitis*. In many cases, where neither of the viridans streptococci predominated, the pH had dropped to three and the total viable count was lower. When counts were high and pH very low (2 or less), it was assumed that the pH change was recent and that the organisms would soon be reduced.

The pattern of distribution of the organisms was changed when the pH dropped. The trend was for other organisms to be more numerous at the lower pH values. This trend is reflected in Table IV, which compares the appearance of various types at low pH (less than 3.0).

TABLE III.—NUMBER OF ORGANISMS PER ML. OF GASTRIC JUICE AT VARIOUS pH RANGES

pH range	*Number of samples*	*Samples with growth*	*Range of counts*	*Mean*
1.0 - 2.0	21	2	10^2 - 10^4	10^3
2.1 - 3.0	59	27	10^2 - 10^6	10^4
3.1 - 4.0	6	4	10^2 - 10^5	10^4
4.1 - 5.0	9	8	10^2 - 10^7	10^6
5.1 - 6.0	6	5	10^3 - 10^7	10^6
6.1 - 7.0	26	26	10^2 - 10^8	10^7
7.1 - 8.0	20	19	10^2 - 10^8	10^7
8.1 - 8.5	5	5	10^6 - 10^8	10^7

Effect of Gastric pH on Bacterial Growth

Table III shows the breakdown of the viable counts of organisms per ml. of gastric juice found at pH ranges in increments of one pH unit. The counts below 10^2 organisms per ml. were discounted in this analysis. Listed are the number of samples in each range and the samples which showed growth of 10^2 or greater. This table demonstrates that, above pH 2.0, there is an increase in the number of samples showing growth and a concomitant increase in the total count. The majority of samples with a count of 10^4 or greater show the same general distribution of types of organisms (Table II). However, at low pH there is a selection of organisms. The maximum count is of the order of 10^8. The counts from saliva samples, determined simultaneously, are always of the order of 10^1 or 10^2 times greater than those in the corresponding gastric samples. It is of interest to note that the greatest number of samples was in the pH range 2.1-3.0. The ranges from 1-3 and 6-8 contained 80% of all samples tested.

The pH range of all samples was between 1.0 and 8.5. Of the 154 samples, 81 (53%) had a pH of less than 3.0 while in the remaining 73 cases (47%) the pH was higher than 3.0; in 33% a pH of 6.0 or greater was found. Of the samples with a pH less than 3.0, 30% contained greater than 10^2 organisms per millilitre. Active growth in 30% of the samples with a pH less than 3.0 indicates that the drop in pH had occurred just before intubation. This raises the question of duration of survival of the organisms under adverse conditions; our samples were obtained from ambulatory individuals in the early morning after 12 hours of fasting.

TABLE IV.—EFFECT OF GASTRIC pH ON BACTERIAL GROWTH: GROWTH AT pH LESS THAN 3.0, COMPARED TO pH GREATER THAN 3.0

Organisms	*Positive samples at pH less than 3.0 showing growth of individual organisms*	*Comparison with samples at pH greater than 3.0*
	%	%
S. mitis	23	50
S. salivarius	27	73
Yeasts	30	50
S. aureus (3)	9	8
S. epidermidis (8)	23	50
Lactobacilli (9)	27	50
Diplococci (2)	6	3
Sarcina (1)	3	—
Bacteroides Fusobacterium (4)	12	3
Anaerobic diphtheroids (2)	6	—
Peptostreptococcus (2)	6	1
Diphtheroids (9)	27	19
Neisseria (3)	9	25
Bacillus (3)	9	5
Veillonella (1)	3	3
Proteus (1)	3	1
Streptococceus pyogenes (1)	3	8

Table IV includes all growth observed and is the only table showing growth of less than 10^2 organisms/ml. The figures are based on isolation of strains from each specimen regardless of viable counts and may differ from those in Table I.

Organisms found more frequently at pH less than 3.0 included the sarcina, peptostreptococcus, Bacteroides spp., Fusobacterium spp., Bacillus spp. and diphtheroids. At pH less than 3.0, a marked reduction in the frequency of standard organisms (Group 1), *Streptococcus pyogenes* and Neisseria spp. was observed. With many organisms such as Veillonella spp., *Staphylococcus aureus* and Proteus spp., the rate of appearance is approximately the same, regardless of pH (Table IV). The most noteworthy difference was observed in the Neisseria which appeared in moderate numbers (25% of the specimens); however, at pH 3.0 or less the rate dropped to 9%.

Age Distribution of Positive Gastric Cultures

The age of individuals studied ranged from 8 to 79 years. The age distribution is shown in Table V. Only four were below the age of 20; 33 were 20 to 39 years old; 76, 40 to 59 years old; and 34, 60 to 79 years old. The pH ranges of each of these groups were similar; however, some differ-

ences can be observed. The 60-79 age group showed a higher mean pH value, which is shown in the column listing the number of samples in each age group with a pH less than 3.0; this figure is much lower in the older age group. The analysis of the samples of pH 6.0 or greater shows very little variation in the different age groups. The age range of the subjects with the more alkaline samples (i.e. greater than pH 6.0) was 20-76 years. The average age of these individuals was 50, only slightly above the total group average.

TABLE V.—ANALYSIS OF pH AND GROWTH AS RELATED TO AGE

Age range (yr.)	*pH range*	*Mean pH*	*Total*	*Total specimens with growth*	*Total specimens pH less than 3.0*	*Specimens of pH less than 3.0 with growth*	*Specimens of pH 6 or more*	*Specimens of pH 6 or more with growth*
20-39	1.3-8.4	4.4	34	18 (53%)	17 (53%)	4 (23%)	13 (41%)	12 (92%)
40-59	1.2-8.5	4.0	78	45 (57%)	41 (52%)	13 (32%)	21 (25%)	20 (95%)
60-79	1.2-8.4	5.0	33	25 (73%)	12 (35%)	5 (41%)	18 (53%)	16 (89%)

COMMENTS AND CONCLUSIONS

Several aspects of this study suggest that some revision of current views on gastric bacterial flora is necessary.

With respect to the frequency of positive gastric cultures, the studies were done in fasting human subjects whose stomachs were empty except for constant drainage of nasopharyngeal and salivary secretions. Therefore these specimens represent natural gastric flora. As 82% of the total specimens were positive, it is possible that, with food intake, the frequency of positive gastric cultures would be further increased. Studies on non-fasting subjects are now in progress in this laboratory.

As far as is known, the members of the viridans group, *Streptococcus salivarius* and *Streptococcus mitis*, are not pathogenic in the gastrointestinal tract. They are the most common salivary organisms and are pathogenic in the blood stream. *Staphylococcus epidermidis* and Micrococcus spp. are not pathogenic, while Lactobacillus spp. is known to play a role in dental caries. Yeasts (Candida), although present in relatively small numbers, are potentially pathogenic. If a focus of infection has already been established and the growth of bacteria normally present is inhibited, as by indiscriminate antibiotic therapy, yeasts can maintain a chronic inflammatory state. It appears that such extraneous factors must be present before yeasts are pathogenic in the stomach. In gastric cultures in which other bacteria of Group 1 were present in small numbers, there was no massive overgrowth of yeasts; this may be related to the longer generation time of yeasts in culture.

The Corynebacterium species found are not potentially pathogenic, with the possible exception of *Corynebacterium pyogenes* which has been isolated from skin pustules. *Staphylococcus aureus, Streptococcus pyogenes* and *Diplococcus pneumoniae* are all potentially pathogenic. It appears that the carrier rate of these organisms among the general population is higher than their rate of occurrence in gastric cultures. It is generally agreed that Group A streptococci and *D. pneumoniae* have been recovered often enough to be considered as indigenous.[8] The Bacteroides group found in 7% of positive gastric cultures has been isolated from patients with appendicitis, diverticulitis and other disorders of the gastrointestinal tract where there is stasis, but their etiological significance remains obscure.

Organisms which comprise the normal biota of the intestinal tract were included in our studies as Group 3. *Aerobacter aerogenes,* Achromobacter spp. and Peptostreptococcus spp. are not usually considered to be part of the normal intestinal flora, but are frequently seen.[8] In the gastric specimens we observed them only rarely, although many specimens contained regurgitated bile. Few enterococci were observed in the gastric juice, and they were also uncommon in the nose or throat cultures. *E. coli* was found in only three gastric specimens. One of these patients had had a gastric resection, and in this situation, direct passage of intestinal bacteria from the jejunum into the stomach was more likely to occur.

Cultures of gastric juice in which growth was very heavy were similar to those of saliva. That the introduction of the nasogastric tube interfered very little with the accurate bacteriological evaluation of the gastric juice is attested to by the sterile gastric cultures, and the frequent finding of bacterial species in the nasopharynx that were not encountered in gastric cultures. In many of these instances conditions for growth were optimal for all organisms, but certain organisms had been selected out from the saliva. Examination of specimens with sparse growth at pH greater than 3.0 showed that the organisms most frequently found were also those most frequently found at low pH, although here also there has been selection because the range of frequency of these organisms was 23-30%.

Methods of culture had also given rise to another type of selection; organisms which grow *in vitro* only with elaborate cultural procedures were seldom or never seen in culture. However, they were commonly seen in stained smears of the gastric juice itself. These included Borrelia and fusiform bacteria. Special media for the isolation of the fastidious *Hemophilus influenzae* were also not used in this study.

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Little is known about the metabolic products of oral micro-organisms in the human stomach, although it is known that these organisms can produce toxins and enzymes that are potentially harmful. Also it is not known whether these toxic products come into contact with and penetrate the gastric mucosa.

The gastric mucin is considered to be a first line of defence of the cells of the gastric mucosa. Neuraminidase, by removing the sialic acid groups of gastric-gel mucin, renders it more susceptible to proteolytic breakdown;[9] in this way, neuraminidase partially reduces the protective effect of mucin. Thonard, Hefflin and Steinberg[10] demonstrated that 24 of 30 mixed oral cultures had a high neuraminidase activity on orosomucoid, and Lindstedt, Lindstedt and Gustafsson[11] found that a full intestinal flora degraded mucus *in vitro*. Both groups reported that a mixed flora was necessary before a significant degree of enzyme activity could be demonstrated.

Many of the salivary organisms in the resting stomach are potential toxin producers. Mergenhagen, Hampp and Scherp[12] have shown that six species of oral anaerobes—Veillonella spp., *Selenomonas sputigena, Bacteroides melaningenicus, Fusobacterium nucleatum, Borrelia buccalis* and *B. vincenti*—produce endotoxins and that the oral mucosa is sensitive to these endotoxins. When the stomach is in the resting state and the gastric juice contains a high enough level of pepsin and hydrochloric acid, masses of micro-organisms are presumably destroyed in a relatively short period. This then leads to a concomitant release of the bacterial endotoxins, a potentially important irritant.

The frequent appearance of bacteria within intact cells sloughed off from the mucosa into the gastric lumen suggests that mucin does not completely protect the mucosa from bacterial invasion but provides a protective envelope for the bacteria present in the gastric lumen. A similar observation regarding the role of mucus in the intestine was made some years ago by Florey.[13] He reported that mucus did not cover the epithelial cells completely and that there were many organisms inside the cells. Florey also demonstrated experimentally the ability of bacteria to penetrate the intestinal mucosa.

The undisputed presence of viable organisms in the stomach raises the tantalizing question whether there is a mechanism to protect micro-organisms. The most obvious possibility is the gastric mucin. Olitski[14] reviewed the role of mucin in the enhancement of the infectious process that follows intraperitoneal inoculation. He suggested the hypothesis, sustained for many years, that mucin simply coated the organisms, thus obstructing the antibacterial mechanisms of the host. Scherr[15] has presented new evidence which indicates that the effect is not simply one of a coating but may involve the reticuloendothelial system. Scherr also has pointed out that the most effective mucins have a large amount of ash, particularly of aluminium. *In vitro* studies by Sims[16] have demonstrated that lactobacilli and streptococci survive for considerably longer periods in mucin solutions. The mechanisms of action of the mucin in any of the processes mentioned are not clear. However, it is evident that the viscous, gastric gel mucin could coat the bacteria and protect the organisms from the deleterious effect of gastric acid and proteolytic enzymes.

The successful passage of the coli-dysentery-typhoid group through the stomach into the intestine is well established. In his original studies Knott[4] reported that mycobacteria and bacterial spores are unaffected by residence in the gastric lumen. However, many oral organisms have been found in the transient jejunal flora.[17, 18] These organisms survived the transit from the oral cavity to the jejunum. Where then is the great acid-barrier? Obviously a wider concept of the conditions governing the survival of micro-organisms in the human stomach is necessary; this need has been taken into account in proposing a *gastric let* mechanism, which will encompass all inhibitory and promoting factors[19] affecting natural gastric flora.

Little of the literature on gastric flora deals with studies on patients without gastrointestinal disease. In some studies, the bacteria found were disregarded as being oral;[20, 21] in others, the numbers of samples taken were too small to give a true representation. The inadequate bacteriological investigation in the older works further complicated an accurate evaluation of the fate of bacterial flora in the stomach.

It is possible that even minor changes in the gastrointestinal physiology disrupt the "normal" clearing of the flora. However, in disease states we have ample evidence that two effects are important: Firstly, the gastric flora is not adequately destroyed; and, secondly, the upper gastrointestinal tract is invaded by organisms from the colon. Barber and Franklin[22] studied the bacteria found in the stomach and duodenum in patients with gastric, duodenal and jejunal ulcer, and gastric cancer. They observed that tissues surrounding a chronic peptic ulcer are often edematous, the neighbouring lymph nodes are enlarged and inflammation is present. They postulated that bacteria may play a role in peptic ulcer. The organisms found were predominantly of the oral type and were of the same species as those reported here. They also reported that bacteria were present without achlorhydria and concluded that bacteria are a hazard at operation. Gastric juice samples from 11 of 27 patients with gastric ulcer contained viable organisms; all 12 of the samples from duodenal ulcer cases were sterile.

The upward migration of colonic flora in disease states was demonstrated by Goldstein, Wirts and Kramer[23] in patients with steatorrhea following subtotal gastrectomy. Many subjects in this group

developed an increased bacterial flora due to the establishment of intestinal organisms in the afferent limbs; simultaneously, the numbers of organisms in the stomach increased and colonic organisms predominated in the gastric juice.

Tamura[24] studied samples of gastric secretion obtained at laparotomy from patients with gastric cancer and reported rapid proliferation of streptococci, bacteroides, staphylococci, Candida, enterococci, enterobacteriaceae, lactobacilli, Veillonella and Fusobacterium. These findings indicate that a mixed oral and colonic flora can become permanent in the stomach, probably owing to stasis. Shiner, Waters and Cray[2] also found an increase in the numbers of Gram-negative bacilli in both stomach and jejunal juice in culture studies of patients with idiopathic steatorrhea. In our investigation, we have noted that individuals carrying species in the stomach that are normally resident in the intestine did not have these organisms in the nose or throat. In addition, when there were intestinal organisms in the stomach, they were present in large numbers.

The evidence presented in this communication demonstrates amply that the resting gastric secretion contains high counts of viable micro-organisms and that these are in quantities which cannot be disregarded. In the normal course of events, it is likely that a large proportion of these micro-organisms are eliminated by the digestive processes. However, the constant ingestion of saliva and drainage from the nasopharyngeal area provides the stomach with a rich and varied microflora. The organisms survive in relatively large numbers as long as the pH does not drop below 3.0; in the normal individual, this situation prevails for approximately one-third of the day. Further studies are needed to elucidate the time of and conditions for survival of the organisms in the stomach, with reference to the protective effect of gastric mucin on bacterial species isolated from the lumen.

SUMMARY

A total of 154 samples of gastric juice was obtained through a nasogastric tube from 149 fasting subjects ranging in age from 8 to 79 years; nose and throat swabs and a sample of saliva were also obtained. Eighty-two per cent of the gastric cultures were positive. Natural gastric flora was classified into three groups according to the frequency of occurrence of each species. The relative frequency of viable strains in each specimen was designated as *predominant, equally distributed* or *second in predominance.* The study of the effects of gastric pH has demonstrated an increase in the number of samples showing bacterial growth above pH 2.0 and a concomitant increase in the total bacterial growth. At low pH, certain organisms were selected out. The age distribution of subjects with positive gastric cultures was also studied.

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