GASTRIC FLORA OF FASTING HEALTHY SUBJECTS AND ITS RELATIONS TO pH

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Abstract

Samples of gastric juice were collected from 60 fasting subjects ranging from 10 to 80 years of age. Seventy-six per cent of the gastric cultures were positive. Natural gastric flora were classified into those organisms found frequently and sporadically. Effect of gastric pH on bacterial growth has indicated an increase in the number of samples showing growth above pH 5. Yeast was the only organism showing maximum growth at low pH. The age distribution of subjects in relation to positive gastric cultures was also studied (JPMA 30:113, 1980).

Introduction

A relationship between pH of gastric juice and positive gastric cultures has been reported by Drasar et al (1969). pH of gastric juice is intimately associated with both the quality and quantity of the gastric flora (Gray and Shiner, 1967). With the rise in pH, an increase in bac terial cultures was observed (Franklin and Skoryna, 1971). Presence of profuse bacterial flora including coliforms is found in markedly acid deficient individuals but in normal subjects it co-relates well with the absence of bactericidal activity (Giannella et al., 1972). Medications which decrease gastric acidity favour an increase in the growth of gastric bacteria while substances which lower the pH may decrease bacterial growth (Franklin and Skoryna, 1971).

This study was undertaken to determine the pH of fasting gastric juice from normal subjects and enumerate qualitatively and quantitatively the normal gastric flora of healthy subjects and its relation to pH.

Material and Methods

Collection of Specimer.

Samples of gastric juice were obtained from 60 fasting healthy subjects ranging from 14 to 80 years of age. In the morning after a 10-12 hours fast, a Ryle's tube was passed into the stomach and the sample was aspirated. The first sample was discarded and a second aspiration was performed and the specimen was processed either immediately or within 30 to 60 minutes after collection. Endoscopy was done in all the subjects to exclude any gastric pathology.

Determination of pH

The pH of the gastric juice was measured with a glass electrode pH meter.

Direct smear of gastric juice was made and stained by Gram's method.

Microbiological Techniques

Qualitative and quantitative aerobic and anaerobic bacterial counts were done on samples of gastric juice. Serial tenfold dilutions in sterile were prepared and streaked on the media listed in the table 1.

Table I

Organism	*Medium*	*Incubation*
Total aerobes	Tryptose Blood Agar	Aerobic 24 hrs 37°C
Total anaerobes	Tryptic soy Agar 5% sheep blood	Anaerobic 48-72 hrs 37°C
Coliforms	MacConkey's Agar	Aerobic 24 hrs 37°C
Lactobacilli	Rogosa S.L. Agar	Aerobic 96 hrs 37°C
Streptococci	Mitis Salivarius Agar	Aerobic 24-48 hrs 37°C
Staphylococci	Mannitol Salt Agar	Aerobic 24-48 hrs 37°C
Yeasts	Sabouraud dextrose Agar	Aerobic 72 hrs 28°C

Samples of 0.1 ml undiluted gastric juice were also streaked on the same media.
All bacteria isolated were subcultured to obtain a pure culture. Microorganisms were identified by colony characteristic, grams staining and biochemical reactions.

Results

The gastric flora obtained from sixty apparently healthy fasting subjects were studied.
In 46 samples (76.6 per cent) bacterial growth was demonstrated.
On direct microscopic examination nearly all the specimens of gastric juice contained many gram positive cocci and bacilli of varying shapes and sizes. Yeast cells were also frequently encountered.

Table II: Gastric Bacterial Flora of Fasting Subjects

Orgarisms	*BACTERIAL COUNT*		
	Mean	*S.D.*	*S.E.*
T. anaerobes	2.26	1.0	0.29
T. aerobes	2.61	0.94	0.23
Streptococci	3.00	1.39	0.62
Lactobacilli	2.86	1.19	0.42
Coliforms	2.28	0.70	0.31
Yeasts	2.19	0.96	0.18
Staphylococci	2.15	0.98	0.21

Table II shows the gastric bacterial flora of sixty fasting subjects with their mean and standard deviation and standard error. The low standard deviation indicated little variability of samples for each individual group of organisms.

The microorganism found frequently and sporadically in gastric juice are shown in Table III.

Table III: Frequency of Occurrence of Bacterial Species in Fasting Human Subjects

Organisms found frequently in gastric specimen 15-16%	Organisms found sporadically in gastric specimens (2-15%)
Yeasts 53%	Streptococci 13%
Staphylococci 36%	Coliforms 8%
Lactobacilli 15%	Diptheroids 8%

Group I includes the frequently isolated bacteria found in 15-60 per cent of specimens. Yeast, Staphylococci and Lactobacilli were included in this group. Organisms found sporadically were Streptococci, Coliforms and Dip-theroid isolated in group II which comprised of 2-15 per cent of positive cases.

Table IV: Gastric Juice at Different pH Range

pH range	*No. of samples*	*Samples with growth*	*Range of counts*
1.0-2.0	26	12	Undiluted-10^1
2.1-3.0	5	4	Undiluted-10^1
3.1-4.0	2	1	Undiluted 10^1
4.1-5.0	6	4	Undiluted-10^2
5.1-6.0	8	8	Undiluted-10^1
6.1-7.0	13	13	Undiluted-10^4
7.1-8.0	—	—	—

Table IV shows the viable growth of organism per ml of gastric juice found at pH ranges in increments of one pH unit. The ranges from 1-2 and 6-7 contained 86.6 per cent of all samples tested. At pH 5-7 all the samples isolated were positive for growth.

Table V: Effect of Gastric pH on Bacterial Growth

Organisms	*Positive sample at pH less than 3 showing growth*	*Positive sample at pH more than 3 showing growth*
T. aerobes	13 3%	58 6%
T. anaerobes	13 3%	38 4%
Yeasts	93.3%	73.1%
Staphylococci	33.3%	57.6%
Streptococci	0%	23.0%
Lactobacilli	0%	26.9%
Coliforms	0%	23.0%

Table V includes growth observed in gastric specimen and is based on isolation of strains from each sample regardless of viable counts. Organism found more frequently at pH less than 3 were yeast, while the most note worthy difference was found in Lactobacilli, Coliforms and Streptococci which were not isolated at this pH. Staphylococci, total aerobes and total anaerobes showed a decrease at pH 3. The microorganism isolated from the three sites of the gastrointestinal tract i.e., saliva, gastric juice and feces are shown in table VI.

Table VI: Bacterial Flora of Saliva, Gastric Juice and Feces from Healthy Subjects

No.	Age	Sex	Saliva	Gastric Juice	Stool
			pH culture	pH culture	pH culture
1.	45	F	6.4 Strept. Staph	1.2 Sterile	5.5 Strept, Staph, Coliform, Yeast
2.	30	M	6.0 Strept, Staph	1.5 Sterile	6.2 Strept, Staph, Coliform
3.	20	M	5.8 Strept, Staph	1.6 Sterile	6.0 Strept, Coliform
4.	40	M	6.9 Strept, Coliform, Staph	1.6 Yeast	6.2 Strept, Coliform, Staph, Yeast, Lactobacilli
5.	34	M	6.2 Strept, Staph, Coliform, Yeast Lactobacilli	1.7 Sterile	4.7 Strept, Staph, Coliform, Yeast
6.	45	F	7.0 Yeast, Staph, Coliform	2.1 Yeast, Staph	7.0 Yeast, Staph, Coliform, Strept
7.	75	M	5.7 Strept, Staph, Yeast	3.4 Strept, Staph, Yeast	6.3 Strept, Yeast, Coliform
8.	45	M	7.3 Yeast, Staph, Coliform, Strept, Lacto	4.7 Sterile	6.2 Coliform, Strept, Lacto-bacil
9.	35	F	6.4 Strept, Staph, Coliform	5.2 Strept, Staph	6.1 Strept, Coliform
10.	26	M	6.7 Strept, Staph, Lactobacilli, Coliform, Yeast	6.2 Strept Lactobacilli Coliform Yeast, Staph	6.2 Strept, Lactobacilli, Coli-form, Yeast

Samples of gastric juice with a pH below 2 were mostly sterile or showed the presence of acid resistant organism. With the increase in pH nearly all microorganisms isolated from the saliva were also found in the gastric juice ana feces of the same individual and those subjects whose gastric juice contained yeast also had yeast in their saliva.

The distribution of gastric flora in relation to age is shown in Table VII.

Table VII: Analysis of pH and Growth as Related to Age

Age range year	pH range	Mean pH	Total	Total specimen with growth	Total specimen pH less than 3.0	Specimen of pH less than 3.0 with growth	Specimen of pH 6 or more	Specimen of pH 6 or more with growth
10-29	1.4—6.7	3.58	23	15 (65.2%)	12 (52.2%)	3 (25%)	7 (30.4%)	7 (100%)
30-49	1.2—6.7	3.10	26	17 (65.3%)	14 (53.8%)	7 (50%)	5 (11.5%)	5 (100%)
50-69	1.8—6.7	4.82	7	7 (100%)	2 (28.5%)	2 (28.5%)	3 (42.8%)	3 (100%)
70-89	1.5—5.1	3.32	4	3 (75%)	2 (50%)	1 (50%)	—	—

Maximum number of samples were collected between the age groups of 10-49 years. pH range of each of these groups was not very different but in the age group of 70-89 years a slight difference was observed.

Discussion

The microorganisms isolated from the gastric juice in the present study were similar to those reported by Franklin and Skoryna (1966). Positive gastric cultures were obtained in 76.6 per cent of the subjects studied while Franklin and Skoryna (1971) reported positive cultures in 75 per cent and Bhat et al (1972) in 50 per cent of the control subjects.

Organisms found frequently in gastric samples were yeast, Staphylococci and Lactobaccilli. Other organisms such as Streptococci, Coliforms and Diptheroids were found sporadically. Scho-nebeck (1968) reported yeast like fungi in 57 per cent of normal subjects while yeast in this study was isolated in 53 per cent of individuals.

Giannella et al (1972) demonstrated strict pH dependence of the bactericidal activity of gastric juice. When pH of gastric juice was raised to greater than 4.0 no bactericidal activity was detected. Classification of organisms growing at pH greater than 3 indicated that survival of organisms in the gastric juice is greatly influenced by pH as shown in Table V. High pH of the acid deficient stomach permits oral and ingested bacteria to survive and multiply (Gorbach et al., 1967; Bhat et al., 1972). In normal subjects Drasar et al (1969) stated that gastric acidity is apparently influenced by the passage of bacteria in the stomach.

Evidence presented in this communication demonstrates that resting gastric juice contains viable microorganism and these are in quantities which cannot be disregarded. In the normal course of events it is likely that large proportion of these microorganism are eliminated by the digestive piocess. Constant ingestion of saliva and drainage from the nasal area provide the stomach with a rich and varied microflora. Sack et al (1972) speculated that normal gastric acidity was a natural barrier to infection and that pre-existing achlorhydria predisposed in- dividuals to infection. Steffen (1977) cited from (Editorial, 1978) reviews of the published work on achlorhydria as a factor in susceptibility to infection by the alimentary route and stated that this question should be kept in mind and indiscriminate use of antacids should be avoided as reports have indicated that decrease in acidity of gastric juice will lead to intestinal infection with Salmonella (Giannella et al., 1971), Escherichia coli (Dupont et al., 1971) and Brucella (Editorial, 1978).

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