Some tests of exocrine pancreatic function are based on the estimation of intestinal chymotryptic activity by using specific substrates such as N-benzoyl-L-tyrosyl-PABA (BT-P). In this study the action of a number of bacteria isolated from human or monkey intestines on BT-P was investigated. The monkeys studied had an intestinal flora closely resembling that of man. Except for Bacteroides, Proteus vulgaris No. 73, Proteus morgani No. 202, and Pseudomonas aeruginosa No. 76, significant splitting of BT-P did not take place when the common enteric microorganisms were tested. It was concluded that with the possible exception of bacterial overgrowth of the small bowel, microbial chymotryptic activity is not of importance.

Reviews of the pertinent literature (1–3) contain scant information concerning chymotrypsin-like activity of intestinal bacteria. Two methods of assessing exocrine pancreatic function had been described (4–6) which are based on the measurement of intestinal or fecal chymotrypsin activity by using specific substrates. The question arose as to whether intestinal bacteria commonly present in the intestine can split these substrates, particularly N-benzoyl-L-tyrosyl-p-aminobenzoic acid (BT-P). This peptide has been recently used as a new oral test of exocrine pancreatic function (PFT) (5–8). In the PFT, the tracer, p-aminobenzoic acid (PABA), is split from BT-P by pancreatic chymotrypsin in the small intestine. It is rapidly absorbed from the gut and excreted in the urine. Splitting of the compound by bacterial enzymes could potentially interfere with this test and cause false normal results when exocrine pancreatic deficiency is present (9). The present communication is the result of an examination of the action of some enteropathogenic and nonpathogenic bacteria isolated from man and nonhuman primates that could influence the outcome of the BT-P test. The possible participation of these bacteria in the total intestinal chymotryptic activity was also evaluated.

MATERIALS AND METHODS

Bacteria

If not specified, all organisms were isolated from nonhuman primates. Four strains of Aerobacter aerogenes, seven strains of Escherichia coli (serotype 04 and No. 166 of human origin), Proteus vulgaris (No. 73) and two Proteus morgani, four Enterobacter aerogenes, Serratia marcescens No. 78 and Serratia sp. No. 13, two each of Lactobacillus acidophilus (No. 16 of human origin), Bifidobacterium bifidum, Pseudomonas aeruginosa, five each of coagulase-positive nonenteropathogenic Staphylococcus aureus (No. 94 and 88 from human intestine), Enterococcus sp. (No. 88 and 119 from man), three Veillonella parvula and two Veillonella alcalescens (No. 38 and 49 from man), four Vibrio cholerae strains and two Vibrio parahaemolyticus from man, one Bacteroides intestinatis (No. 32 from man), two Bacteroides fragilis and one Bacteroides sp. as well as one strain of Clostridium perfringens were tested. All bacteria were cultured on appropriate media assuring optimal growth, then suspended in 0.14 M NaCl, pH 7.0, to contain $10^6 \pm 17$ (mean \( \pm \) std) bacteria per ml.
The bacterial filtrate was prepared by growing the respective microorganism overnight in appropriate media and environmental conditions. The growth was centrifuged at 3000g at 4°C, and the supernate was used in the experiments.

Gelatin liquefaction ("gelatinase" determination) was performed by growing the microorganisms in 10% gelatin (Difco), pH 7.3, for 3 days and testing for liquefaction after freezing the tube containing the gelatin.

**Chemical Tests**

The amount of liberated PABA was determined by the method of Imondi et al (10). The BT-P contained 32% PABA. One hundred-fifty mg of the substrate were dissolved in freshly prepared 0.2 M Tris (hydroxymethyl)aminomethane (TRIS), pH 7.8. One ml of the bacterial suspension or supernate was added, then 0.9 ml distilled water. After incubation at room temperature 25 ml of 5% trichloracetic acid were admixed. After 10 min, the precipitate was filtered through a Whatman No. 1 filter paper. One ml freshly prepared 0.1% solution of sodium nitrite was added to 10 ml of the filtrate. Four min. later 1 ml of a 0.5% ammonium sulfamate solution and 1 ml of 0.1% N-1 (naphthyl) ethylene diamine dihydrochloride were added. After 10 min, the intensity of the color was measured in the Coleman Junior colorimeter at 550 nm. Dilutions containing known concentrations of PABA were prepared for comparison. The results were expressed in percent of PABA liberated from BT-P.

When initial experiments were carried out to establish the optimal time and number of bacteria, large batches of bacteria or their supernates were mixed with the substrate and samples of 2.5 ml were drawn after 30 min, or 2, 3, 6, 9, and 16 hr incubation at room temperature. The percent of liberated PABA was determined and bacterial counts made by plating. Tenfold dilutions of the samples of bacteria-substrate mixtures were plated to media suitable for the growth of the respective microorganisms and colonies counted after incubation at 37°C.

**RESULTS**

Optimum growth rates were obtained over a concentration range of 10^8-10^6 cells/ml. The data in Table 1 show typical growth data for two bacterial strains, *V. cholerae* Inaba No. 18 and *Bacteroides* sp. No. 43. Although hydrolysis of BT-P was negligible during the initial 6 hr of incubation, approximately 14% and 16% of the BT-P was cleaved within 16 hr.

Table 2 shows the amount of BT-P hydrolyzed by several bacterial strains during a 16-hr incubation period. In each case, the cell-free supernatants were also capable of hydrolyzing BT-P. There were all *Proteus morgani* and three other strains which were unable to liquefy gelatin: *A. aerogenes* No. 18, *E. coli* No. 43, and *E. coli* No. 166.

In addition to the active strains shown in Table 2, there were several organisms which were unable to hydrolyze BT-P, ie, less than 2% hydrolyzed in 16 hr. These included three *Aerobacter*, five *E. coli*, one *Pseudomonas*, four *Enterobacter*, two *Lactobacillus*, two *Flavobacter*, two *Bifidobacterium*, and five each of *Staphylococcus* and *Enterococcus*, and Veillonella strains.

**DISCUSSION**

The splitting of BT-P did not take place to a significant extent when common gram-negative enteric microorganisms were tested, except with some *Pseudomonas*, *Bacteroides*, and *Proteus* strains. The reaction required a considerably longer incubation (6 to 16 hr). Enteropathogenic vibrios that are known to elaborate various proteinases (ie, table 2, No. 11-13) produced hydrolysis of BT-P at a higher rate than bacterial supernates which contained various substances loosely designated as exotoxins (ie, Table 2, No. 1-9).

The small intestinal flora, if present at all, does not normally contain Enterobacteriaceae or anaerobic bacteria (11, 12). Therefore, orally administered BT-P is not likely to be split by bacteria in the small bowel. However, degradation of BT-P by colonic bacteria may be expected if the test period is extended beyond 6 hr as suggested by Bornschein et al (8) and Gyr (5) or if the passage of the chyme containing BT-P is accelerated and reaches the colon and its flora within a short time. Imondi et al (13) could indeed demonstrate that intracecally administered BT-P was degraded to a moderate extent (10%) in rats within the 6-hr test period. Splitting by anaerobes and Enterobacteriaceae is a likely explanation for their results. Various diseases are associated with bacterial contamination of the small bowel such as achlorhydria, diverticulosis of the small intestine, and cirrhosis (11). In this situation, bacterial chymotryptic activity may be of some importance, although serious interference with the PFT is not likely because of the short time of exposure.

There are only sporadic and controversial data on small-intestinal flora in chronic pancreatitis (14). However, this is an important subject which may be relevant to the interpretation of the PFT. Perhaps alterations of the jejunal flora represent one of the reasons for the few false normal PFT in patients with chronic pancreatitis (6). Although the present work addresses itself solely