larity of the second acid grouping to the first suggests that its pKa is similar to that of the first. It seems probable, therefore, that all three species are present to some extent in the pH 7.4 buffer of these investigations.

The UV curve of bishydroxycoumarin at pH 7.4 shows a peak at 303 nm., a shoulder above 310 nm., and a binding to bovine serum albumin. There are a slight peak shift and a diminished absorbance, as shown by the differential UV plot of Fig. 3. This plot shows a large decrease in absorbance on binding for D/P ratios greater than 1.5 at wavelengths near 315 nm. The lack of pKa knowledge and the possibility of tautomerism make the interpretation of the effect of pH on UV data impossible at this time, and the CD peaks cannot be associated with any specific species of bishydroxycoumarin. It is possible that the observations reported here are due to the different binding of the various ionized or unionized forms of bishydroxycoumarin.

Another possible explanation for the CD data is that there is a primary binding of a single molecule of bishydroxycoumarin followed by association of more drug molecules at the binding site, as has been suggested for the binding of dyes to polypeptides (9, 10). However, no new peaks nor wavelength shifts are observed at high D/P ratios, apparently obviating the possibility of self-association involving drug chromophores.

Perhaps a more probable explanation for the break in observed ellipticity against D/P ratio plot and the difference in behavior at the two wavelengths is that there is a primary binding site and a series of other less specific binding sites on the bovine serum albumin molecule, with binding to both types of sites occurring to some extent simultaneously. The less specific nature of this secondary binding may be supported by the optical activity being induced only at the higher wavelengths. It is possible that the secondary binding sites become available only after the primary binding, although there is no major conformational change on drug binding.

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[5] Since this work was completed, C. F. Chignell [*Mol. Pharmacol.*, **6**, 1(1970)] studied the binding of bishydroxycoumarin with human serum albumin by CD. He found that after 3 moles of drug was bound per mole of protein, little or no change in ellipticity was seen. The authors confirmed this finding themselves and it is in sharp contrast to the data reported here for bovine serum albumin. These large species variations are currently being investigated.

Pseudomonas aeruginosa Contamination of Liquid Antacids: A Survey

ELIZABETH P. ROBINSON

Abstract □ In a recent survey, 279 retail packages of liquid antacid containing magnesium hydroxide as an active ingredient from 11 manufacturers, 21 raw materials, and six in-process samples were examined for *Pseudomonas aeruginosa*. Eighty-five of the finished bottles (30.5%) and two in-process samples (33%) were positive. The aerobic plate count ranged from <100 to 9,300,000 organisms/g. Nineteen of the total samples (6.8%), including one water sample used as a raw material, were contaminated with coliforms or *Alcaligenes* sp. These samples had an aerobic plate count from <100 to 500,000 organisms/g.

Keyphrases □ *Pseudomonas aeruginosa* contamination—liquid antacids, survey □ Antacids, liquid—contamination by *Pseudomonas aeruginosa*, survey □ Contamination, *Pseudomonas aeruginosa*—in liquid antacids

Contamination of nonsterile pharmaceuticals by pathogenic yeasts, molds, and bacteria is rapidly becoming a matter of worldwide concern (1–7). This laboratory recently encountered bacterial contamination in liquid antacids manufactured by pharmaceutical firms in northeastern United States. Previous experience with contamination of liquid antacids had revealed the presence of *Pseudomonas aeruginosa*. In a recent survey of antacid products of a similar type, with samples obtained both during manufacturing and from the finished product, the author encountered a high level of bacterial contamination, with *P. aeruginosa* being the predominating organism. Magnesium hydroxide was the common active ingredient in all products examined.

Table I—Tests to Differentiate *Pseudomonas aeruginosa* from *Alcaligenes* sp.

Test	*P. aeruginosa*	*Alcaligenes* sp.
Glucose utilization	Oxidation	Nonutilized
Cytochrome oxidase	+ (>2 min.)	+ (>2 min.)
Nitrate reduction	NO_2 or N_2	NO_2 or N_2
Gelatin liquefication	+	−
Arginine dihydrolase production	+	−
Growth at 42°	+	+
Motility	+	+
Fluorescence	+	−
Pigment	Pyocyanine Pyorubrin	−
Flagella	1 Polar	Peritricious

EXPERIMENTAL

In this survey, 279 retail packages of liquid antacid representing 11 manufacturers and 34 different labels, 21 raw materials, and six in-process samples were examined.

A sample portion of 10 ml. or 10 g. was thoroughly mixed with

Table II—Results of a Survey for Bacterial Contamination of Liquid Antacids, In-Process Samples, and Raw Materials

Type of Sample	Number Tested	*P. aeruginosa*	*Alcaligenes* sp.	Coliforms
Raw materials	21	0	1	0
In-process	6	2	0	0
Finished bottles	279	85	16	2

A summary of contaminated samples from the three manufacturers is presented in Table III. From the wide aerobic plate-count range, it can be readily seen that *Pseudomonas* contamination is extremely nonuniform, even within a given batch. By enrichment procedures, it is possible to recover small numbers of *Pseudomonas* from contaminated products.

Samples of magnesium hydroxide and other major raw materials used in manufacturing the contaminated batches of antacid were not available for testing. The raw materials examined included six samples of magnesium hydroxide used in the manufacture of non-

Table III—Summary of Contaminated Samples

Manufacturer	Aerobic Plate Count	Total Samples Tested	*P. aeruginosa* Number Positive	*P. aeruginosa* Percent	Other Gram-Negatives Number Positive	Other Gram-Negatives Percent
A	<100–9,300,000	132	65	49.2	16	12.1
B	36,000–165,000	16	5	31.3	0	0
C	100,000–361,000	17	15	88.2	2	11.8

90 ml. of sterile distilled water containing 1% of a surfactant[1], and 10-fold serial dilutions to 1:100,000 were prepared in sterile distilled water. One milliliter of each dilution and 1 ml. of the undiluted product were seeded into both 10 ml. of tryptic soy broth (Difco) and 10 ml. of Koser citrate medium (BBL). After 48 hr. incubation at 35°, each dilution was streaked on plates of desoxycholate citrate agar (Difco) and incubated for 24 hr. Typical colonies were transferred to triple sugar iron agar slants. All cultures giving alkaline slant and butt reactions, with or without hydrogen sulfide production, were transferred to biochemical media for identification.

The cytochrome oxidase test of Ewing and Johnson (8), glucose utilization using OF Medium (BBL) (8), growth at 42° (9), gelatin liquefication (10), arginine dihydrolase production (11), fluorescence under longwave UV light on *Pseudomonas* agar F (Difco) containing 1% glycerol, and production of pyocyanine or pyorubrin water-soluble pigments on Tech agar (BBL) (11) were performed. Difco nitrate broth containing 0.5% bacto-agar was employed for the nitrate reduction test. The 0.5% agar gave tentative motility results, which were substantiated by wet mount preparations and Bailey's flagella stain (10). Cultures giving positive reactions according to the scheme of Sutter (11) were considered to be *P. aeruginosa* (Table I).

Aerobic plate counts were performed as described elsewhere (12), using tryptic soy agar (Difco) as the plating medium.

RESULTS AND DISCUSSION

Eighty-five of the finished product samples (30.5%), all from three manufacturers but representing 24 private labels, and two in-process samples, were positive for *P. aeruginosa* (Table II). The aerobic plate-count range for all contaminated samples was from <100 to 9,300,000 organisms/g. Samples contaminated with other Gram-negative organisms had a plate-count range from <100 to 500,000 organisms/g. All samples from which no Gram-negative organisms were isolated had plate counts of <100 organisms/g.

The original cultures isolated were subjected to more extensive biochemical testing. The tests used proved to be the most valuable in identification along with the consistent presence of pyocyanine and/or pyorubrin pigment in all cultures identified as *P. aeruginosa*. These cultures were also examined by electron microscopy to determine the position of the flagella. Unidentified cultures classed as *Alcaligenes* sp. were late cytochrome oxidase positive (greater than 2 min.), did not utilize glucose, and had peritricious flagella. These were isolated from one sample of water used as a raw material and from 16 finished product samples. Coliforms fermented lactose on desoxycholate citrate agar or gave an acid reaction on triple sugar iron and were isolated from five finished product samples.

[1] Tergitol Anionic 7, Union Carbide.

contaminated samples (aerobic plate counts of <100 organisms/g.).

The effect that *P. aeruginosa* has on the composition, and possibly on the therapeutic activity, of antacid preparations still needs to be studied. No changes in color or odor were noted in the *Pseudomonas*-contaminated samples tested. However, two batch samples contaminated with coliforms had a strong ammonia-like odor rather than the characteristic odor of peppermint, suggesting that some product decomposition had occurred.

The high level of contamination with a potential pathogen in commercial antacids from a few manufacturers indicates that a need exists for control of all raw materials (including the manufacture of extracted natural materials such as magnesium hydroxide from sea water), manufacturing processes of nonsterile drugs, and finished product material.

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