the variation of dosage form mass were not evaluated in this investigation.

SUMMARY

The theories of Kakemi *et al.* (1) that relate the dielectric constants of water-miscible vehicles to rectal absorption have been extended to include a water-immiscible vehicle and have been applied to a practical formulation-absorption problem in humans.

REFERENCES

(1) K. Kakemi, T. Arita, and S. Muranishi, *Chem. Pharm. Bull.*, **13**, 969(1965).
(2) A. N. Paruta and S. A. Irani, *J. Pharm. Sci.*, **54**, 1334 (1965).
(3) M. Hobel and M. Talebian, *Arzneim.-Forsch.*, **10**, 653 (1960).
(4) K. Salzmann, *Therapiewoche*, **12**, 1034(1962).
(5) E. Nelson and T. Marioka, *J. Pharm. Sci.*, **52**, 864(1963).
(6) A. J. Cummings, M. L. King, and B. K. Martin, *Brit. J. Pharmacol. Chemother.*, **29**, 150(1967).
(7) "Handbook of Chemistry and Physics," 39th ed., Chemical Rubber Pub. Co., Cleveland, Ohio, 1958, p. 2343.
(8) *Ibid.*, p. 2335.
(9) *Ibid.*, p. 2340.
(10) W. D. Walkling and R. F. Shangraw, *J. Pharm. Sci.*, **57**, 1927(1968).
(11) B. B. Brodie and J. Axelrod, *J. Pharmacol. Exp. Ther.*, **94**, 22(1948).
(12) "The National Formulary," 13th ed., Mack Publishing Co., Easton, Pa., 1970, p. 882.
(13) *Ibid.*, p. 16.
(14) K. T. Koshy and J. L. Lach, *J. Pharm. Sci.*, **50**, 113(1961).

ACKNOWLEDGMENTS AND ADDRESSES

Received August 17, 1970, from the *School of Pharmacy, University of Maryland, Baltimore, MD 21201*

Accepted for publication October 7, 1970.

The authors acknowledge the financial assistance of the American Viscose Division, FMC Corp., Marcus Hook, Pa.; Eli Lilly and Co., Indianapolis, Ind.; and the Yager Drug Company, Baltimore, Md.

*Graduate Fellow of the American Foundation for Pharmaceutical Education.

Extrinsic Cotton Effects of Bishydroxycoumarin when Bound to Bovine Serum Albumin

J. H. PERRIN and P. IDSVOOG

Abstract □ The binding of bishydroxycoumarin to bovine serum albumin at pH 7.4 was investigated by circular dichroism and differential absorption. No major conformational changes were observed on binding, but optical activity was induced at wavelengths above 290 nm. and investigated for various drug-to-protein ratios. Several possible explanations for the experimental observations are made.

Keyphrases □ Bishydroxycoumarin—extrinsic Cotton effects when bound to bovine serum albumin □ Albumin, bovine serum—bound bishydroxycoumarin, extrinsic Cotton effects □ Circular dichroism—analysis □ Differential absorption—analysis

Bishydroxycoumarin[1] was found to be over 99% bound to human plasma proteins, even when the concentration in whole blood was 1000 mg./l. (1). Paper electrophoresis studies suggest that the drug is entirely bound to the albumin fraction (2). In this report the binding of bishydroxycoumarin to bovine serum albumin in 0.1 *M* pH 7.4 phosphate buffers is investigated. Other highly protein bound drugs which have shown extrinsic Cotton effects include the *N*-arylanthranilates (3) and the pyrazolone analgesics (4, 5).

MATERIAL AND METHODS

Bishydroxycoumarin USP was used as supplied[2]. The bovine serum albumin was crystallized and lyophilized[3]. All buffer materials were reagent grade. The circular dichroism (CD) spectra were obtained at 25° in 5-, 10-, or 20-mm. cells, using the 6002 attachment to a Cary 60 spectropolarimeter[4]. The dynode voltage was never allowed to exceed 0.35, and the signal-to-noise

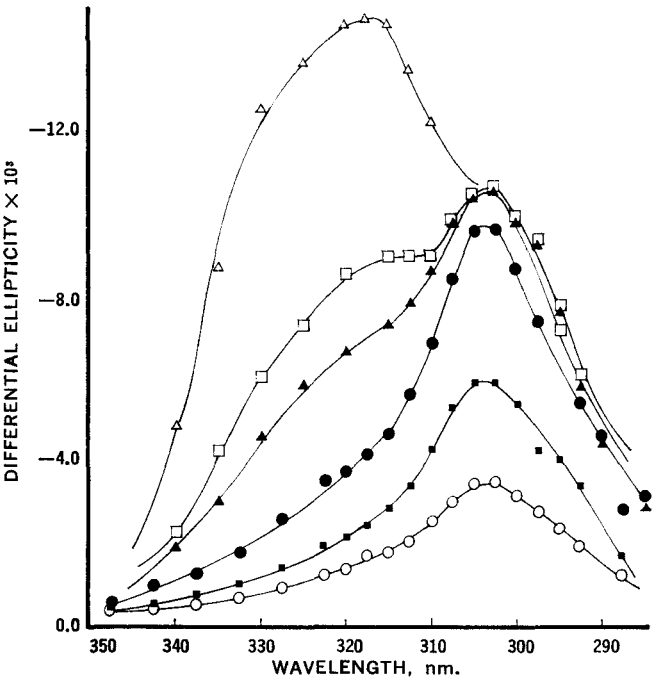

Figure 1—*Extrinsic CD curves for bishydroxycoumarin binding to bovine serum albumin measured in 0.1* M *phosphate buffer, pH 7.4, corrected to 1-cm. pathlength. D/P molar ratios are:* △, *10.26;* □, *6.16;* ▲, *5.13;* ●, *1.44;* ■, *0.82; and* ○, *0.4.*

[1] Dicumarol.
[2] Abbott Laboratories, North Chicago, Ill.
[3] Sigma Chemical Co., St. Louis, Mo.
[4] Cary Instruments, Monrovia, Calif.

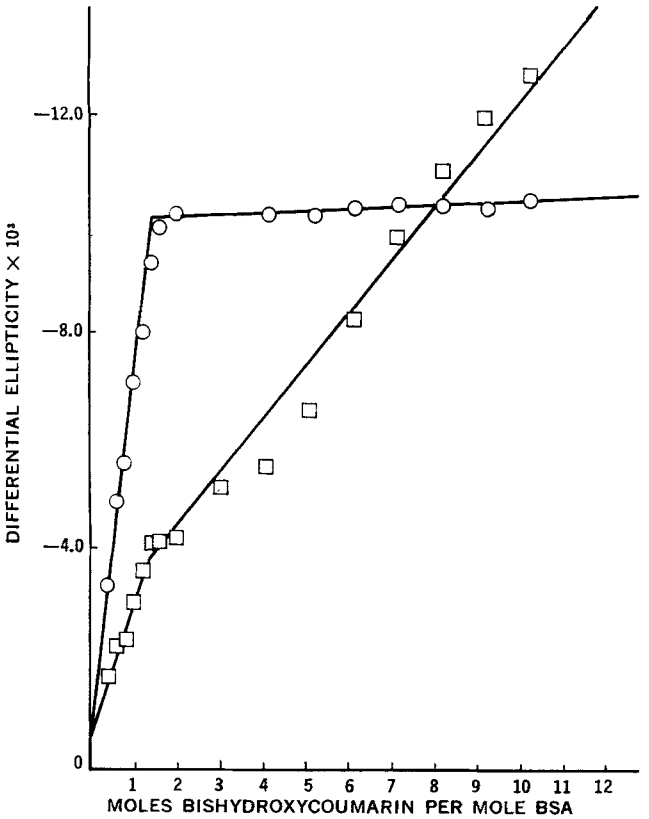

Figure 2—*Plot of observed ellipticity against D/P ratio for bishydroxycoumarin binding to bovine serum albumin. Key:* ○, *wavelength of 303 nm.; and* □, *wavelength of 318 nm. corrected to 1-cm. pathlength.*

ratio was always greater than 40 for the optically clear solutions. CD data are presented as differential ellipticity, which is taken as the observed ellipticity minus the ellipticity due to the constant concentration of serum albumin at the same wavelength. The differential UV spectra were obtained by determining the spectra of the drug alone, the protein alone, and then the mixture, using the buffer as the reference in a Cary 14 spectrophotometer.

A 1.45×10^{-5} *M* bovine serum albumin concentration (mol. wt. 69,000) and drug concentrations up to 1.49×10^{-4} *M* were used in these investigations.

RESULTS AND DISCUSSION

The extrinsic Cotton effects produced above 290 nm. are shown in Fig. 1; any small contribution of the protein was subtracted from these observed ellipticities. Below 290 nm., the ellipticity of the protein becomes dominant and the absorbance of the solutions rises so that good quantitation is impossible and, as such, is not reported. Investigations in shorter pathlength cells and on diluted solutions, down to 200 nm., suggest that there is little or no departure from the α-helical structure associated with bovine serum albumin at pH 7.4.

Various drug-to-protein (D/P) ratios were used, and the shapes as well as the magnitudes of the induced CD curves were dependent upon this ratio (Fig. 1). At low D/P ratios, there is a peak at 303 nm. and a slight shoulder at 318 nm. However, at higher D/P ratios, this shoulder develops into a dominant peak. Figure 2 shows the plot of observed ellipticity against D/P ratio at fixed wavelengths of 303 and 318 nm. Both plots show a break at a D/P ratio of approximately 1.5; there is a leveling off of induced ellipticity at 303 nm. above this D/P ratio, but there is a continued increase in ellipticity at 318 nm. with all solutions investigated.

The explanations of these differences and the break at the D/P ratio of 1.5 are complicated by the lack of information of the pKa's of bishydroxycoumarin. Bishydroxycoumarin is a dibasic acid whose pKa's are extremely difficult to measure because of poor aqueous solubility and instability in extremes of pH. However, values of pKa_1 have been reported as 5.7 (6) and 6.5 (7); the simi-

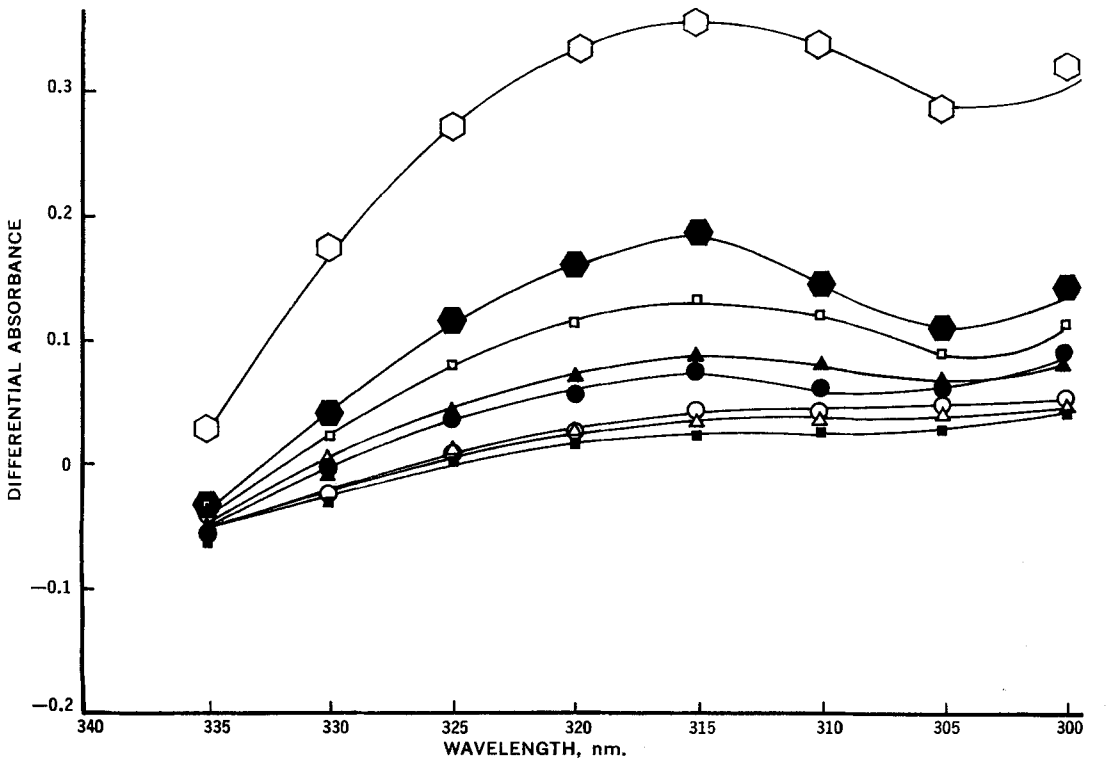

Figure 3—*Differential UV plot for binding of bishydroxycoumarin to bovine serum albumin. Differential absorbance = absorbance of drug alone + absorbance of protein alone − absorbance of mixture of drug and protein in 0.1* M *phosphate buffer, pH 7.4, in 1-cm. cells. Key:* ○, *5.13;* ●, *4.10;* □, *3.08;* ▲, *2.46;* ●, *2.05;* ○, *1.44;* △, *1.03; and* ■, *0.82.*

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.

REFERENCES[5]

(1) M. Weiner, S. Shapiro, J. Axelrod, J. R. Cooper, and B. B. Brodie, *J. Pharmacol. Exp. Ther.*, **99**, 409(1950).

(2) R. A. O'Reilly, P. M. Aggeler, and L. S. Leong, *Thromb. Diath. Haemorrh.*, **11**, 1(1964).

(3) C. F. Chignell, *Mol. Pharmacol.*, **5**, 455(1969a).

(4) C. F. Chignell, *Life Sci.*, **7**, 1181(1968).

(5) C. F. Chignell, *Mol. Pharmacol.*, **5**, 244(1969b).

(6) J. J. Burns, S. Wexler, and B. B. Brodie, *J. Amer. Chem. Soc.*, **75**, 2345(1953).

(7) J. Axelrod, J. R. Cooper, and B. B. Brodie, *Proc. Soc. Exp. Biol. Med.*, **70**, 693(1949).

(8) R. Nagashima, G. Levy, and E. Nelson, *J. Pharm. Sci.*, **57**, 58(1968).

(9) E. R. Blout and L. Stryer, *Proc. Nat. Acad. Sci., USA*, **45**, 1591(1959).

(10) E. R. Blout, *Biopolym. Symp.*, **1**, 397(1964).

ACKNOWLEDGMENTS AND ADDRESSES

Received April 13, 1970, from the *School of Pharmacy, University of Wisconsin, Madison, WI 53706*

Accepted for publication October 6, 1970.

[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