# DIFFERENCES IN THE CIRCULAR DICHROIC SPECTRA OF VARIOUS SPECIES SERUM ALBUMIN COMPLEXES WITH NOVOBIOCIN*

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SUMMARY

The circular dichroic spectra of 8 different species albumin-novobiocin complexes appear to be unique. In the spectral region of interest a chromophore of the novobiocin is perturbed by the complexing with the albumin. Each species albumin imparts a different amount of perturbation which results in a unique circular dichroic spectrum. At least a part of this perturbation is a conformational change induced in the drug.

Although a vast number of studies have been carried out on and with serum albumin there is still only a small amount of information available concerning species differences. Chemically, most albumins behave similarly in their reactions with ions and molecules of numerous types, although the extents may be somewhat different. Much of the early work was done with bovine serum albumin because of its accessibility in crystalline form, and many generalizations have resulted from these studies (1).

More recently optical rotatory dispersion measurements and the related circular dichroic spectra have been used to show differences among various species albumins. Blauer, et al. (2,3) have shown that the bilirubin complexes of human and bovine albumins give vastly different extrinsic Cotton effects. Chignell (4) used the drug flufenamic acid complexed with various albumins and showed by means of the Cotton effects that human, bovine, porcine and equine albumins possess similar binding sites which were different from those of canine, ovine, and rabbit albumins.

We would like to report here some interesting observations in the circular dichroic spectra of the drug novobiocin, a coumarin derivative, and of

its complexes with various species albumins. The initial observation that the circular dichroic spectrum of the human albumin-novobiocin complex was vastly different from that of the bovine albumin-novobiocin complex although their respective ultraviolet absorption spectra were very similar has already been reported (5). The circular dichroic spectra of the novobiocin compounds of 6 other species albumins have been measured, and each spectrum appears to be unique.

## MATERIALS

Sheep, canine, rabbit, horse and porcine Cohn fraction V albumins were obtained from Pentex Corp. Crystalline bovine albumin was obtained from Pentex and crystalline human albumin from Grand Island Biochemical Co. All the albumins were purified further by successive dialyses against 0.5M NaCl, 0.1M NaCl and deionized water. Rat albumin was isolated from rat serum by the acid-methanol procedure (6) and passed through a Sephadex G-200 column (7) to isolate the monomer. Rabbit and porcine albumins were deionized according to the method of Dintzis (8).

The novobiocin sodium, lot ZD-692, was the generous gift of Dr. G. B. Whitfield of the Upjohn Co., Kalamazoo, Michigan and was used without further purification. This drug exhibited a molar extinction coefficient at 304 nm in 0.1M sodium phosphate buffer, pH 7.4 of $2.10 \times 10^4$ 1/M per cm. All other chemicals were reagent grade.

## RESULTS

The circular dichroic spectra of the 8 different albumins and of their novobiocin complexes are shown in Figure 1. The observed ellipticity is reported because the molecular weights of some of the albumins are not well documented. Since the drug itself has a non-zero ellipticity in the region of interest (5), any amount of drug uncomplexed in solution with the drug-albumin complex will contribute to the observed ellipticity. Consequently, after using ultrafiltration to determine the concentration

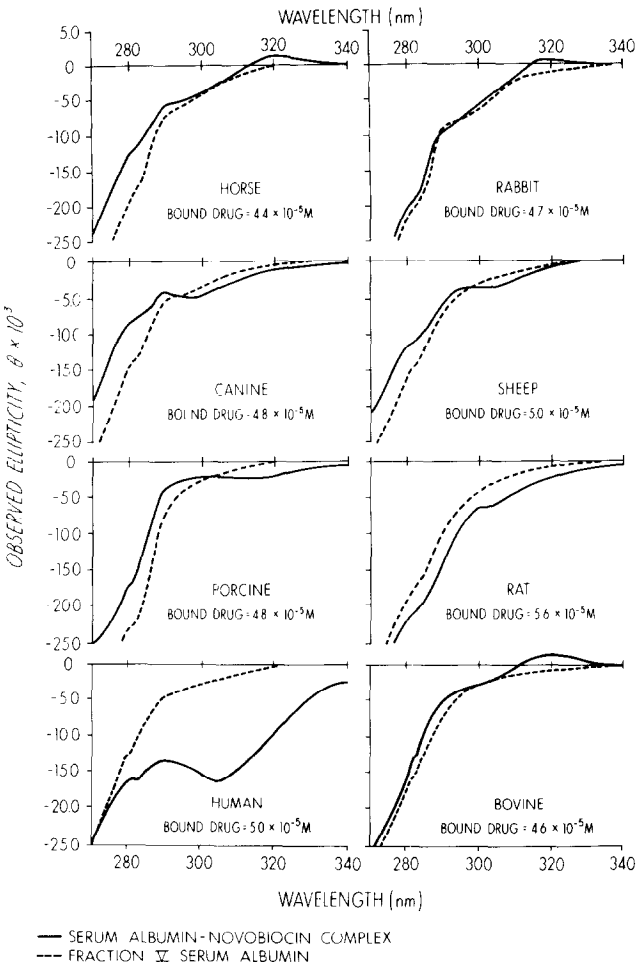


Figure 1. Circular dichroic spectra of several serum albumins and their respective novobiocin complexes. Protein concentration for all curves was 0.24%. Total novobiocin, bound plus free, was $6.0 \times 10^{-5}$ M. The amount of drug bound to each albumin was determined by ultrafiltration and appears on each spectrum. The ellipticity due to the uncomplexed drug has been subtracted from the observed ellipticity of the mixture in order to obtain the observed ellipticity of the complex only (solid line above). All measurements were taken in 0.1M sodium phosphate buffer, pH 7.4, 38°C.

of free drug in the novobiocin-albumin mixture, the contribution to the observed ellipticity of the mixture due to the free drug was subtracted out of the raw datum curve to obtain the spectrum of the complex. All of the albumins free in solution give the same general shape spectra (the dashed curves). The horse, bovine and rabbit complexes exhibit a positive

peak centered around 318 nm to 320 nm. This peak corresponds to the intense transition of the drug which appears at 304 nm. In contrast to these two, the other five complexes show the 304 nm transition to be inverted with the largest difference in observed ellipticity between the free albumin and the complex occurring around 304 nm. For the porcine complex, however, this negative peak occurs at 316 nm.

If a molecular weight of 69,000 were assumed for these albumins, the average ratio of bound drug to protein in Figure 1 would be 1.4 The spectra of higher ratio novobiocin to albumin complexes were also examined for four of the albumins, and three of these are shown in Figure 2. These are higher ratio complexes for bovine serum albumin, purified monomer deionized rabbit serum albumin and purified monomer deionized porcine serum albumin. Novobiocin complexed with human albumin at higher ratios produced only a small increase in rotation with no further change in the character of the spectrum of the complex. Its spectrum is not shown. The spectra in Figure 2 show that higher ratios of drug to albumin than those shown in Figure 1 result in further qualitative and quantitative changes of the spectra particularly below 300 nm. Those changes could be indicative of heterogeneity of the binding sites.

## DISCUSSION

The sign (+ or -) of the ellipticities in the circular dichroic spectra gives an additional dimension to show differences. Where similarities appear at low binding ratios of drug to albumin, differences may be enhanced at higher binding ratios. This may be seen in comparing the spectra of rabbit albumin and bovine albumin complexes at higher novobocin ratio shown in Figure 2. Here the two spectra may be seen to be considerably different where they are quite similar when the ratio of drug to albumin is near unity. It is of further interest to note that none of the bound drug at the ratios tested exhibited a circular dichroic spectrum similar in position and intensities to that of the unbound drug (5).

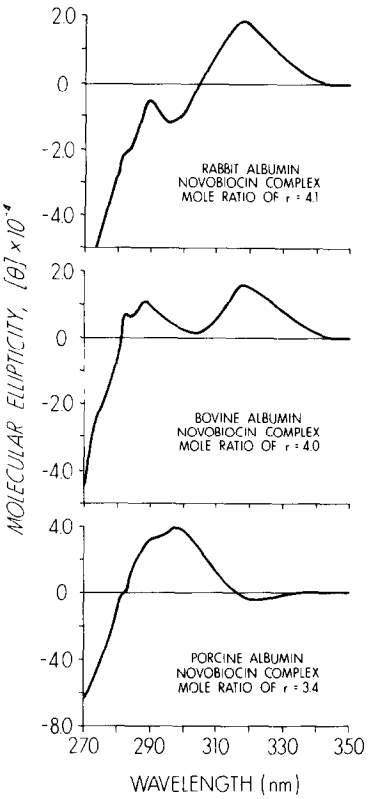


Figure 2. Circular dichroic spectra of three high ratio novobiocin-albumin complexes. Protein concentration was 7.2 X $10^{-5}$M (69,000 mol. wt.) while total novobiocin, bound plus free, was 3.6 X $10^{-4}$M. The mole ratio of bound drug to albumin appears on each spectrum. The ellipticity due to the uncomplexed drug was subtracted from the observed ellipticity of the mixture before calculation of the molecular ellipticity of the complex. Solvent was 0.1M sodium phosphate buffer, pH 7.4, 30°C.

Much of the published work on the binding of albumin to drugs with symmetric chromophores have attributed the appearance of circular dichroism to an extrinsic effect in which the protein perturbs the chromophore. In the case of novobiocin, it has been demonstrated that the circular dichroic spectrum of the drug alone in solution could be perturbed by a change in the pH of the solution (5). Solvent polarity also seems to affect its conformation (5). A change in pH from 7 to 1 brought about an inversion in sign of the ellipticity as well as a bathochromic shift in the peak. Space filling models of novobiocin show that rotation of a benzamido group could

affect the chromophoric group intrinsicly. It is postulated here that reactions of novobiocin with the albumins from various species force the benzamido group into positions unique to each albumin. This would qualitatively explain the unique spectra. This does not rule out additional extrinsic effects which might be caused by perturbing groups of the protein.

We have demonstrated a technique and a reagent which may be used to distinguish albumins from different species. Further investigation is necessary to yield information which will implicate specific portions of the albumin molecules.

NOTE: We are including a circular dichroic spectrum of Novobiocin alone at pH 7.4 for the benefit of the referees. Since this spectrum has already been published we feel that it should not be included in this paper.

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