# Protein Binding of Benz[a]anthracene and Benzo[a]pyrene

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**Abstract** □ Fluorometric studies on the binding of benz[a]anthracene and benzo[a]pyrene to human serum albumin are described. The protein molecule appears to have one binding site for the hydrocarbons, but all of the sites on the protein are not fully occupied even in relatively large hydrocarbon concentrations. Equilibrium studies showed that both hydrocarbons bind to the protein to the same extent. Evidence for the energy transfer from the tryptophan residue of the protein to bound hydrocarbons is examined. By using Förster's theory, the mean distance between the tryptophan residue and bound ligand was found to be 15.2 Å for benz[a]anthracene and 19.6 Å for benzo[a]pyrene. It is concluded that the two hydrocarbons may bind to the same general area on the protein molecule near the tryptophan residue but at different sites. The structural differences of the hydrocarbons, which may greatly affect their orientations on the protein molecule, affect mainly the selection of the binding site rather than the binding equilibrium.

**Keyphrases** □ Benz[a]anthracene—binding to human serum albumin, fluorometric analysis □ Benzo[a]pyrene—binding to human serum albumin, fluorometric analysis □ Binding, protein—benz[a]anthracene and benzo[a]pyrene to human serum albumin, fluorometric analysis □ Fluorometry—binding of benz[a]anthracene and benzo[a]pyrene to human serum albumin □ Hydrocarbons—benz[a]anthracene and benzo[a]pyrene, binding to human serum albumin, fluorometric analysis □ Carcinogens—benz[a]anthracene and benzo[a]pyrene, binding to human serum albumin, fluorometric analysis

Carcinogenic hydrocarbons are biologically active but chemically inert. Because of the aromatic system, these hydrocarbons are capable of forming complexes with macromolecules *via* hydrophobic stacking forces. The hydrophobic interaction is not uncommon in biological systems. For example, a nucleic acid possesses profound hydrophobic stacking interaction between its bases (1, 2), and the binding of protein to nucleic acid involves the stacking interaction between the tryptophan residues of the protein and the nucleic acid bases (3, 4).

The hydrophobic binding of the carcinogenic hydrocarbons with proteins or other macromolecules is important for several reasons. Carcinogenesis may be initiated by the covalent binding of the carcinogens with cell constituents (5–7). Therefore, the hydrophobic binding represents the initial step toward carcinogenesis. Transport of the carcinogens to active receptor sites and the activation of parent carcinogens are also necessary for chemical reaction.

Furthermore, because of the extensive, delocalized $\pi$-electron system, these hydrocarbons can be easily activated into long-lived free radicals (8), which may efficiently form covalent bonds with macromolecules if a strong hydrophobic stacking interaction occurs. Therefore, binding information such as the nature of the binding sites on the macromolecule should help in understanding the mechanism of carcinogenic action. Besides the plausible involvement of carcinogenesis, the interaction of these hydrocarbons with macromolecules may result in other serious biological effects yet to be known, since such binding may effectively change the conformations of the macromolecules and, consequently, alter the precision of their physiological functions.

The carcinogenicity of the polycyclic aromatic hydrocarbons is sensitive to trivial structural changes. For example, 7,12-dimethylbenz[a]anthracene is the most potent of the known chemical carcinogens, whereas 7,12-diethylbenz[a]anthracene does not evoke cancer; benz[a]anthracene is only a weak carcinogen, whereas benzo[a]pyrene is strongly carcinogenic. The structural differences may have a profound effect on the orientation of these compounds on a macromolecule, resulting in binding at different sites. Thus, the measurement of binding affinity alone is insufficient for the correlation of binding with biological effects.

Fluorescence spectroscopy is one of the most useful tools for energy transfer studies. In a protein interaction, the tryptophan residue of the protein may transfer its electronic energy to a bound ligand if, among other conditions, the two units are closely located on the macromolecule. The energy transfer data permit calculation of the mean distance between the energy donor and the acceptor, which can be used to differentiate the binding sites for compounds with similar structures.

This study was an attempt to provide additional insight into the interaction of carcinogenic hydrocarbons with proteins. By using the fluorescence technique, studies were made on the binding of benz[a]anthracene and benzo[a]pyrene to human serum albumin. Emphasis was placed on the nature and quantitative aspects of the binding and the difference in binding between benz[a]anthracene and benzo[a]pyrene.

## EXPERIMENTAL

**Materials**—Benz[a]anthracene[1], benzo[a]pyrene[1], L-tryptophan[2], crystalline human serum albumin[3], and soluble ribonucleic acid from liver[4] were purchased from commercial sources. Further purification of benz[a]anthracene and benzo[a]pyrene was made by recrystallization from methanol. The solvents were spectroscopic grade, and all other chemicals were reagent grade.

**Instruments and Methods**—Fluorescence measurements were made with a spectrophotofluorometer[5] equipped with a 150-w xenon lamp and a 1P21 photomultiplier tube. Fluorescence spectra were recorded with an *x–y* recorder[6]. The entrance slit of the excitation light and the exit slit of the fluorescence emission were 4 and 5 mm, respectively. Absorption spectra and measurements were made with a UV–visible spectrophotometer[7]. Except in the solvent and pH studies, all measurements were carried out in pH 7 phosphate buffer (0.5 *M*). The temperature was controlled at 25 ± 0.5°.

Human serum albumin was freeze dried to constant weight before use. The concentration of the protein was calculated with a molecular weight of 69,000. The concentrations of benz[a]anthracene and benzo[a]pyrene used were $10^{-7}$–$10^{-5}$ *M*; microliter amounts of stock solution ($10^{-3}$ or $10^{-4}$ *M* in acetone) were transferred, using microsyringes, into a fluorescence cell containing 2 or 3 ml of a protein or other solution.

**Binding Studies**—The binding of benz[a]anthracene and benzo-

[1] Eastman Kodak Co.
[2] Nutritional Biochemicals Corp.
[3] Lot 5868, Nutritional Biochemicals Corp.
[4] Lot 9020, Nutritional Biochemicals Corp.
[5] Aminco-Bowman, American Instrument Co., Silver Spring, Md.
[6] Omnigraphic, Houston Instrument, Bellaire, Tex.
[7] Cary 118, model C, Varian Instrument Division, Palo Alto, Calif.

Table I—Fluorescence Data of $10^{-6}$ *M* Benz[*a*]anthracene and Benzo[*a*]pyrene in Various Solvents

| Solvent | Benz[*a*]anthracene | | | Benzo[*a*]pyrene | | |
|---|---|---|---|---|---|---|
| | A[a] | B[b] | C[c] | A | B | C |
| pH 7 Phosphate buffer | 300 | 410 | 5 | 310 | 420 | 2 |
| | 300 | 500 | 15 | 310 | 505 | 10 |
| | 372 | 500 | 20 | 393 | 505 | 13 |
| Methanol | 288 | 403 | 54 | 300 | 420 | 80 |
| | 342 | 403 | 18 | 382 | 420 | 115 |
| Acetone | 350 | 403 | 18 | 384 | 420 | 118 |
| Cyclohexane | 288 | 403 | 59 | 300 | 420 | 85 |
| | 342 | 403 | 18 | 384 | 420 | 122 |

[a] Excitation maximum in nanometers. [b] Emission maximum in nanometers. [c] Relative fluorescence intensity of the emission.

[*a*]pyrene to human serum albumin was determined by the Scatchard equation:

$$\overline{V}/D_f = nK - \overline{V}K \qquad \text{(Eq. 1)}$$

where $D$ represents the hydrocarbons, $D_f$ is the concentration of unbound $D$, $\overline{V}$ is the ratio of bound $D$ per protein concentration, $n$ is the number of binding sites on the protein, and $K$ is the binding constant. The values of $\overline{V}$ and $D_f$ were measured by fluorescence quenching titration (9). In this method, a protein solution is titrated with successive additions of $D$, and the quenching of the native fluorescence of the protein is recorded after each addition. The quenched fluorescence, expressed as a percentage of the initial fluorescence of the protein, is then plotted as a function of the concentration ratio of $D$ and the protein.

The method requires titration curves at several protein concentrations. Above a certain protein concentration, the titration curves are superimposable since $D$ is all bound. This superimposed curve represents the degree of quenching as a function of $\overline{V}$. In a solution of lower protein concentration, $D$ is only partially bound, and $\overline{V}$ is equal to the concentration ratio on the superimposed curve at the same degree of quenching.

The quenching of the fluorescence of human serum albumin by benz[*a*]anthracene and benzo[*a*]pyrene was measured at the emission wavelength of 348 nm with the excitation at 280 nm. The binding constants were calculated for solutions containing $2.5 \times 10^{-6}$ *M* human serum albumin and varying concentrations of the hydrocarbons in the $5 \times 10^{-7}$–$6 \times 10^{-6}$ *M* range. The high protein concentration, in which the hydrocarbons were all bound, was $2.5 \times 10^{-5}$ *M*.

**Energy Transfer Theory**—The quenching of the fluorescence of human serum albumin by benz[*a*]anthracene and benzo[*a*]pyrene, like that observed in other protein interactions (10, 11), is due to energy transfer from the excited-state tryptophan residue (energy donor) to the bound ligand (energy acceptor). The energy transfer information allows calculation of the mean distance, $R$, between the donor and acceptor by the equation (12):

$$X/(1 - X) = (R_0/R)^6 \qquad \text{(Eq. 2)}$$

where $X$ is the fraction of the absorbed photons transferred, and $R_0$ is the characteristic distance at which the probability of energy transfer is equal to the probability of fluorescence. The degree of energy transfer may be measured by the degree of quenching of the protein fluorescence.

According to Förster's theory (13):

$$R_0 = (1.69 \times 10^{-33}\, \tau J_v / r^2 \bar{v}_0^2)^{1/6} \text{ cm} \qquad \text{(Eq. 3)}$$

where $\tau$ is the fluorescence lifetime of the donor in the absence of transfer, $r$ is the refractive index of the solvent, $\bar{v}_0$ is the mean of the wave numbers of maximum absorption of the acceptor and the emission of the donor, and $J_v$ is the overlap integral. To determine $\bar{v}_0$ and $J_v$, Eq. 4 may be used:

$$J_v = \int_0^\infty E(v)F(v)dv \qquad \text{(Eq. 4)}$$

where $E(v)$ is the molar absorptivity of the acceptor, and $F(v)$ is the molar emission coefficient of the donor. The molar emission function, $F(v)$, represents the photospectral distribution of the fluorescence in units dependent on $F(v)$ at maximum emission being equal to the molar absorptivity of the donor. By redrawing the absorption and emission data of the acceptor and donor in terms of the wave number scale, $J_v$ and $\bar{v}_0$ can be determined by graphical integration.

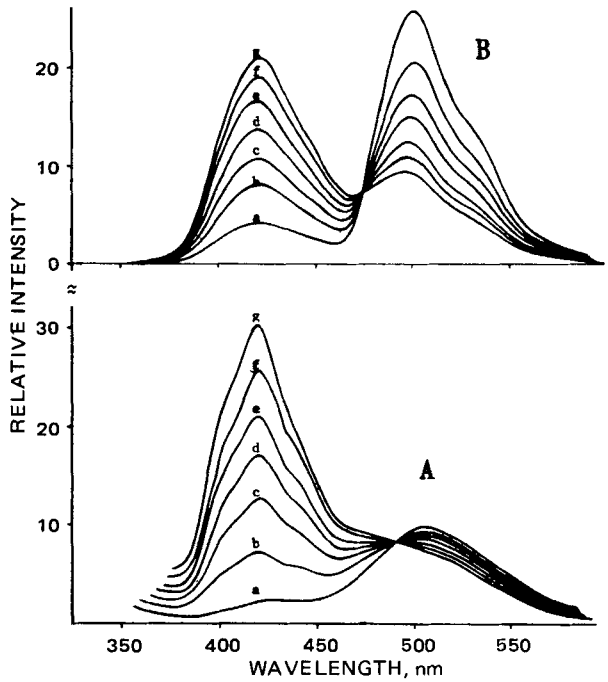


**Figure 1**—*Effect of human serum albumin on the fluorescence spectrum of (A) $10^{-6}$ M benzo[a]pyrene (excited at 310 nm) and (B) $10^{-6}$ M benz[a]anthracene (excited at 300 nm) in pH 7 buffer. The concentration of the protein (a to g) was 0–3 × $10^{-6}$ M with an increment of 0.5 × $10^{-6}$ M. In B, a polarizing film was used in the emission slit slide to diminish the protein fluorescence.*

## RESULTS

The fluorescence data of $10^{-6}$ *M* benz[*a*]anthracene (I) and of benzo[*a*]pyrene (II) measured in various solvents are shown in Table I. In pH 7 phosphate buffer, the emission maxima of the hydrocarbons were found to be in two regions: the A region bands at 500 and 505 nm for I and II, respectively, and the relatively weak fluorescence bands in the B region at 395–420 nm. In organic solvents, the A region bands were diminished and strong fluorescence bands were found in the B region. Despite the spectral difference as observed in aqueous and organic solvents, the emission maxima of the hydrocarbons did not shift according to the solvent polarity. The spectra obtained in cyclohexane and in methanol were almost identical. Thus, the spectral change cannot be explained by the dipole–dipole interaction theory (14). Except in acetone, two excitation maxima were observed for the hydrocarbons. Acetone was able to eliminate the short wavelength excitation bands (320 nm or shorter) of many fluorescent compounds. For example, salicylic acid was fluorescent in water when excited at 300 nm but nonfluorescent in acetone when excited at any wavelength.

Figure 1A shows the effect of human serum albumin on the fluorescence spectrum of benzo[*a*]pyrene in pH 7 buffer. The excitation wavelength was set at 310 nm. The binding of the hydrocarbon to the protein was clearly indicated by the spectral change and the appearance of an isosbestic point, indicating the existence of two species in the solution, *i.e.*, the bound and unbound benzo[*a*]pyrenes. The spectra of the enhanced B region fluorescence were similar to those observed in organic solvents.

The effect of human serum albumin on the fluorescence spectrum of benz[*a*]anthracene, excited at 300 nm, at neutral pH is shown in Fig. 1B. Since the excitation wavelength was quite close to that of the protein fluorescence, interference of the B region fluorescence of the hydrocarbon by the fluorescence of the protein was expected. To eliminate the interference, the spectra of Fig. 1B were recorded using a polarizing film in the emission slit slide. Although the B region fluorescence spectrum was slightly changed and shifted toward longer wavelength, the effect of the protein on the spectrum of the hydrocarbon clearly indicated the binding.

The enhancement of the B region fluorescence of the hydrocarbons by the protein was stronger in high pH than in low pH solutions. Below pH 4, where most serum albumins undergo molecular unfolding (9), no significant enhancement was observed. These results suggest a strong interaction between the protein and the hydrocarbons in basic solution,

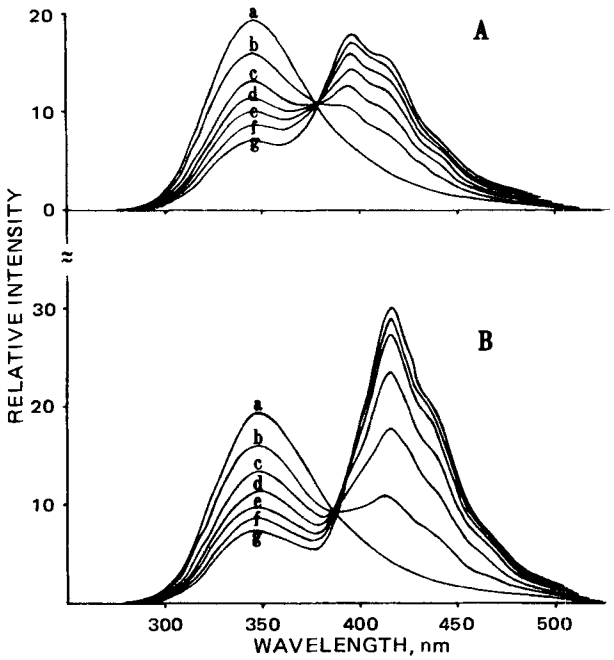


**Figure 2**—*Effect of benz[a]anthracene (A) and benzo[a]pyrene (B) on the fluorescence spectrum of 2.5 × $10^{-5}$ M human serum albumin (excited at 310 nm) at neutral pH. The concentration of the hydrocarbons (a to g) was 0–3 × $10^{-6}$ M with an increment of 0.5 × $10^{-6}$ M.*

presumably due to a conformational change of the protein upon changing pH. The fluorescence of the hydrocarbons was not affected by solution pH.

Figure 2 shows the effect of I and II on the fluorescence spectrum of human serum albumin at neutral pH. The excitation wavelength was set at 280 nm. The quenching of the protein fluorescence was again accompanied by the enhancement of the B region fluorescence.

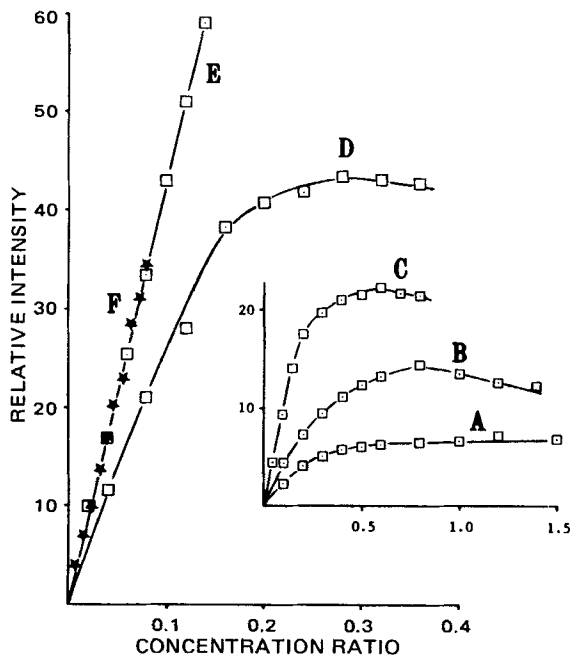


**Figure 3**—*Plots of the fluorescence enhancement at 420 nm (excited at 310 nm) for benzo[a]pyrene as a function of the concentration ratio of benzo[a]pyrene and human serum albumin at several protein concentration levels. The protein concentrations were: A, 1.25 × $10^{-6}$ M; B, 2.5 × $10^{-6}$ M; C, 5 × $10^{-6}$ M; D, 1.25 × $10^{-5}$ M; E, 2.5 × $10^{-5}$ M; and F, 5 × $10^{-5}$ M. The data were obtained by titrating the protein solutions in pH 7 buffer with successive additions of benzo[a]pyrene. Corrections of the residue fluorescence of the protein and the hydrocarbon were made before the plot.*

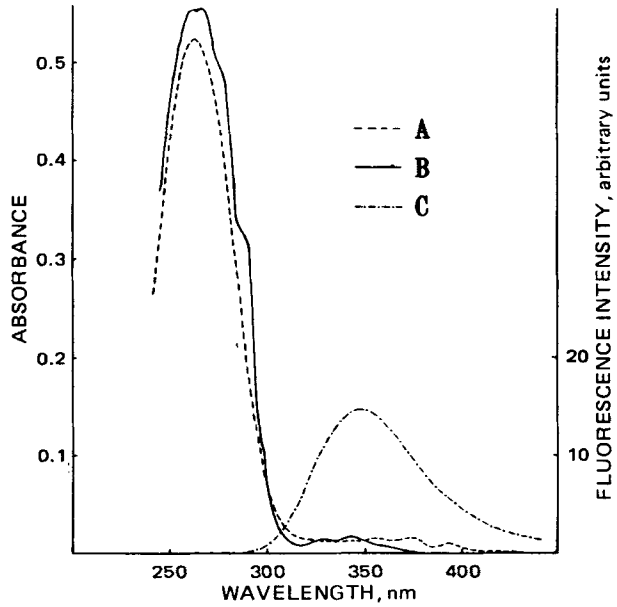


**Figure 4**—*(A) Absorption spectrum of 2 × $10^{-6}$ M benz[a]anthracene. (B) Absorption spectrum of 2 × $10^{-6}$ M benz[a]anthracene in the presence of 2.5 × $10^{-5}$ M human serum albumin. The same amount of protein was also present in the reference cell. In this spectrum, the hydrocarbon is all bound. (C) Fluorescence spectrum of 2.5 × $10^{-5}$ M human serum albumin (excited at 280 nm). All solutions were prepared in pH 7 phosphate buffer.*

Figure 3 shows the plots of the fluorescence enhancement at 420 nm *versus* the concentration ratio of benzo[a]pyrene and the protein at several protein concentration levels. Corrections of the residue fluorescence of the protein and benzo[a]pyrene were made prior to the plots. In curve A, where the protein concentration was 1.25 × $10^{-6}$ *M*, the titration curve plateaued when the concentration ratio was one or larger. This finding suggests that the protein molecule has one binding site for benzo[a]pyrene. The same is true for benz[a]anthracene. At higher protein concentrations, the fluorescence enhancement reached a maximum with the concentration ratio well less than one. Further addition of benzo[a]pyrene led to fluorescence quenching.

Although the reason for the quenching is not clear, the results suggest that the binding sites of the protein are not fully occupied by the hydrocarbon at high protein concentrations. This result is possible since, at high concentrations, the protein molecules tend to self-associate and the binding of hydrocarbon may even aid in the association by forming complexes with more than one protein molecule. When the protein concentration was 2.5 × $10^{-5}$ *M* or larger, the hydrocarbon in the concentration range shown in Fig. 5 was all bound. This result is indicated by the superimposed straight-line plot and by the absence of the A region fluorescence. Similar results were obtained for benz[a]anthracene.

To elucidate the enhancement of B region fluorescence by the protein, similar studies were made with tryptophan and soluble ribonucleic acid. Tryptophan also can induce the B region fluorescence of the hydrocarbons, but the concentration of tryptophan has to be much larger than that of the protein containing one tryptophan residue per molecule. On the other hand, the addition of soluble ribonucleic acid did not enhance the B region fluorescence; instead, the fluorescence of the hydrocarbons was strongly quenched by an interaction with nucleic acid. These results suggest that the B region fluorescence in protein interaction is due to energy transfer from the tryptophan residue to bound hydrocarbons. The absence of the B region fluorescence in the nucleic acid interaction may be attributed to the lack of an energy donor in the nucleic acid molecule.

A prerequisite for energy transfer is that the absorption spectrum of the energy acceptor must overlap the fluorescence spectrum of the donor. Figures 4 and 5 show that this prerequisite is met in the binding of the hydrocarbons to human serum albumin. In binding with the protein, the visible absorption bands of the hydrocarbons shifted toward high energy or shorter wavelength. The difference spectra of bound hydrocarbons (spectrum B of Figs. 4 and 5), in which the protein absorption was canceled using a double-beam technique, exhibited shoulder bands in the 280–300-nm region, indicating the involvement of the tryptophan residue

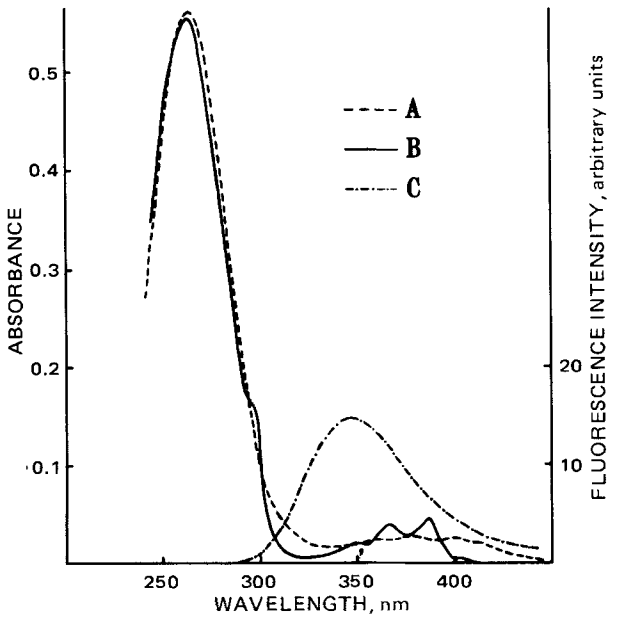


**Figure 5**—*(A) Absorption spectrum of 2 × $10^{-6}$ M benzo[a]pyrene. (B) Absorption spectrum of 2 × $10^{-6}$ M benzo[a]pyrene in the presence of 2.5 × $10^{-5}$ M human serum albumin. In this solution, the hydrocarbon is all bound. The protein absorption is canceled using a double-beam method. (C) Fluorescence spectrum of 2.5 × $10^{-5}$ M human serum albumin. All solutions were prepared in pH 7 phosphate buffer.*

in the binding. This finding is particularly true for benz[a]anthracene, whose binding site may be in the vicinity of the tryptophan residue.

The energy transfer data for the binding of I and II to human serum albumin are shown in Table II. The characteristic distances, $R_0$, were 25.9 and 32.0 Å for I and II, respectively, and were calculated from the following data: $r$ = 1.36, $\tau$ = 2.64 nsec, $\bar{\nu}_0$ = 2.90 × $10^4$ $cm^{-1}$ for I and 2.72 × $10^4$ $cm^{-1}$ for II, and $J_\nu$ = 1.05 × $10^{11}$ $cm^3$-$mmole^{-2}$ for I and 3.25 × $10^{11}$ $cm^3$-$mmole^{-2}$ for II. The value of $\tau$ was calculated from the equation: $\tau = Q\tau_n/P$, where $Q$ is the quantum yield of human serum albumin; $\tau_n$ is the natural fluorescence lifetime of tryptophan, which was taken to be 12 nsec (15); and $P$ is the fraction of protein absorption due to the tryptophan residue, which was reported to be 19.5% for human serum albumin (16).

The quantum yield of human serum albumin in pH 7 phosphate buffer was determined by comparison of the emission peak areas of the protein and the tryptophan solutions of equal absorbance at 280 nm. The quantum yield of tryptophan was taken to be 0.13 (17). The values of $\bar{\nu}_0$ and $J_\nu$ were graphically determined, according to Eq. 4, by redrawing the data of Figs. 4 and 5 in terms of the wave number scale. The unit of the molar emission function, $F(\nu)$, was set so that $F(\nu)$ at maximum emission was equal to 0.59 × $10^4$ $cm^2$-$mmole^{-1}$, the molar absorptivity of tryptophan at 280 nm in pH 7 phosphate buffer. Although the characteristic distance, $R_0$, calculated in this manner is an approximation because of the complicated protein system, the results show the difference in binding sites for the two hydrocarbons.

Figure 6 shows the fluorescence quenching titrations of human serum albumin with I and II. The data were obtained by titrating the protein solutions with successive additions of the hydrocarbons, with the excitation and emission wavelengths set at 280 and 348 nm, respectively. In a high protein concentration (2.5 × $10^{-5}$ $M$), about 95% of the protein fluorescence was quenched as a result of energy transfer. According to Eq. 2, the mean distances between the tryptophan residue of the protein and bound hydrocarbons were 15.2 and 19.6 Å for I and II, respectively.

**Table II—Energy Transfer Data for the Binding of Benz[a]anthracene and Benzo[a]pyrene to Human Serum Albumin**

| | $J_\nu$, $cm^3$-$mmole^{-2}$ | $R_0$, Å | $R$, Å | $K^a$, $M^{-1}$ |
|---|---|---|---|---|
| Benz[a]anthracene | 1.05 × $10^{11}$ | 25.9 | 15.2 | 1.56 × $10^5$ |
| Benzo[a]pyrene | 3.25 × $10^{11}$ | 32.0 | 19.6 | 1.46 × $10^5$ |

[a] Binding constant.

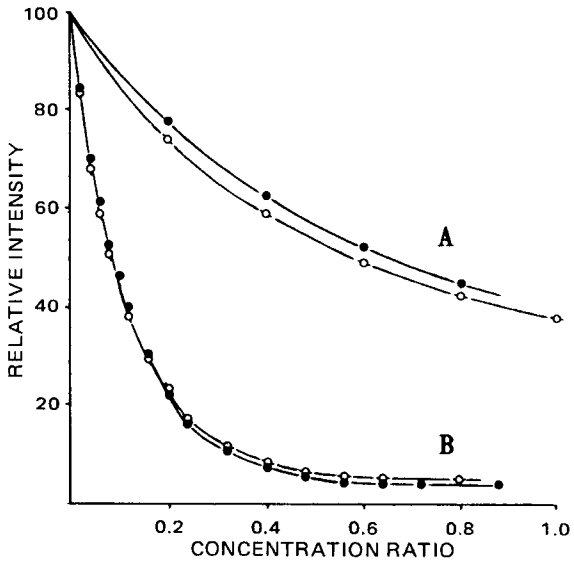


**Figure 6**—*Quenching of the fluorescence of human serum albumin by benz[a]anthracene and benzo[a]pyrene. The quenched fluorescence is plotted as a function of the concentration ratio of the hydrocarbons and the protein. Key: ●, benz[a]anthracene; ○, benzo[a]pyrene; A, 2.5 × $10^{-6}$ M protein concentration; and B, 2.5 × $10^{-5}$ M protein concentration.*

The Scatchard plots for the binding of I and II to human serum albumin are shown in Fig. 7. In a protein solution of 2.5 × $10^{-6}$ $M$, the number of binding sites was 0.5 for both compounds. The binding constants for I and II were 1.56 × $10^5$ and 1.46 × $10^5$ $M^{-1}$, respectively. Despite the structural difference of the two hydrocarbons, their binding affinities to the protein were approximately equal. In view of the energy transfer and binding data, it is concluded that the two hydrocarbons bind in the same general area on the protein molecule but at different sites. The structural factor, which may greatly affect the orientations of the hydrocarbons on the protein molecule, affects mainly the energy transfer distance rather than the binding equilibrium.

## DISCUSSION

The quenching of the fluorescence of human serum albumin by benz[a]anthracene and benzo[a]pyrene, as well as the strong enhancement of the B region fluorescence of the hydrocarbons, indicates the transfer of electronic energy from the tryptophan residue to bound ligands. The conditions necessary for energy transfer are: (*a*) the donor must be a fluorescent group with a sufficiently long lifetime, (*b*) the absorption spectrum of the acceptor must overlap the emission spectrum of the donor, (*c*) the relative orientations of the oscillators must permit a strong interaction, and (*d*) the donor and the acceptor must be within a certain distance for a given efficiency of energy transfer (18). The results of the present study are in excellent agreement with these conditions.

As shown in Figs. 4 and 5, the protein fluorescence band strongly ov-

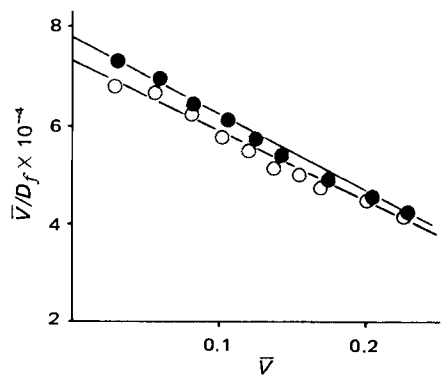


**Figure 7**—*Scatchard plots for the binding of benz[a]anthracene (●) and benzo[a]pyrene (○) to 2.5 × $10^{-6}$ M human serum albumin at neutral pH.*

erlapped the absorption bands of the hydrocarbons. The absorption spectra of the protein-bound hydrocarbons exhibited shoulder bands in the 280–300-nm region, indicating the involvement of the tryptophan residue in the interaction. The strong enhancement of the B region fluorescence for the hydrocarbons in binding with the protein represents a transition resulting from an energy transfer process because the binding of these compounds with soluble ribonucleic acid, a macromolecule without an energy donor, does not induce the B region fluorescence.

Excluding the fact of energy transfer, the fluorescence of the hydrocarbons is strongly quenched by interacting with the protein and soluble ribonucleic acid. This result probably indicates a stacking interaction between the hydrocarbon and the aromatic moieties of the macromolecules. The association of fluorescence quenching with stacking interactions was reported previously. For example, the binding of protein to nucleic acid always results in strong quenching of the protein fluorescence, which has been shown to be due to the stacking interaction between the tryptophan residues and the nucleic acid bases (3, 4). In a fluorescence study of compounds in which an indole group is connected to nucleic acid bases by a three- or four-atom bridge, Mutai *et al.* (19) showed that the stacked form, in contrast to the fluorescent unstacked form, is nearly nonfluorescent. Benz[*a*]anthracene and benzo[*a*]pyrene are planar molecules. In binding with nucleic acid, benzo[*a*]pyrene intercalates with the nucleic acid bases (8). In protein interactions, it is possible that the binding allows plane parallelism between the tryptophan residue and the hydrocarbon and, therefore, results in fluorescence quenching of both units.

The fact that only bound benz[*a*]anthracene and benzo[*a*]pyrene can receive energy is indicated by the fluorescence studies of tryptophan with the hydrocarbons. Human serum albumin has one tryptophan residue per molecule. However, the addition of tryptophan, in the same concentration range as that of the protein (Fig. 1), does not induce the B region fluorescence. Only in high tryptophan concentrations do the spectra of the hydrocarbons become similar to those observed in protein solutions, since the two units are then close enough for the efficiency of energy transfer. These results suggest that the binding sites for the hydrocarbons are quite close to the tryptophan residue and that the hydrocarbons are favorably oriented with the tryptophan residue to permit interaction and energy transfer. The small $R$ value for benz[*a*]anthracene indeed suggests that the binding site is in the vicinity of the tryptophan residue.

The energy transfer information is important in understanding the interaction of carcinogenic hydrocarbons with proteins. Benz[*a*]anthracene and benzo[*a*]pyrene interact with human serum albumin to approximately the same extent as indicated by the binding constants. This is expected since these compounds are chemically inert and the binding is of a hydrophobic nature. The difference in structure of the two would probably make little difference in their binding affinities. The difference in ring structure, however, may have a profound effect on their orientations on the protein molecule. Conclusions based on binding strength alone could be misleading. Benzo[*a*]pyrene is strongly carcinogenic, whereas benz[*a*]anthracene is not. While the binding constants of the two suggest similar binding, their protein binding sites are distinguished by energy transfer data.

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# Rapid Sensitive Fluorometric Analysis of Cephalosporin Antibiotics


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**Abstract** □ A rapid and sensitive fluorometric analysis for cephalosporins, which can also be applied to penicillins, is presented. The method involves reaction with 0.1 *N* sodium hydroxide at 100°, producing stable fluorescent products. This method was applied to cephalexin and ampicillin with detection at concentrations as low as 0.01 μg/ml.



**Keyphrases** □ Cephalosporins, various—fluorometric analysis, commercial samples in aqueous solutions □ Fluorometry—analysis, various cephalosporins, commercial samples in aqueous solutions □ Antibacterials—various cephalosporins, fluorometric analysis, commercial samples in aqueous solutions


Several methods for the quantitative measurement of cephalosporins in aqueous solution have been described. They utilize such techniques as microbiological assay (1), GLC (2), formol titration (3), colorimetry (4–7), liquid chromatography (8, 9), iodometry (4, 10), reaction with hydroxylamine (4), and polarography (11). The nonmi-