Anal. Calcd. for $C_6H_5O_2N_5$: N, 39.11. Found: N, 39.24.

Acetone from 7-Acetonylxanthopterin (XVI).—Under similar treatment as mentioned for 6-acetonylisoxanthopterin (V), 66 mg. (66%) of acetone 2,4-dinitrophenylhydrazone was obtained from 7-acetonylxanthopterin (XVI). The residual xanthopterin being in the reductive state had no fluorescence, but addition of MnO_2 regenerated the fluorescence and xanthopterin was identified through paper chromatography.

Miscellaneous.—Acetone was also obtained in yields of 75% and 66% from 2,4,6-trihydroxy-7-acetonylpteridine (XIX) and 2,4,7-trihydroxy-6-acetonylpteridine (XXII), respectively. Xanthopterincarboxylic acid (XV), 2,4,6-trihydroxypteridyl-7-carboxylic acid (XVIII) and 2,4,7-trihydroxypteridyl-6-carboxylic acid (XXI) were also decarboxylated under the influence of Al–Hg to give xanthopterin (XIV), 2,4,6-trihydroxypteridine (XVII) and 2,4,7-trihydroxypteridine (XX), respectively.

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NAGOYA, JAPAN

[CONTRIBUTION FROM THE DEPARTMENT OF INDUSTRIAL MEDICINE, NEW YORK UNIVERSITY POST-GRADUATE MEDICAL SCHOOL]

The Interaction of Dinitrobenzene Derivatives with Bovine Serum Albumin[1,2]

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The reversible interaction of a series of ionic and non-ionic substituted 2,4-dinitrobenzenes with bovine serum albumin has been studied at two temperatures. They differed from each other only in the substituent at carbon one. Included in the series were two 2,4-dinitrophenyl amino acids. The nature of the substituent at carbon one was found to modify considerably the binding affinity. Relative binding affinities were obtained graphically and energies of interaction (ΔH^0) computed. Chloride ion inhibits the binding of dinitrophenol by bovine serum albumin but not that of dinitroaniline. Dinitrotoluene also inhibits binding of dinitrophenol. Binding to bovine serum albumin results in shifts in the absorption spectra of ε-dinitrophenyl-aminocaproic acid, dinitroaniline and dinitrophenol.

The interactions of inorganic and organic ions with serum albumins have been studied extensively. Little attention, however, has been given until very recently to the binding of neutral organic molecules.[3] We have studied the reversible interactions of bovine serum albumin with a series of ionic and non-ionic substituted 2,4-dinitrobenzenes which differ only in the substituent at carbon one. Included in this series were some 2,4-dinitrophenyl (DNP) amino acids. The compounds studied made it possible to evaluate the contribution of the various substituents to the binding and, in particular, to obtain information on the ionic contribution to the binding. The binding of an ionic compound in the presence of a non-ionic homolog has made it possible to gain some insight into the specificity of binding sites.

Furthermore, substituted 2,4-dinitrobenzenes are of considerable interest from an immunologic viewpoint. Some of these compounds, under appropriate conditions, give rise to a wide range of allergic reactions.[4] The interaction of these compounds with serum albumin provides a basis for interpreting some of these biological phenomena[5] and is of particular interest in relation to corresponding interactions involving antibodies specific for the 2,4-dinitrophenyl group. The latter problem is under study in this Laboratory.

The present paper records measurements of the binding of the following 2,4-dinitrobenzene derivatives by bovine serum albumin: 2,4-dinitrophenol, 2,4-dinitrobromobenzene, 2,4-dinitroaniline, 2,4-dinitrotoluene, *m*-dinitrobenzene, ε-N-dinitrophenyllysine (ε-DNP-lysine) and ε-dinitrophenyl-aminocaproic acid (ε-DNP-aminocaproic acid). Competition of various compounds for the binding sites of the albumin molecule was studied in the following pairs: dinitrophenol and dinitrotoluene, dinitrophenol and chloride ion, dinitroaniline and chloride ion. The absorption spectra of dinitrophenol, dinitroaniline and ε-DNP-aminocaproic acid bound to serum albumin were obtained. Under the experimental conditions, dinitrophenol and ε-DNP-aminocaproic acid were in anionic form, while ε-DNP-lysine was a dipolar ion.

Experimental

Materials and Methods.—Crystalline bovine serum albumin, obtained from Armour and Company, was used. Protein concentrations were measured in a model DU Beckman spectrophotometer. The spectrophotometer reading was calibrated with micro-Kjeldahl nitrogen values for a bovine serum albumin solution. The protein concentration was calculated, using a value of 16.07% nitrogen[6] and a molecular weight of 69,000.[7]

Commercial samples of dinitrophenol, dinitrobromobenzene, dinitroaniline, dinitrotoluene and *m*-dinitrobenzene were twice recrystallized. ε-DNP-lysine was prepared according to the method of Porter.[8] Its purity was indicated chromatographically and by a melting point of 182° (literature 180°[8]).

ε-DNP-Aminocaproic acid was prepared from ε-aminocaproic acid according to the method given by Sanger[9] for DNP-phenylalanine. Precipitation of ε-DNP-aminocaproic acid by acidification was repeated three times to remove un-

(1) The support of this work by grants from the United States Public Health Service and from the Standard Oil Co. (N. J.), New York City, is gratefully acknowledged.

(2) A preliminary report was presented at the meeting of the American Society of Biological Chemists: *Federation Proc.*, **12**, 187 (1953).

(3) I. M. Klotz and J. Ayers, THIS JOURNAL, **74**, 6178 (1952).

(4) K. Landsteiner and M. W. Chase, *J. Exp. Med.*, **66**, 337 (1937).

(5) H. N. Eisen, L. Orris and S. Belman, *ibid.*, **95**, 473 (1952).

(6) E. Brand, *Ann. N. Y. Acad. Sci.*, **47**, 187 (1946).

(7) E. J. Cohn, W. L. Hughes, Jr., and J. H. Weare, THIS JOURNAL, **69**, 1753 (1947).

(8) R. R. Porter in "Methods of Medical Research," Vol. 3, The Year Book Publishers, Inc., Chicago, Ill., 1950, p. 256.

(9) F. Sanger, *Biochem. J.*, **39**, 507 (1945).

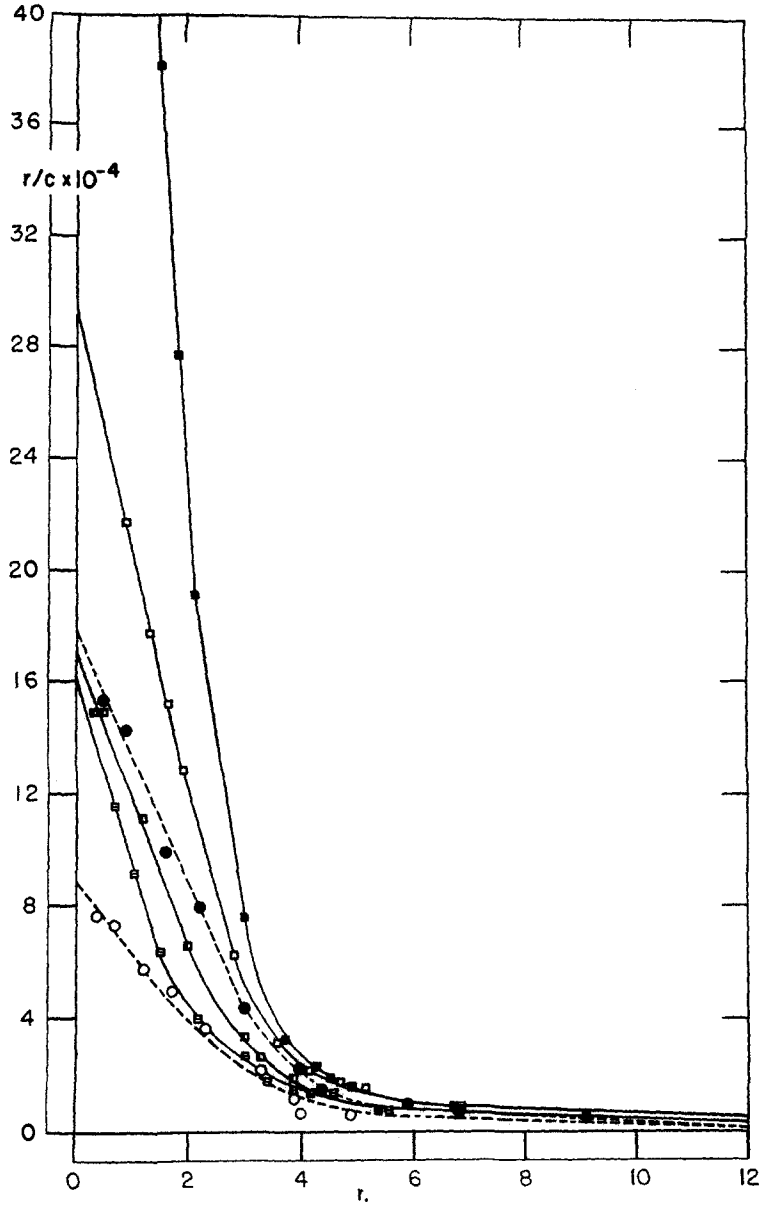

Fig. 1.—Binding of dinitrophenol and of ε-DNP-aminocaproic acid by bovine serum albumin in 0.05 *M* phosphate buffer, *p*H 7.4. Dinitrophenol at 5°, ■—■; at 25°, □—□; with 0.16 *M* NaCl at 25°, ▯—▯; with 61.6×10^{-5} *M* dinitrotoluene at 25°, ▮—▯; ε-DNP-aminocaproic acid at 5°, ●----●, and at 25°, O----O.

reacted aminocaproic acid. The compound was crystallized from methanol–water (1:1). The crystals were washed repeatedly with hot ether–ligroin (1:1), recrystallized from methanol–water and dried *in vacuo;* m.p. 134° (dec.). The compound was found pure by chromatography on a silica gel column[9] using chloroform–0.5 *M* phosphate buffer, *p*H 6.7. *Anal.*[10] Calcd.: C, 48.5; H, 5.1; N, 14.1. Found: C, 48.4; H, 5.0; N, 13.9.

The concentrations of the dinitrobenzene derivatives were estimated from measurements of their optical density in 0.05 *M* phosphate buffer, *p*H 7.4, in the spectrophotometer. The molar extinction coefficients calculated at their respective absorption maxima are given in Table I.

Dialysis Experiments.—The extent of binding was measured by the method of equilibrium dialysis. All experiments were carried out in 0.05 *M* phosphate buffer, *p*H 7.4 ± 0.05, because this concentration of phosphate is large enough to make the Donnan correction negligible. A protein concentration of 3×10^{-5} *M* was used, except in the case of dinitroaniline at 25° where 6×10^{-5} *M* was also used; with dinitrobromobenzene the protein concentrations were 6×10^{-5} *M* at 25° and 4×10^{-5} *M* at 5°. The initial concentrations of dinitrobenzene derivatives ranged from 2×10^{-3} to 1.1×10^{-5} *M* except in those cases where solubility of the compound limited the use of high concentrations (see Figs. 1 and 2).

Ten ml. of protein solution, in a bag of Visking sausage casing, was equilibrated with 10 ml. of solution of the respective dinitrobenzene derivative of varying concentration in screw-cap tubes. As some of the spectrophotometric analyses were done in the ultraviolet region, it was found necessary to treat the sausage casing according to the procedure of Teresi and Luck.[11] The tubes were rocked gently (2 r.p.m.) in a constant temperature water-bath. Equilibration was complete after 6 hours at 25.0 ± 0.1° and after 16 hours at 5.0 ± 0.1°. All experiments were performed in duplicate.

The amount of dinitrobenzene derivative bound per mole of serum albumin was calculated from the concentration of free dinitrobenzene derivative at equilibrium after correcting for casing adsorption.

While the binding was reversible in all cases, some derivatization of the albumin was found with dinitrobromobenzene which reacts with proteins[5] in a manner similar to dinitrofluorobenzene.[9] The amount of derivatization, however, is small under the conditions used here. It was determined by measurements of the optical density of the equilibrated albumin solutions at 365 mμ, the absorption maximum of proteins derivatized by halogen substituted 2,4-dinitrobenzenes. Assuming an extinction coefficient of 17,400, *i.e.*, that of ε-DNP-lysine, and correcting for the contribution of serum albumin and of free dinitrobromobenzene, the amount of dinitrobromobenzene combined in this manner was calculated and an appropriate correction made in the free dinitrobromobenzene concentration. As the small contribution of dinitrobromobenzene reversibly bound to albumin was neglected, the corrections applied are maximal. For the experiment at 5° the correction at the highest initial dinitrobromobenzene concentration amounted to 1.2% and at 25° to 3.6%. These figures correspond to derivatization of the protein to the extent of 0.1 and 0.2 group, respectively, per mole protein. This amount of derivatization cannot be evaluated with respect to binding properties of the protein and has, therefore, been ignored.

TABLE I

MOLAR EXTINCTION COEFFICIENTS OF 2,4-DINITROBENZENE DERIVATIVES IN 0.05 *M* PHOSPHATE BUFFER, *p*H 7.4

Substituent at carbon one	λ_{max}, mμ	ε at λ_{max}
–OH	358	14,900
–ε-Aminocaproic acid	365	17,800
–Br	265	10,300
$-NH_2$	345	14,000
$-CH_3$	250	13,300
–H	244	14,300
–ε-N-lysine	360	17,400

In the competition experiments, the competitor (dinitrotoluene or NaCl) at a constant concentration was placed in

(10) Elemental analyses were done by F. Schwarzkopf Microanalytical Laboratory, Middle Village, N. Y.

(11) J. D. Teresi and J. M. Luck, *J. Biol. Chem.*, **174**, 653 (1948).

the bag with the protein. The concentration of substituted dinitrobenzene in the outside solution was varied as indicated before. The initial concentrations of dinitrotoluene and NaCl were 1.23×10^{-3} and 0.32 M, respectively. When dinitrotoluene was competing with dinitrophenol, the equilibrium concentrations of both compounds were calculated from optical density readings at two wave lengths (358 and 310 mμ). Thus it was possible to calculate the moles bound per mole protein for each compound.

Absorption Spectra.—Absorption spectra of dinitrophenol, ϵ-DNP-aminocaproic acid and dinitroaniline bound to serum albumin were determined in 0.05 M phosphate buffer, pH 7.4, and 3×10^{-5} M albumin at $r = 1$, where r is the average number of molecules of the respective derivative bound per protein molecule. The total concentration of each dinitrobenzene derivative was chosen on the basis of the binding curves at 25° so as to attain a value of $r = 1$. These concentrations were 3.56×10^{-5}, 4.63×10^{-5} and 11.8×10^{-5} M, respectively. Optical density readings were corrected for the small protein contribution. In the case of dinitroaniline, a reduced light path (5 mm.) was necessary because of the high optical density of the system. The spectra were computed as indicated by Karush.[12]

Results and Discussion

The experimental results are given in Figs. 1 and 2. r/c is plotted as a function of r, where r is the average number of molecules of dinitrobenzene derivative bound per protein molecule at free dinitrobenzene derivative concentration c. Smooth curves were drawn to fit the experimental points and were extrapolated to obtain the intercept on the r/c axis.

From the law of mass action it follows that

$$r = \sum_i \frac{K_i c}{1 + K_i c} \quad (i = 1,2,\ldots,n) \qquad (1)$$

assuming n binding sites per protein molecule with intrinsic association constants K_i, and assuming no interaction among bound ions.[13] For the case of equal K_i's, according to Scatchard[14]

$$r/c = nK - rK \qquad (2)$$

When r/c is plotted as a function of r a straight line should result. The data plotted in Figs. 1 and 2 are obviously not straight lines. Deviations from linearity may be due to electrostatic interaction or to unequal K_i's. Electrostatic interaction, however, has been found previously not to account for non-linear binding curves obtained with ions.[13,15] It would seem even less relevant with neutral molecules.

From the shape of the curves obtained it appears that the binding of dinitrobenzene derivatives could be more adequately described by an equation of the form of (1) which assumes two kinds of sites, each associated with a different value of K, as used by Scatchard, Scheinberg and Armstrong,[16] and by Karush.[13] For dinitrophenol a remarkably good fit of the data was obtained when theoretical curves were calculated using values of $n_1 = 3$ and $n_2 = 11$, where n_1 and n_2 represent the number of sites of each kind. However, when theoretical curves for the other compounds were calculated, it was found that, with decreasing binding affinities, smaller values for n_1 were required to fit the curves.

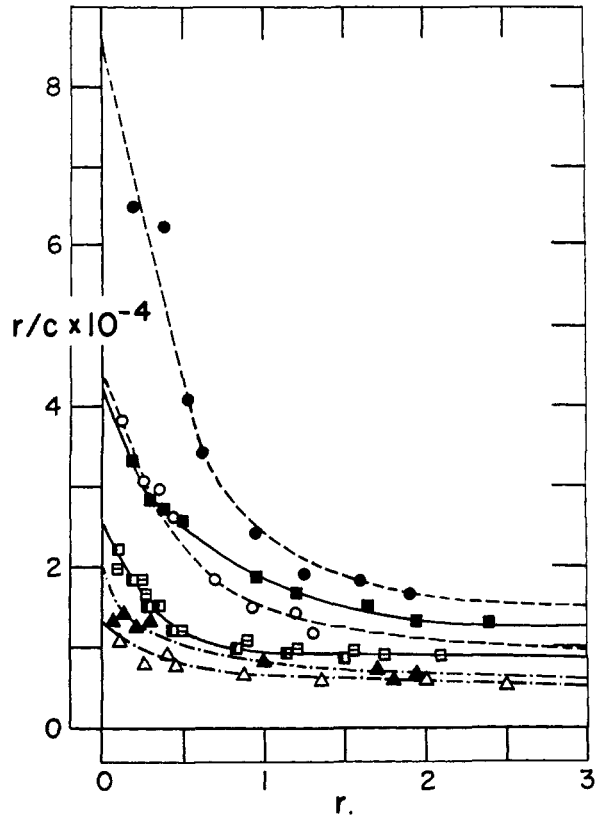

Fig. 2.—Binding of dinitrobromobenzene, dinitroaniline and dinitrotoluene by bovine serum albumin in 0.05 M phosphate buffer, pH 7.4. Substituent in dinitrobenzene: Br at 5°, ●---●, and at 25°, O---O; NH_2 at 5°, ■——■, and at 25°, □——□; with 0.16 M NaCl at 25°, ◧——◧; CH_3 at 5°, ▲—·—·—▲, and at 25°, △—·—·—△.

Eventually n_1 not only was less than one, but also was smaller at high than at low temperature. Although curves can be fitted in this manner, they do not seem to have any simple interpretation. Thus, it can be inferred that the K_i's change over the whole range of binding described by the experimental data. The values for successive K_i's become smaller with increasing concentration of free dinitrobenzene derivative. It is tempting to suggest that one of the reasons for this is competitive interaction of small molecules with intramolecular bonds in the protein. This is consistent with the viewpoint of Karush that structural changes take place in the protein when it binds anions.[12]

In spite of the non-linearity of the binding curve, the intercept on the r axis still corresponds to the value of n according to equation (1), but the error in extrapolation is large. Because of the decreasing values of K_i's, it seems difficult to reach a limiting value for n experimentally and has never been accomplished with serum albumin. A wide variation for values of n, obtained by extrapolation, is reported in the literature.[17]

We have, therefore, not attempted to determine the value of n, but have confined ourselves to comparing relative binding affinities obtained from

(12) F. Karush, *J. Phys. Chem.*, **56**, 70 (1952).
(13) F. Karush, THIS JOURNAL, **72**, 2705 (1950).
(14) G. Scatchard, *Ann. N. Y. Acad. Sci.*, **51**, 660 (1949).
(15) F. Karush and M. Sonenberg, THIS JOURNAL, **71**, 1369 (1949).
(16) G. Scatchard, J. H. Scheinberg and S. H. Armstrong, Jr., *ibid.*, **72**, 535 (1950).
(17) I. M. Klotz, *Cold Spr. Harb. Symposium Quant. Biol.*, **14**, 97 (1950).

extrapolations to the r/c axis. As follows from equation (1)[13]

$$\lim_{c \to 0} r/c = \sum_i K_i \qquad (3)$$

This is numerically equal to nK_0 where K_0 is an average binding constant. As the value of nK_0 includes two parameters, capacity and affinity, thermodynamic constants such as ΔF° and ΔS° cannot be calculated. Hence, we only have computed values of ΔH° as a measure of the energy of binding (see Table II). The only assumptions made in these calculations are: that n, the binding capacity, is constant for the binding of each compound at different temperatures, and that the competitive effect of phosphate ion is constant at different temperatures as well. The ΔH° values in Table II are higher than those reported in the literature for the binding of anions,[17] but agree well with that for *p*-aminoazobenzene.[3]

TABLE II

BINDING AFFINITIES AND BINDING ENERGIES OF 2,4-DINITROBENZENE DERIVATIVES WITH BOVINE SERUM ALBUMIN IN 0.05 *M* PHOSPHATE BUFFER, *p*H 7.4

Substituent at carbon one	$\sum_i K_i \times 10^{-4}$ 5°	$\sum_i K_i \times 10^{-4}$ 25°	ΔH^0, kcal./mole
–OH	70.0	29.6	−7.1
–ε-Aminocaproic acid	18.4	8.8	−6.1
–Br	8.6	4.4	−5.5
$-NH_2$	4.2	2.5	−4.4
$-CH_3$	2.0	1.3	−3.6
–H	0.45	0.3	−3
–ε-N-lysine	0.4	0.3	−2

Relative Binding Affinities.—The sequence of binding affinity of the substances tested to bovine serum albumin is, in decreasing order (listing only the substituent at carbon one): –OH, –ε-aminocaproic acid, –Br, $-NH_2$, $-CH_3$, –H, –ε-N-lysine (Table II). Inasmuch as the binding of the last two compounds is rather weak, the error in calculation is so large that only approximate figures are given. No change in binding of dinitroaniline per mole protein took place at 25° when the protein concentration was doubled.

The two anionic substances, dinitrophenol and ε-DNP-aminocaproic acid, are the most strongly bound (Table II) although the ionic contribution to the binding energy is relatively small. As dinitrophenol and *m*-dinitrobenzene differ from one another only in the ionized hydroxyl group it can be assumed that the difference in the free energy of binding (ΔF^0) is due only to this group. This group may form, both, a semi-ionic bond and, through its oxygen atom, a hydrogen bond with serum albumin. If n is the same for the binding of dinitrophenol and of *m*-dinitrobenzene, the contribution of the ionized phenolic hydroxyl group to the binding, calculated from the difference in the free energy of binding, amounts to 2.8 kcal./mole. This figure does not include a correction taking into consideration that only the anion is repelled by the net negative charge on the protein. An approximate calculation of this repulsion was made with the relation[18,19] developed from the Debye–Hückel theory using a value of 5 Å. for the radius of dinitrophenol, of 30 Å. for b, the radius of the protein, 31.5 Å. for a, the distance of closest approach, a net charge of −15 for the protein[20] and an ionic strength of 0.13. Applying this correction the contribution of the ionized phenolic hydroxyl group will be higher by about 0.3 kcal./mole.

As can be seen from Figs. 1 and 2, all compounds are more strongly bound at low than at high temperature. The pronounced temperature effect with these compounds seems to be characteristic of binding of neutral molecules[3] and indicates the large non-ionic contribution to the binding energy. Probably van der Waals interaction with the aromatic ring, hydrogen bonding through the strongly polar nitro groups, and specific effects of the substituent groups contribute to the interaction energy. The order of binding affinity of the non-ionic substances with the substituents –Br, $-NH_2$, $-CH_3$ agrees with that calculated for substituted hapten interaction with antibody by Pauling and Pressman.[21] Their calculation is based on electronic van der Waals interaction resulting from polarizability and van der Waals radii of the substituent groups. According to their calculation, the compound with the –OH substituent is most weakly bound. Hence, the stronger binding in our experiments of dinitrophenol which has a pK' of 4.0[22] must be attributed to its ionic interaction and hydrogen bond formation with bovine serum albumin.

The dipolar ion ε-DNP-lysine showed very little binding affinity. As the homologous compound ε-DNP-aminocaproic acid is quite strongly bound, it is apparent that the introduction of the positively charged α-amino group interferes with the interaction. The stronger binding of the smaller ion dinitrophenol than of the larger ion ε-DNP-aminocaproic acid, which one would expect to exhibit van der Waals binding through its carbon chain, may reflect a closer approach of the negative charge of the former to the positive site of the protein than is possible for the negative charge of the carboxyl group. Alternately, it may suggest the importance of spatial relationships of the binding sites in the protein molecule. Possibly the separation of the anionic group from the dinitrophenyl group makes access to the binding sites of the protein molecule more difficult. While commonly binding affinity increases with the length of the aliphatic side chain,[15] results similar to ours have been reported for methyl and butyl orange binding to human serum albumin.[23]

Absorption Spectra.—Absorption spectra of dinitrophenol, dinitroaniline and ε-DNP-amino-

(18) D. A. MacInnes, "Principles of Electrochemistry," Reinhold Publ. Corp., New York, N. Y., 1939, p. 146.

(19) G. Scatchard in E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publ. Corp., New York, N. Y., 1943, pp. 52–59.

(20) G. Scatchard, A. C. Batchelder and A. Brown, THIS JOURNAL, **68**, 2320 (1946).

(21) L. Pauling and D. Pressman, *ibid.*, **67**, 1003 (1945).

(22) L. Pauling, "The Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 1948, p. 206.

(23) I. M. Klotz, R. K. Burkhard and J. M. Urquhart, THIS JOURNAL, **74**, 202 (1952).

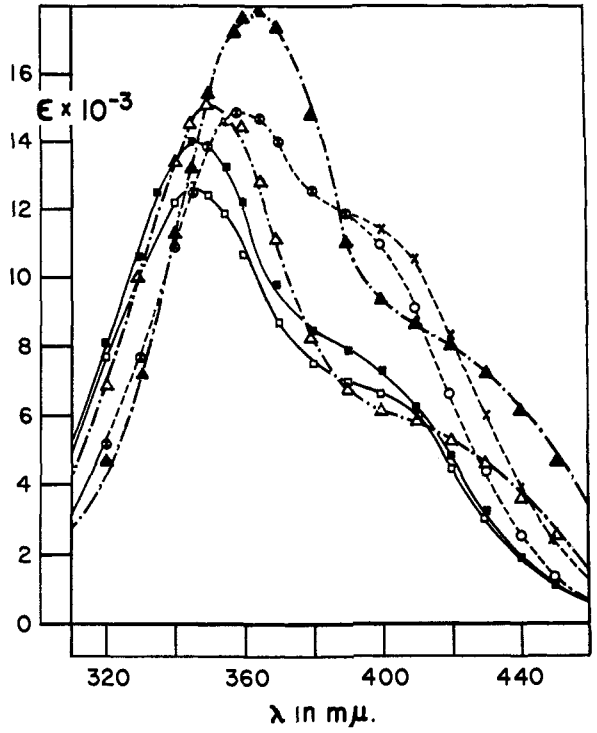

Fig. 3.—Absorption spectra of dinitrophenol (free, ×----×, and albumin-bound, O----O); ε-DNP-aminocaproic acid (free, ▲—.—.—▲, and albumin-bound, Δ—·—·—Δ); dinitroaniline (free, ■——■, and albumin-bound, □——□).

caproic acid, bound to bovine serum albumin, are compared with those of the corresponding free compounds in Fig. 3. Molar extinction coefficients of the albumin-bound form of these compounds are given in Table III. For the other dinitrobenzene derivatives no such spectral measurements were made as the error involved in calculation would be great due to the large protein contribution at the wave length of their maximum absorption.

TABLE III

SPECTRAL CHARACTERISTICS OF 2,4-DINITROBENZENE DERIVATIVES BOUND TO BOVINE SERUM ALBUMIN IN 0.05 *M* PHOSPHATE BUFFER, *p*H 7.4

Substituent at carbon one	Total concn., *M* × 10⁵	Free, %	*r*	λmax, mμ	ε at λmax
-OH	3.56	12.6	1.03	358	15,000 ± 170
-ε-Aminocaproic acid	4.63	33.1	1.01	350	15,100 ± 200
-NH_2	11.8	74.4	1.02	340–350	12,400 ± 600

It is of interest to note that binding to bovine serum albumin results in spectral shifts for the three compounds tested. This shift is most marked for ε-DNP-aminocaproic acid where the absorption maximum is displaced to shorter wave length and the extinction coefficient is lowered. In the case of dinitroaniline there is also a lowering of the extinction coefficient, while a shift of the absorption maximum cannot be ascertained because of the relatively large error in the calculation, introduced by the large amount of free dinitroaniline present (see Table III). The albumin-bound form of dinitrophenol shows a displacement of the absorption curve to shorter wave length only in the region of 400 mμ, an effect shown also by

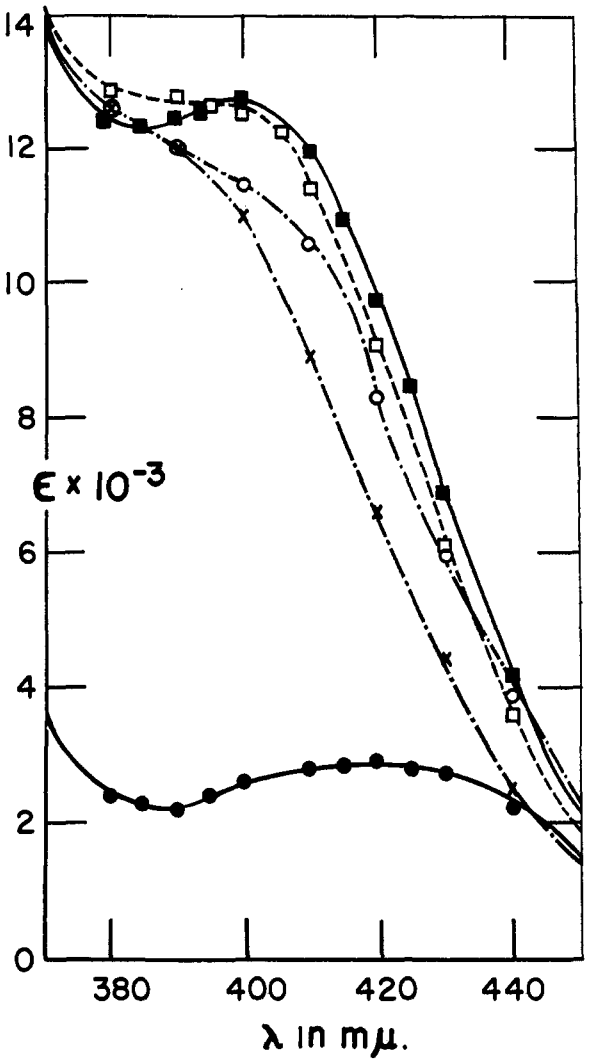

Fig. 4.—Absorption spectra of dinitrophenol in several solvents and of albumin-bound dinitrophenol: 1,4-dioxane, ●——●; butanol, ■——■; ethanol, □----□; water, *p*H 7.4, free, O—.—.—O, and albumin-bound ×—.—.—×.

ε-DNP-aminocaproic acid and dinitroaniline. Similar effects have been described previously for other compounds.[12,24,25] The spectral displacements suggest a change in the small molecule when it is bound to serum albumin.

In order to determine whether hydrogen bonding has an effect on the spectra and correspondingly plays an important role in the binding of the substances in question to serum albumin, the spectrum of the sodium salt of dinitrophenol was obtained in several organic solvents and compared with the spectrum of dinitrophenol in phosphate buffer, *p*H 7.4, and with the spectrum of the albumin-bound dinitrophenol (see Fig. 4). It can be seen that the spectrum in ethanol shows a plateau in the region from 380 to 400 mμ. In butanol there is a distinct peak at 400 mμ with an extinction minimum at 385 mμ. In 1,4-dioxane the peak is even more distinct, and the maximum is displaced to 420 mμ. Apparently the extinction peak of dinitrophenol in the

(24) I. Klotz, *Chem. Revs.*, **41**, 373 (1947).

(25) I. M. Klotz, R. K. Burkhard and J. M. Urquhart, *J. Phys. Chem.*, **56**, 77 (1952).

region around 400 mμ disappears as one goes from dioxane to butanol, to ethanol, to water and to the albumin-bound form of dinitrophenol. The direction of this shift is in accord with the findings of Ungnade[26] for monosubstituted benzene derivatives with electron donor substituents in a variety of solvents. This seems to indicate hydrogen bond formation between dinitrophenol and serum albumin through the oxygen atom of dinitrophenol.

Competitive Binding.—As shown in Figs. 1 and 2, chloride ion at 0.16 *M* concentration inhibits the interaction of bovine serum albumin with dinitrophenol. As non-ionized dinitrotoluene also interferes with the binding of dinitrophenol (see below), it is apparent that both ionic and non-ionic interactions are effective in forming a stable complex of serum albumin with an ionic compound such as dinitrophenol. The binding of dinitroaniline was not affected by the presence of chloride ion at the concentration used. It is of interest that Klotz and Ayers[3] reported displacement of aminoazobenzene from bovine serum albumin by SCN^- ion but not by Cl^- ion. The small effect of chloride ion on the binding of dinitrophenol and the lack of an effect on the binding of dinitroaniline is at least partly due to the small affinity of bovine serum albumin for Cl^- ion. It might also indicate that chloride ion is bound to sites in the protein other than those involved in the interaction with an organic molecule.

In order to test whether there are structural differences among the binding sites in the protein with regard to organic anions and organic molecules, an experiment was set up in which dinitrophenol and dinitrotoluene were equilibrated simultaneously with serum albumin. It can be seen from Fig. 1 that dinitrophenol and dinitrotoluene compete for some common albumin sites, since the amount of dinitrophenol bound is reduced in the presence of dinitrotoluene. At the same free dinitrophenol concentration the total number of moles of dinitrophenol + dinitrotoluene bound exceeds the number of moles of dinitrophenol bound in the absence of dinitrotoluene, until 6 moles is bound (Table IV).

TABLE IV

EFFECT OF DINITROTOLUENE (61.6×10^{-6} *M*) ON THE BINDING OF DINITROPHENOL BY BOVINE SERUM ALBUMIN IN 0.05 *M* PHOSPHATE BUFFER, *p*H 7.4, 25°

Free concn. of dinitrophenol, $c \times 10^5$	A[a] Dinitrophenol in the absence of dinitrotoluene	B Dinitrophenol in the presence of dinitrotoluene	C[b] Dinitrotoluene in the presence of dinitrophenol
71.1	6.20	5.40	1.0
31.4	4.90	4.24	2.0
21.6	4.44	3.91	2.1
12.7	3.69	3.26	2.4
9.1	3.39	3.00	2.5
3.13	2.52	2.03	2.8
1.07	1.60	1.19	3.3
0.427	.86	.64	3.4
0.344	.70	.51	3.7

[a] Interpolated from binding curve (see Fig. 1). [b] The values in this column are subject to error ranging from 10% at low values of *c* to 35% at high values of *c*. Nevertheless, even with this error, the sum of B + C exceeds A for all free concentrations of dinitrophenol up to 31.4×10^{-5} *M*.

This finding suggests that, on the average, some of the sites preferentially bind dinitrophenol while others preferentially bind dinitrotoluene. It may be inferred, therefore, that some specific differences occur in the binding sites of bovine serum albumin despite its great adaptability in the binding of molecules and ions of varying structures.

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[CONTRIBUTION FROM THE RESEARCH DIVISION, ELECTROCHEMICALS DEPARTMENT OF E. I. DU PONT DE NEMOURS AND COMPANY]

A New Synthesis of the Thiazole Fragment of Vitamin B_1[1]

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The thiazole fragment of vitamin B_1, 4-methyl-5-(β-hydroxyethyl)-thiazole, has been prepared by a new 4-step synthesis from 2-methylfuran.

In a study of derivatives of furfural, a new and improved synthesis of 4-methyl-5-(β-hydroxyethyl)-thiazole (V) has been developed. 2-Methylfuran (I), which was employed as the starting material for this route, can be obtained in high yield by the hydrogenation of furfural.[2-4] The thiazole moiety of vitamin B_1 was obtained from I by the four-step synthesis shown below.

2-Methylfuran (I) has previously been converted to 5-hydroxy-2-pentanone (II) by a process equivalent to partial hydrogenation followed by hydrolysis.[4-6] Significant improvement in yields of II was obtained through a detailed study of reaction variables. We have developed a continuous process for the cyclodehydration of II in the presence of acid catalysts to give III in reproducible yields of 86% or more. Although high yields of III had been reported by distillation of II,[4] we

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