

# Comparison between Theoretical and Experimental Electric Dipole Moments of Selected *N,N*-Dimethylaniline Derivatives

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**Abstract** □ Electric dipole moments of nine *N,N*-dimethylaniline derivatives were determined experimentally in benzene solution according to literature methods. The theoretical dipole moments of these compounds were calculated from molecular orbital approximations according to the Del Re  $\sigma$ -electron treatment, the Hückel  $\pi$ -electron method, the Pariser-Parr-Pople treatment, the complete neglect of differential overlap method, and selected combinations of these methods. Parameters not previously reported were determined for the nitro group by reproduction of experimental dipole moments for use in the Del Re calculations. Comparisons between the experimental and theoretical moments indicate that the combination of the Del Re  $\sigma$ -electron and Hückel  $\pi$ -electron methods gave the best reproduction of the experimental dipole moment values while being considerably less expensive in terms of computer size and time, as well as requiring less time in preparing the data than more sophisticated methods examined.

**Keyphrases** □ *N,N*-Dimethylaniline derivatives—comparison between theoretical and experimental electric dipole moments □ Electric dipole moments, *N,N*-dimethylaniline derivatives—comparison between theoretical and experimental values □ Dipole moments, electric, *N,N*-dimethylaniline derivatives—comparison between theoretical and experimental values □ Electronic parameters—comparison between theoretical and experimental electric dipole moments for *N,N*-dimethylaniline derivatives □ Quantum mechanics—comparison between theoretical and experimental electric dipole moments for *N,N*-dimethylaniline derivatives

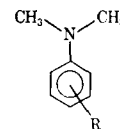
In view of the current interest in correlations between selected physicochemical properties and biological activities of diverse series of compounds (1-5), several investigators have also become interested in the potential applications of quantum mechanical indexes in such studies (6-12). Due to the variety of available methods and levels of sophistication of quantum mechanical calculations, interest was generated in determining the method of choice for the calculation of selected electronic parameters for a series of compounds for subsequent use in structure-activity studies. The basis for selection of a method of choice for this study consisted of the following three criteria: (a) the accuracy of the method; (b) the labor involved in the calculation, including the availability of the parameters required for each method and the relative ease of parameter determination as well as the complexity of setting up the data required for each method; and (c) the cost per calculation for each compound relative to the accuracy, which is primarily dependent, of course, upon the computer size and the computer time required for each calculation.

As Pullman suggested (13), the comparison of charges *per se* is: "a very complicated problem if only because their theoretical definitions are not

equivalent in the different procedures." Also, the charges are not directly measurable quantities. Since the interest of this study is primarily in parameters based on the distribution of electronic charges, the basis selected for relative comparison of the methods was the closely related measurement more readily accessible to a direct experimental check, the molecular electric dipole moments. Pullman (13) carried out a somewhat similar study for the nucleic acid bases. There is, however, a great difference in the nature of the compounds studied, particularly since the compounds of interest in this study contain both electron-withdrawing and electron-releasing substituent groups.

The theoretical methods selected for comparison were: (a) the Del Re  $\sigma$ -charge calculations, (b) the Hückel  $\pi$ -electron molecular orbital approximations, (c) a summation of the Del Re  $\sigma$ - and Hückel  $\pi$ -electron calculations, (d) the Pariser-Parr-Pople self-consistent-field  $\pi$ -electron calculations, (e) a combination of the Del Re  $\sigma$ - and Pariser-Parr-Pople  $\pi$ -electron methods, and (f) the complete neglect of differential overlap all-electron treatment (CNDO/II) method.

The compounds selected for this comparative study were the *N,N*-dimethylaniline series shown in Table I. These compounds present simple, conjugated  $\pi$ -electron systems which allow for a relatively easy and complete  $\pi$ -electron theoretical calculation. Since this series also consists of electron-releasing and electron-withdrawing substituent groups, the



**Table I**—Experimental Electric Dipole Moments of Selected *N,N*-Dimethylanilines<sup>a</sup>

Compound	R	$\mu_s^b$	$\mu_G^c$	Experimental Error
I	H	1.59	1.59	$\pm 0.02$
II	<i>m</i> -CH <sub>3</sub>	1.51	1.52	$\pm 0.003$
III	<i>p</i> -CH <sub>3</sub>	1.12	1.13	$\pm 0.02$
IV	<i>m</i> -OCH <sub>3</sub>	2.18	2.18	$\pm 0.02$
V	<i>p</i> -OCH <sub>3</sub>	1.77	1.78	$\pm 0.04$
VI	<i>m</i> -NO <sub>2</sub>	5.27	5.30	$\pm 0.02$
VII	<i>p</i> -NO <sub>2</sub>	7.09	7.13	$\pm 0.03$
VIII	<i>m</i> -Cl	3.19	3.19	$\pm 0.02$
IX	<i>p</i> -Cl	3.32	3.32	$\pm 0.03$

<sup>a</sup> In benzene solution at  $25 \pm 0.01^\circ$ . <sup>b</sup> Dipole moment calculated by the method of Smith (14). <sup>c</sup> Dipole moment calculated by the method of Guggenheim (15).

study encompasses a fairly wide range of electronic properties.

For comparison with theoretical values, experimental electric dipole moment values for the subject compounds were determined (Table I). The range in electronic properties over the series is exemplified by the range in dipole moment values of 1.12–7.10 Debyes.

## EXPERIMENTAL

**Measurement of Electric Dipole Moments**—The dielectric constants of the series of *N,N*-dimethylaniline compounds in Table I were measured in benzene solution by the heterodyne beat method (16, 17). The compounds were very carefully purified and dried *in vacuo* at 25° over phosphorus pentoxide for 48 hr. Criteria for purity included constant melting points on three successive recrystallizations, single peak on the gas chromatograph, and constant refractive indexes on successive distillations. Seven solutions of approximately 50 ml each and ranging from approximately 0.007 to 0.10% (w/w) in spectroscopic grade benzene<sup>1</sup> were prepared from each compound. Weighings were made to 10<sup>-5</sup> g on a semimicro balance<sup>2</sup>. Flasks containing the solutions were kept in a desiccator over anhydrous calcium sulfate<sup>3</sup> until used. All solutions were measured within 2 hr after preparation.

Dielectric constants of the solutions were measured at 25.00 ± 0.01° on a dipolemeter<sup>4</sup> with the cell thermostated with a circulator<sup>5</sup>. The 20-ml jacketed, gold-plated (DFL 1) cell was calibrated with benzene, carbon tetrachloride, and cyclohexane at 20° using dielectric constant values (18) of 2.2832, 2.2368, and 2.0230, respectively. Refractive indexes were measured at 25.00 ± 0.01° using the D line of sodium on a precision laboratory refractometer<sup>6</sup> thermostated with a circulator<sup>5</sup>. All temperatures were measured with thermometers calibrated against a National Bureau of Standards certified thermometer.

From the indexes of refraction, dielectric constants, and weight fractions of the seven solutions and the solvent, the electric dipole moment ( $\mu$ ) of each compound was calculated according to the methods of Smith (14) ( $\mu_S$ ) and Guggenheim (15) ( $\mu_G$ ).

**Theoretical Electric Dipole Moments**—Net atomic charges were determined from the electron charge densities of the atoms calculated by the various methods according to Eq. 1:

$$Q_r = N - q_r \quad (\text{Eq. 1})$$

where  $Q_r$  is the net charge on atom  $r$ ,  $N$  is the number of electrons contained in the valence orbital of the atom prior to its bonding with other atoms, and  $q_r$  is the charge density at atom  $r$ . The summations of the products of the value of  $Q$  at each atom and the approximate component of its molecular geometry ( $d_x$ ,  $d_y$ , and  $d_z$ ) multiplied by a conversion constant (4.77) to obtain Debye units yield the three-dimensional components ( $\mu_x$ ,  $\mu_y$ , and  $\mu_z$ ) of the dipole moment (Eqs. 2–4):

$$\mu_x = 4.77 \sum(Q_r d_x) \quad (\text{Eq. 2})$$

$$\mu_y = 4.77 \sum(Q_r d_y) \quad (\text{Eq. 3})$$

$$\mu_z = 4.77 \sum(Q_r d_z) \quad (\text{Eq. 4})$$

The resultant theoretical moment for each compound was then calculated according to Eq. 5:

$$\mu = \sqrt{\mu_x^2 + \mu_y^2 + \mu_z^2} \quad (\text{Eq. 5})$$

## RESULTS AND DISCUSSION

Initial calculations were made on the compounds in the series using the simplest of methods, the Del Re (19)  $\sigma$ -electron treatment. By this method, only the  $\sigma$ -electrons are treated. The parameters used in the calculations were those of Del Re (19) and

Table II—Del Re Parameterization of the Nitro Group

Compound	Experimental Dipole Moment, Debyes <sup>a</sup>	Calculated Dipole Moment, Debyes <sup>b</sup>	$\Delta\mu^c$
Nitrobenzene	4.25	4.16	-0.09
<i>m</i> -Nitroaniline	4.90	4.99	+0.09
<i>p</i> -Nitroaniline	5.60	5.60	0.00

<sup>a</sup> Taken from McClellan (21). <sup>b</sup> Calculated from Hückel molecular orbital  $\pi$ -electron charge densities using reported parameters (20, 23) and from Del Re  $\sigma$ -electron charge densities using reported parameters (19, 20). <sup>c</sup>  $\Delta\mu =$  experimental dipole moment - calculated dipole moment.

Berthod and Pullman (20) with the exception of those for the nitro group.

Since the bond parameters ( $\gamma_p$  and  $\epsilon_{pq}$ ) necessary for treatment of the nitro group (C—N and N—O bonds) were not available in the literature, parameterization of this group was necessary. Since these parameters are based on their ability to reproduce experimental electric dipole moment values, closely related molecules (namely, nitrobenzene, *m*-nitroaniline, and *p*-nitroaniline) whose dipole moments were reported previously (Table II) (21), were selected for use in the parameterization of this group. These molecules were selected because of their simplicity and similarity to the molecules to be studied with the derived parameters.

Since  $\gamma_p$  is an empirical measure of the ability of atom  $p$  to polarize atom  $q$  and should not change with the change in bond type, the value of  $\gamma_p$  (0.10) was selected to be the same as that for other C—N and C—O bonds. Therefore, the values of  $\epsilon_{pq}$  were varied to obtain a consistent theoretical reproduction of the experimental dipole moments of all three molecules. Parameters used in these calculations were those reported by Berthod and Pullman (20) and Berthod *et al.* (23) for the Hückel  $\pi$ -electron molecular orbital calculations and by Del Re (19) and Berthod and Pullman (20) for the Del Re calculations. Theoretical dipole moments were calculated as already described<sup>7</sup>.

These calculations resulted in the evaluation of  $\epsilon_{C-N}$  and  $\epsilon_{N-O}$  to be 0.50 and 1.00, respectively. As shown in Table II, all calculated dipole moments are in excellent agreement with the experimental values.

The  $\sigma$ -electron dipole moments ( $\mu_\sigma$ ) for the series of dimethylanilines were then calculated from the atomic net charges obtained through these calculations (Table III). As should be anticipated, the overall agreement between the observed ( $\mu_{obs}$ ) and calculated moments is generally poor. The deviation between these values ( $\Delta\%\mu_\sigma$ ) varies from a low value of 21% for the *p*-methoxy compound to a high value of 98% for the *p*-methyl derivative. The average deviation for the series is 64%.

The second level of calculation was the Hückel  $\pi$ -electron molecular orbital treatment (HMO) (22). According to this method, only the  $\pi$ -electrons are treated. The parameters used in these calculations<sup>8</sup> were those determined by Berthod and Pullman (20) and Berthod *et al.* (23). These values were selected in order to use parameters consistent with those used in the Del Re study.

Again, the  $\pi$ -electron electric dipole moments ( $\mu_{\pi(HMO)}$ ) were calculated from the atomic net charges (Table III). The overall deviations between the calculated and observed dipole moments are somewhat lower from Hückel molecular orbital  $\pi$ -electron calculations than from the  $\sigma$ -electron calculations. This indicates a greater contribution of the  $\pi$ -electrons to the overall dipole moment than that of the  $\sigma$ -electrons. The deviations ( $\Delta\%\mu_{\pi(HMO)}$ ) range from a low of 1% for the *p*-methyl derivative to a high of 91% for the *p*-methoxy derivative. The average deviation for this method is 46%.

A third treatment of the data was the calculation of the dipole moments from a summation of the Del Re  $\sigma$ - and the Hückel  $\pi$ -

<sup>1</sup> Matheson, Coleman and Bell.

<sup>2</sup> Sartorius Selectra.

<sup>3</sup> Drierite.

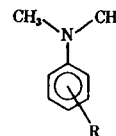
<sup>4</sup> Wissenschaftlich-Technische Werkstätten DM01.

<sup>5</sup> Haake NBe.

<sup>6</sup> Bausch & Lomb Inc.

<sup>7</sup> All calculations were made on the IBM 1620<sup>11</sup> computer using programs written by Dr. G. E. Bass and Dr. K. Sundaram and the All-India Institute of Medical Sciences.

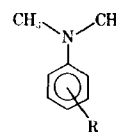
<sup>8</sup> Calculations were performed on the IBM 1620<sup>11</sup> computer using a program written by Dr. K. Sundaram and modified by Dr. G. E. Bass.



**Table III**—Theoretical Electric Dipole Moments of *N,N*-Dimethylanilines Calculated from the Del Re  $\sigma$ -Electron and Hückel Molecular Orbital  $\pi$ -Electron Charges and Comparison with Observed Values

R	$\mu_{\text{obs}}^a$	$\mu_{\sigma}$	$\Delta\% \mu_{\sigma}^b$	$\mu_{\pi}(\text{HMO})$	$\Delta\% \mu_{\pi}(\text{HMO})^b$	$\mu_{\sigma+\pi}(\text{HMO})$	$\Delta\% \mu_{\sigma+\pi}(\text{HMO})^b$
H	1.59	0.06	96	1.13	29	1.19	25
<i>m</i> -CH <sub>3</sub>	1.51	0.08	95	1.13	25	1.15	24
<i>p</i> -CH <sub>3</sub>	1.12	0.02	98	1.13	1	1.11	1
<i>m</i> -OCH <sub>3</sub>	2.18	1.43	34	1.16	47	2.12	3
<i>p</i> -OCH <sub>3</sub>	1.77	1.40	21	0.16	91	1.31	26
<i>m</i> -NO <sub>2</sub>	5.28	1.41	73	3.44	35	4.82	9
<i>p</i> -NO <sub>2</sub>	7.10	1.45	80	3.96	44	5.40	24
<i>m</i> -Cl	3.19	1.90	40	0.99	69	2.35	26
<i>p</i> -Cl	3.32	1.93	42	0.79	76	2.72	18

<sup>a</sup> Observed dipole moments were measured in benzene solution at  $25 \pm 0.01^\circ$ . <sup>b</sup>  $\Delta\% \mu = (\mu_{\text{obs}} - \mu_{\text{calc}}) / \mu_{\text{obs}} \times 100$ .



**Table IV**—Theoretical Electric Dipole Moments of *N,N*-Dimethylanilines Calculated from Del Re  $\sigma$ -Electron and Pariser-Parr-Pople  $\pi$ -Electron Charges and Comparison with Observed Values

R	$\mu_{\text{obs}}^a$	$\mu_{\sigma}$	$\mu_{\pi}(\text{PPP})$	$\Delta\% \mu_{\pi}(\text{PPP})^b$	$\mu_{\sigma+\pi}(\text{PPP})$	$\Delta\% \mu_{\sigma+\pi}(\text{PPP})^c$
H	1.59	0.06	0.79	50	0.85	46
<i>m</i> -CH <sub>3</sub>	1.51	0.08	0.79	48	0.81	46
<i>p</i> -CH <sub>3</sub>	1.12	0.02	0.79	29	0.77	31
<i>m</i> -OCH <sub>3</sub>	2.18	1.43	0.68	69	2.01	8
<i>p</i> -OCH <sub>3</sub>	1.77	1.40	0.33	81	1.62	8
<i>m</i> -Cl	3.19	1.90	0.79	75	2.41	24
<i>p</i> -Cl	3.32	1.93	0.79	76	2.72	18

<sup>a</sup> Observed moments were measured in benzene solution at  $25 \pm 0.01^\circ$ . <sup>b</sup>  $\Delta\% \mu = (\mu_{\text{obs}} - \mu_{\text{calc}}) / \mu_{\text{obs}} \times 100$ .

electron net charges ( $\mu_{\sigma+\pi}(\text{HMO})$ ). The results of this treatment are also shown in Table III. By this method, the calculated dipole moments are in very good agreement with the observed values; the range of the deviations ( $\Delta\% \mu_{\sigma+\pi}(\text{HMO})$ ) goes from 1% for the *p*-methyl derivative to 26% for the *m*-chloro compound. The overall average deviation for the series is only 17%.

The next level of treatment used was the Pariser-Parr-Pople self-consistent-field (PPP-SCF)  $\pi$ -electron method (24-27). Only the  $\pi$ -electrons are treated in this method. The parameters used in the calculations<sup>9</sup> were developed by Nagata *et al.* (28). The geometry of each molecule was derived from the bond distances and bond angles reported by Pople and Gordon (29). Two-center Coulomb integrals were calculated by the Mataga-Nishimito approximation (30).

Similar to the other treatments, dipole moments ( $\mu_{\pi}(\text{PPP})$ ) were calculated from the atomic net charges (Table IV). Missing in this treatment are the nitro derivatives of the series which were deleted due to a convergence problem in the calculations. By this method, the deviations between the observed and calculated values ( $\Delta\% \mu_{\pi}(\text{PPP})$ ) range from 29% for the *p*-methyl compound to 81% for the *p*-methoxy congener. The average deviation for the series was 61%, indicating poor agreement for the moments calculated by this method and considerably poorer agreement than that obtained for the Hückel molecular orbital  $\pi$ -electron moments.

Similar to the Hückel treatment, the Del Re  $\sigma$ -electron charges were summed with the Pariser-Parr-Pople  $\pi$ -electron charges, and the dipole moments ( $\mu_{\sigma+\pi}(\text{PPP})$ ) were recalculated from these values. Very good agreement (Table IV) between the observed and calculated values was obtained. The deviations ( $\Delta\% \mu_{\sigma+\pi}(\text{PPP})$ ) range from a low of 8% for the *m*-methoxy and *p*-methoxy compounds to a high of 46% for the unsubstituted and *m*-methyl derivatives. The overall average deviation for the series by this method is 26%, indicating relatively good agreement between the calculated and observed moments.

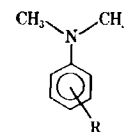
The highest level of sophistication used in this study was the complete neglect of differential overlap treatment (CNDO/II) of Pople and Segal (32, 33). This treatment is essentially an extension of the Pariser-Parr-Pople self-consistent-field formalism to include all valence electrons ( $\pi$  and  $\sigma$ ). The approximations and assumptions used in this method are for the most part those used in the solutions by the Pariser-Parr-Pople method, with the exception that the molecules need not be planar for the complete neglect of differential overlap treatment. Although they are used in a slightly different manner for inclusion in the integrals of the Roothaan equations, the parameters necessary for solution by this method are those used in the Pariser-Parr-Pople formalism. The parameters used in these calculations<sup>10</sup> were those reported by Nagata *et al.* (28). The three-dimensional geometries of the molecules were calculated using the bond distances and bond angles reported by Pople and Gordon (29).

The chloro derivatives were not included in this treatment since the available facilities are not capable of handling second-row elements. From these calculations, the electric dipole moments were calculated in two ways. The first method is similar to the other calculations in that the atomic net charges were used to determine the theoretical moments ( $\mu_{\text{CNDO/II}(\text{ac})}$ ) (Table V). By this method, the overall agreement between the calculated and observed values appears to be very good with the largest difference ( $\Delta\% \mu_{(\text{ac})}$ ) being 55% for the *m*-methyl derivative and the smallest difference being 4% for the *m*-nitro congener. The average percentage deviation for the series is 37%.

The second method is denoted as the "complete dipole." By this method, the dipole moment calculated from the atomic charges is corrected for the atomic polarization resulting from the  $2s$  and  $2p$  orbital mixing (33). This method is considered to be one of the most accurate of the crude theoretical dipole moment calculations. By this method the calculated moments ( $\mu_{\text{CNDO/II}(\text{cd})}$ ) are in excellent agreement with the observed values (Table V). The largest deviation ( $\Delta\% \mu_{(\text{cd})}$ ) between the two is

<sup>9</sup> Calculations were carried out on the IBM 360/40 computer using a program written by Dr. T. Janiszewski (31) and modified by Dr. G. E. Bass.

<sup>10</sup> Calculations were carried out on the Sigma 9 computer using a program written by Dr. G. A. Segal (34).



**Table V**—Theoretical Electric Dipole Moments of *N,N*-Dimethylanilines Calculated from the Complete Neglect of Differential Overlap Treatment and Comparison with Observed Values

R	$\mu_{\text{obs}}^a$	$\mu_{\text{CNDO/II(ac)}}^b$	$\Delta\% \mu_{(\text{ac})}^c$	$\mu_{\text{CNDO/II(cd)}}^d$	$\Delta\% \mu_{(\text{cd})}^e$
H	1.59	0.80	50	0.88	45
<i>m</i> -CH <sub>3</sub>	1.51	0.68	55	0.72	52
<i>p</i> -CH <sub>3</sub>	1.12	0.68	39	0.76	32
<i>m</i> -OCH <sub>3</sub>	2.18	1.25	43	2.39	10
<i>p</i> -OCH <sub>3</sub>	1.77	0.89	50	1.93	9
<i>m</i> -NO <sub>2</sub>	5.28	5.04	4	5.90	12
<i>p</i> -NO <sub>2</sub>	7.10	5.92	17	6.81	4

<sup>a</sup> Observed dipole moments were measured in benzene solution at  $25 \pm 0.01^\circ$ . <sup>b</sup> (ac) indicates that the dipole moment was calculated from the atomic charges. <sup>c</sup>  $\Delta\% \mu = (\mu_{\text{obs}} - \mu_{\text{calc}}) / \mu_{\text{obs}} \times 100$ . <sup>d</sup> (cd) indicates that the dipole moment is the calculated "complete dipole."

52% for the *m*-methyl compound, and the smallest deviation is 4% for the *p*-nitro derivative. The small overall average deviation (23%) by these calculations indicates that this method gave the second best agreement of those used in this comparative study.

A summary of the results of this comparative study is presented in Table VI. Listed beside the method used in the dipole moment calculations is the average percentage deviation between the theoretical and observed electric dipole moments. On this basis of comparison, the highest level of sophistication of the calculations (complete neglect of differential overlap) gave only the second best agreement between the values (second lowest deviation), while a combination of the much less sophisticated treatments (Del Re plus Hückel molecular orbital) led to a slightly better agreement (average deviation of 17 versus 23%). As a result of this study, it is concluded that the less sophisticated methods gave the best "accuracy" (correlation with experimental electric dipole moments) in the treatment of this series of compounds while being considerably less expensive in terms of computer size and time as well as in the time required to prepare the data.

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**Table VI**—Average Deviations between Theoretical and Observed Electric Dipole Moments of Selected *N,N*-Dimethylanilines

Method of Calculation	Average Deviation <sup>a</sup> , %
Del Re $\sigma$ -electron net charges	64
Hückel molecular orbital $\pi$ -electron net charges	46
Pariser-Parr-Pople $\pi$ -electron net charges	61
Summation of Del Re $\sigma$ - and Hückel molecular orbital $\pi$ -electron net charges	17
Summation of Del Re $\sigma$ - and Pariser-Parr-Pople $\pi$ -electron net charges	26
Complete neglect of differential overlap atomic net charges	37
Complete neglect of differential overlap complete dipole	23

<sup>a</sup> Average of  $(\mu_{\text{obs}} - \mu_{\text{calc}}) / \mu_{\text{obs}} \times 100$  for the series.

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## Kinetic Approach to Drug-Protein Binding

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**Abstract** □ Since estimates in the literature for the fundamental binding parameters of dicumarol to bovine serum albumin show wide disparity, these phenomena were reinvestigated, using a nonequilibrium partitioning technique. A three-phase system was used consisting of an aqueous drug-protein phase and a separate aqueous receiving phase, both in mutual contact with a third overlying immiscible organic phase. The phases were contained in a partitioned, temperature-controlled cylindrical glass cell which could be rotated along its horizontal axis to effect gentle yet thorough mixing and drug transfer. Free (unbound) drug diffused from the protein phase sequentially into the other phases where it was analyzed. This technique allowed estimates of free and bound drug to be made over a very wide range of drug-protein ratios, using a single drug-protein solution, in a short time. Data analysis using an iterative, nonlinear, least-squares computer program indicated that there were two classes of binding sites with the following characteristics;  $n_1 = 2.3 \pm 0.15$ ,  $K_1 = (1.8 \pm 0.23) \times 10^5 M^{-1}$ ;  $n_2 = 14.0 \pm 0.71$ ,  $K_2 = (3.0 \pm 0.36) \times 10^8 M^{-1}$ . A computer-solved theoretical treatment of drug transfer within the cell showed good correlation with the experimental results. The rotating cell technique possesses a flexibility in adjustment of operational parameters which should suit it to the study of the binding characteristics of various compounds.

**Keyphrases** □ Dicumarol—reinvestigation of binding to bovine serum albumin, nonequilibrium partitioning technique, three-phase system □ Bovine serum albumin binding of dicumarol—reinvestigated using nonequilibrium partitioning technique, three-phase system □ Drug-protein binding—dicumarol and bovine serum albumin, kinetics determined using nonequilibrium partitioning technique □ Binding of dicumarol to bovine serum albumin—kinetics determined using nonequilibrium partitioning technique, three-phase system

Methods used to study drug-protein interactions can be grouped under four headings: subtractive, direct, electrostatic, and other (1). All methods have their advantages and disadvantages, and the selection of a particular approach depends mainly on the system under study and the aims of the experiment.

The major drawback of direct methods, where both free and bound drug concentrations are determined in the presence of protein, is that the protein can create assay problems. Therefore, most reports are based on the subtractive approach, in which free drug is separated from the protein prior to assay; by subtraction of free drug from the total quantity of drug added to the system, bound drug is obtained. Such methods include equilibrium dialysis, equilibrium partitioning, ultrafiltration, ultracentrifugation,

and gel filtration. Of these, equilibrium dialysis is the most frequently used.

Equilibrium methods can involve considerable labor and cost of chemicals since they require the preparation of many drug-protein samples to cover an adequate range of binding ratios—each sample provides but one data point. There is also a time delay inherent in this approach, which may allow protein denaturation or microbial growth. Recently, there has been a growing interest in kinetic methods to circumvent some of these limitations.

Stein (2) introduced a dynamic dialysis method based on the use of an automated analyzer which reduced the experimental time from hours to minutes. He reported that the binding data of methyl orange to bovine serum albumin obtained by this technique were similar to those obtained (3) using equilibrium dialysis. While this work pioneered the kinetic approach, only one binding system was investigated over a very limited range. Although the technique eliminated prolonged waiting time, individual drug-protein solutions covering all the binding ratios still had to be prepared.

A flow dialysis technique was reported (4-6) which both reduced experimental time and enabled a number of data points to be obtained from a single drug-protein solution. The binding of a number of drugs was studied, and membrane effects were a disturbing factor.

Meyer and Guttman (7) reported preliminary work on a nonequilibrium dialytic method. They later published details of their studies on the various factors influencing the method, and they investigated the binding behavior of a number of compounds including phenol red, methyl orange, warfarin, caffeine, 8-nitrotheophylline, 8-chlorotheophylline; and salicylic acid (8, 9). In their technique, the drug and protein were placed together inside a membrane sac supported in drug-free buffer; unbound drug diffused into the buffer solution at a rate proportional to its concentration in the sac. The concentration of drug in the external buffer phase was kept at a low level by sampling at selected time intervals and replacement with fresh buffer. This method allowed rapid, easy generation of a large series of free and bound