Pharmacokinetics of Chlorpromazine-Induced Miotic Response in Rabbits

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Abstract □ The time variation of changes in the chlorpromazine-induced pupil diameter decrease was studied following varying bolus and slowly infused intravenous doses administered to rabbits. The observed pharmacological response data were converted, *via* the use of a dose–effect curve, to values theoretically corresponding to relative biophasic drug levels. These values were, in turn, used to construct a linear pharmacokinetic model of the drug bioavailability input ⇆ pharmacological response output dynamics of the system. The use of a time domain, MULTIFIT, computerized method of fitting the data to obtain a pharmacokinetic model was compared to the use of a frequency response, PLTEST, approach. The fidelity of the model in quantitatively relating the time course of systemic drug bioavailability to observed pupil response was verified by the satisfactory agreement obtained by directly comparing experimentally known amounts of drug intravenously infused with corresponding values computed from observed changes in pupil size. The applicability of using pharmacological data for quantitative bioavailability and pharmacokinetic analysis of chlorpromazine is demonstrated. This finding is particularly significant because no suitable chemical or radiological direct assay technique exists for determining levels of chlorpromazine, except for high doses, in body fluids.

Keyphrases □ Chlorpromazine—pharmacokinetic model relating induced miotic response to systemic bioavailability, rabbits □ Pharmacokinetic models—chlorpromazine-induced miotic response related to systemic bioavailability, rabbits □ Bioavailability, systemic—related to chlorpromazine-induced miotic response, pharmacokinetic model, rabbits □ Miotic response—chlorpromazine induced, related to systemic bioavailability, pharmacokinetic model, rabbits □ Tranquilizers—chlorpromazine, pharmacokinetic model relating induced miotic response to systemic bioavailability, rabbits

One principal objective of pharmacokinetic research involves the development of mathematical models to characterize the dynamics of drug transfer and drug effects in pharmacologically responding systems. For such models to be of maximum value for the elucidation of the pharmacokinetic system behavior, they must allow the prediction of the time course of drug-induced responses as a function of the manner in which the drug is made available to the biological system.

To elucidate the operative mechanisms involved in pharmacodynamic systems in terms of mathematical models, one must develop a means by which the transfer of a drug from its site of administration to its site(s) of action can be quantitated. This may be done by methods first made familiar by Wagner and Nelson (1) and further developed by Loo and Riegelman (2) if the drug level at the site of administration or in some body compartment can be periodically determined. Such direct methods are seldom practical for the detection of small quantities of drug, and an adequate direct assay may not exist for certain drugs such as chlorpromazine.

Methods of analysis that rely on the detection of the drug in the body fluids are generally applicable only to systemic routes of administration. However, the appropriate use of graded pharmacological response intensity data, when applicable, permits the bioavailability characteristics of a drug to be determined following its administration by any route and obviates detection by direct assay methods.

A theoretical basis has been developed for the quantitative determination of relative biophasic drug levels at any time following dosing by any route of administration using data obtained from the observation of the time course of pharmacological response intensity (3). Engineering analysis techniques for dynamic systems can be directly applied to describe the pharmacokinetic processes that determine the quantitative nature of drug effects (4).

The concept of a direct and rapidly reversible pharmacological effect implies that a given intensity of response is associated with a particular drug concentration at the site(s) of action (5). Smolen and coworkers, *e.g.*, (6), developed a theoretical basis for a pharmacokinetic analysis of data obtained by observing the time course of pharmacological response intensity following single, multiple, or continuous dosing of a drug by any route of administration. Through the suitable use of experimentally determined dose–effect curves, the observed intensities of pharmacological response could be transferred into relative biophasic drug levels at all times following dosing (7), thereby obviating the postulation of hypothetical models for drug–receptor site interaction. This method is general and does not require the assumptions of earlier methods, other than requiring the dynamics of drug disposition to be linear or at least to be linear in an operational range of interest.

The drug chlorpromazine[1] was chosen because of its known effects on the intraocular pressure and pupil diameter in both animals and humans. The extreme lowering of intraocular pressure was demonstrated in cataractous eyes after preoperative administration of chlorpromazine (8). A brief ocular hypotensive effect was noted with chlorpromazine in experimental animals given 25–100 mg im (9), and the drug also caused miosis in rabbits and mydriasis in cats (9, 10). The adrenolytic and ganglionic blocking actions of chlorpromazine might explain the mechanisms for this response.

THEORETICAL

Pharmacokinetic Models—The biokinetic behavior of a drug can be characterized most concisely in terms of mathematical models, and the input for the construction of such models is provided by data reflecting the time course of variation of drug levels in the biophase. By treating the biophase as a compartment, its level of drug at any time

[1] Thorazine, Smith Kline and French, Philadelphia, Pa.

following dosing is by definition uniform throughout its volume and is responsible for the observed intensity of response to the drug.

Since the biophase is a hypothetical concept and often cannot be assayed directly for its drug content, the use of the time course of change of pharmacological response intensities can be an advantageous alternative to the use of data derived from direct chemical or radiological assay of drugs in plasma or other body fluids. However, a functional relationship must be established between the level of drug in the biophase at any time and the observable pharmacological response to that drug. Once this relationship has been established, the mathematical methods employed for the pharmacokinetic analysis of drug action using pharmacological response data are usually identical to the methods used to treat drug assay data.

The conversion of pharmacological response intensity data into biophasic drug levels (or relative biophasic drug levels) is accomplished using an experimentally determined dose–effect curve or the drug's resolution in the manner of a calibration curve. The theoretical basis for the use of dose–effect curves to transform pharmacological data into relative biophasic drug levels prior to further analysis was described previously (5–7).

Model Determination—The principal objective of pharmacokinetic research is the quantitative characterization of the biokinetic behavior of a drug in terms of mathematical models. Engineering analysis techniques for dynamic systems can be applied directly to describe the pharmacokinetic processes determining the quantitative nature of drug effects (4). These powerful techniques involve the areas of engineering control theory and signal processing. Interest in the pharmacokinetic analysis of pharmacological data has been spurred by the need to understand the rates of transfer of a drug to sites of action; such knowledge can facilitate the assessment of factors that account for individual variations in effects elicited by a drug (11, 12).

Except for the transduction between biophasic drug levels and drug response, most drug transfer systems can be described in terms of linear mathematical models or models that are linear piecewise over an operational range of interest. To characterize a pharmacologically responding system in mathematical terms, it is necessary to establish the relationship between drug input and response output (in terms of biophasic drug levels) for bolus intravenous administration of the drug. Such an injection represents an impulse of drug input. If the time course of pharmacological response is then observed following this impulse input and transformed into the corresponding time course of biophasic drug levels *via* the dose–effect curve, the unit impulse response or transfer function for the system is obtained. By assuming the initial conditions to be zero, the transfer function may be mathematically described as:

$$\frac{Y(s)}{X(s)} = G(s) = \text{transfer function} \qquad \text{(Eq. 1)}$$

where s is the independent variable in the Laplace Transform Domain. When the input function is a unit impulse [*i.e.*, $X(s) = 1$], the response output of the system, $Y(s)$, is equal to the transfer function, $G(s)$.

Several techniques can be used to determine a transfer function model for a system. Traditionally, methods are used to best "fit" the data by the technique of nonlinear least squares, *i.e.*, estimation of model parameters such that they minimize the sum of squares of differences between observed and predicted values.

Other methods of fitting sums of exponentials to data are available but are usually for restricted cases (13). A powerful technique, widely used in engineering analysis (14–17) but which has not been applied to pharmacological data, is the frequency response method. General frequency response methods require the excitation of the system for several sinusoidal functions until a new steady state is achieved. In practice, this is impractical because it requires the system to be disturbed several times for prolonged periods. A more practical approach to obtain frequency response information is a "pulse testing" method. The use of frequency response *via* the pulse testing approach was discussed in detail (18). A digital computer program PLTEST was developed by the authors to perform the computations efficiently. A detailed presentation of this method will be reported separately.

Frequency response methods have certain inherent advantages over nonlinear least-squares-type fitting procedures. The prinicpal advantage of pulse testing is that no *a priori* knowledge of the order of the system is required. For least-squares fitting, a form of the model and the number of terms in it must be assumed. In frequency response techniques, the number of asymptotes required to approximate the Bode diagram give rise to the number of poles and zeros of the transfer function.

Table I—Intensity of Pupillary Diameter Decrease (Miosis) in Rabbits Measured as $(P_0 - P)/P_0 \times 10^4$ as a Function of Five Bolus Intravenous Doses of Chlorpromazine

	Dose, mg/kg iv				
Minutes	4.0	3.0	2.0	1.0	0.5
10	1668	1540	923	967	345
20	2321	2020	1399	1307	937
30	2333	2236	1814	1380	1266
40	2561	2280	1928	1520	1375
50	2533	2280	2047	1500	1424
60	2454	2460	2028	1630	1448
70	2495	2360	2082	1703	1498
80	2442	2110	1987	1703	1438
90	2361	2200	1946	1563	1408
100	2352	2180	1973	1550	1337
110	2360	2150	1927	1497	1320
120	2317	2140	1820	1417	1323
130	2240	2200	1813	1407	1270
140	2176	2180	1830	1410	1197
150	2104	2103	1817	1413	1123
160	2048	2113	1733	1360	1033
170	1986	2007	1677	1320	950
180	1907	1837	1636	1300	867
190	1894	1820	1630	1237	830
200	1886	1790	1620	1180	802
210	1875	1757	1617	1157	777
220	1860	1767	1580	1183	683
230	1846	1720	1553	1147	603
240	1820	1630	1527	1060	529
250	1768	1580	1482	1003	443
260	1706	1553	1435	957	377
270	1640	1530	1377	880	317
280	1610	1457	1282	827	250
290	1580	1367	1180	773	180
300	1556	1280	1090	727	107
310	1532	1283	1050	653	93
320	1522	1283	997	583	80
330	1486	1260	937	527	65
340	1450	1240	847	537	49
350	1410	1123	763	527	37
360	1378	963	680	483	25

Another advantage of this method is that it can handle any general input pulse, whereas fitting procedures usually require data from a specific type of pulse. Furthermore, PLTEST takes only a fraction of the time that MULTIFIT takes for the same order system. This advantage becomes particularly important with higher order systems.

Model Confirmation—The inverse transformation to the time domain of the product of two Laplace transformed functions results in the convolution integral. As an example, the output of a system would be computed using the convolution integral if the system transfer function and the input to the system are known:

$$Y(S) = G(S)^*X(S) \qquad \text{(Eq. 2)}$$

or:

$$Y(t) = \int_0^t g(\tau)X(t-\tau)\,d\tau \qquad \text{(Eq. 3)}$$

In the case of model confirmation, it is assumed that the input function is unknown. With an observed output response and a previously obtained transfer function, the task is now to determine the input that caused the given response. This problem is often termed "deconvolution."

There are several possible methods of performing a deconvolution: (*a*) numerical deconvolution, (*b*) analog deconvolution, and (*c*) digital deconvolution. Each method has its advantages and disadvantages (19). A digital deconvolution scheme has been implemented for this purpose. The method essentially involves converting the deconvolution problem into a system of linear differential equations in vector–matrix notation. The details will be presented in a future report.

For all real systems, the numerator of the transfer function is of a lower order than the denominator. This condition causes problems in deconvolution by the above procedure because it gives rise to in-

Table II—Pupillary Diameter Response Intensities from Table I Transduced to $f(I)$ Values (Relative Biophasic Drug Levels) Using the Dose–Effect Curve as a Calibration Curve and Normalized by Dividing by the Corresponding Dose

	$f(I)$/Dose						
	Dose, mg/kg iv						
Minutes	4.0	3.0	2.0	1.0	0.5	Average	*SEM*
10	0.236	0.258	0.155	0.325	0.166	0.228	0.07
20	0.631	0.555	0.313	0.545	0.630	0.535	0.13
30	0.644	0.758	0.600	0.603	1.026	0.726	0.18
40	1.000	0.800	0.727	0.750	1.206	0.897	0.20
50	0.956	0.800	0.869	0.728	1.296	0.930	0.22
60	0.782	1.053	0.844	0.888	1.350	0.984	0.23
70	0.872	0.894	0.910	1.000	1.456	1.026	0.25
80	0.773	0.629	0.797	1.000	1.320	0.904	0.27
90	0.671	0.713	0.747	0.8000	1.266	0.839	0.24
100	0.663	0.698	0.775	0.788	1.150	0.815	0.19
110	0.671	0.667	0.727	0.728	1.100	0.779	0.18
120	0.631	0.662	0.613	0.640	1.100	0.729	0.21
130	0.569	0.713	0.600	0.633	1.026	0.708	0.19
140	0.524	0.698	0.625	0.638	0.936	0.684	0.15
150	0.469	0.625	0.613	0.638	0.830	0.635	0.13
160	0.434	0.629	0.525	0.588	0.730	0.581	0.11
170	0.399	0.542	0.481	0.550	0.640	0.522	0.09
180	0.350	0.420	0.450	0.538	0.550	0.462	0.08
190	0.344	0.408	0.444	0.493	0.526	0.443	0.07
200	0.342	0.389	0.438	0.455	0.500	0.425	0.06
210	0.335	0.367	0.438	0.440	0.486	0.413	0.06
220	0.325	0.373	0.412	0.455	0.410	0.395	0.05
230	0.319	0.342	0.394	0.428	0.340	0.365	0.04
240	0.306	0.296	0.379	0.380	0.290	0.330	0.05
250	0.280	0.275	0.352	0.348	0.226	0.296	0.05
260	0.250	0.263	0.330	0.323	0.190	0.271	0.06
270	0.225	0.253	0.302	0.285	0.150	0.243	0.06
280	0.215	0.229	0.262	0.263	0.116	0.217	0.06
290	0.206	0.197	0.228	0.238	0.090	0.192	0.06
300	0.197	0.175	0.197	0.218	0.050	0.167	0.07
310	0.189	0.175	0.188	0.188	0.046	0.157	0.06
320	0.188	0.175	0.173	0.160	0.040	0.147	0.06
330	0.176	0.169	0.157	0.145	0.030	0.135	0.06
340	0.169	0.164	0.134	0.150	0.026	0.129	0.06
350	0.160	0.138	0.118	0.145	0.020	0.116	0.06
360	0.151	0.108	0.100	0.128	0.010	0.099	0.05

tegral equations rather than differential equations which are easier to solve digitally. To overcome this problem, the procedure involves the addition of as many zeros as required to make the order of the numerator equal to that of the denominator. Quite obviously, the transfer function of the system is changed. However, this change is minimized by the addition of zeros at frequencies 10 times higher than any other frequency present in the system transfer function. This provision ensures that distortions are minimal; it also permits the representation of the problem by means of a system of differential equations. For example, the system:

$$\frac{Y(S)}{X(S)} = \frac{A}{(S+m_1)(S+m_2)(S+m_3)} \qquad m_1 > m_2 > m_3 \qquad \text{(Eq. 4)}$$

can then be written as:

$$X(S) = \frac{(s+m_1)(s+m_2)(s+m_3)}{A(s+10m_1)(s+10m_1)(s+10m_1)}\,Y(S) \qquad \text{(Eq. 5)}$$

Now, this problem is rewritten in the form:

$$\mathbf{x}(t) = \underline{A}\mathbf{x}(t) + \underline{B}y(t) \qquad \text{(Eq. 6)}$$

where bold face denotes a vector and a single underline denotes a matrix.

This vector equation is integrated using the modified Euler numerical integration scheme. Practically speaking, it is difficult to obtain accurate results digitally if the input function was an impulse function or a pulse of very short duration. Hence, an extra s is introduced in the denominator of Eq. 5 so that the result of the numerical integration gives the cumulative or integrated input. Symbolically:

$$X'(S) = \frac{1}{s}\,\frac{(s+m_1)(s+m_2)(s+m_3)}{A(s+10m_1)(s+10m_1)(s+10m_4)}\,Y(S) \qquad \text{(Eq. 7)}$$

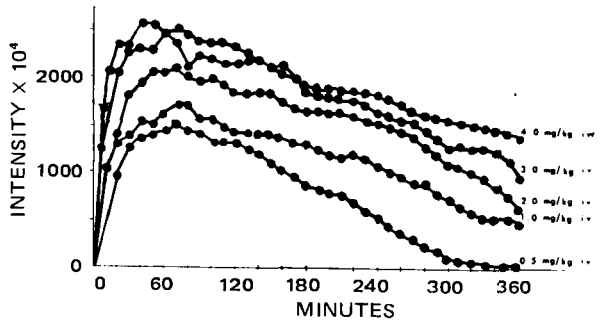

Figure 1—*Intensity of pupillary diameter decrease (miosis) in rabbits measured as* $(P_0 - P)/P_0$, *using a TV pupillometer, as a function of five bolus intravenous doses of chlorpromazine. Each curve represents averaged data from three or four rabbits.*

and:

$$\mathbf{X}'(t) = \underline{A}'x'(t) + \underline{B}y(t) \qquad \text{(Eq. 8)}$$

where:

$$\mathbf{X}'(t) = \int_0^t \mathbf{x}(t)\,dt \qquad \text{(Eq. 9)}$$

A future report will discuss the numerical deconvolution procedure in detail; a simplified overview appeared elsewhere (20, 21).

Experimental and Computational Procedure for Performing a Pharmacokinetic Analysis Using Pharmacological Data—The most commonly applicable procedure for performing a pharmacokinetic analysis of drug action using pharmacological data involves the following steps (20, 21):

1. Administration of several bolus intravenous doses of the drug and monitoring of the time course of pharmacological response intensities. The drug should induce a graded response intensity, and the maximum response resulting from each dose should occur at approximately the same time. If it does not, the assumption of linearity does not hold and an alternative approach must be implemented.

2. Construction of a dose–effect curve by plotting the maximum observed response intensities as a function of dose. The intensity of pharmacological response should be a single-valued, nonhysteretic function of biophasic drug levels (6).

3. Conversion of each I *versus* time–response profile resulting from each dose into a corresponding $f(I)$ *versus* time profile.

4. Dose normalization of the $f(I)$ values for each dose and plotting of all $f(I)/D$ values on the same set of coordinates. All points should scatter around the same curve to confirm the assumption of linearity.

5. Fitting of a sum of exponential equation to the $f(I)/D$ *versus* time curve using various weighted least-squares, regression, computer techniques or other techniques derived from engineering systems analysis such as the pulse testing technique to yield values of equation parameters.

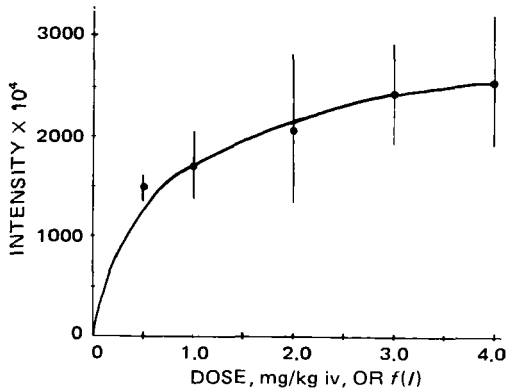

Figure 2—*Intravenous dose–effect curve for chlorpromazine-induced pupillary diameter decrease in rabbits constructed from the maximum response intensities from Fig. 1, with the standard error of the mean indicated for each value. The abscissa is relabeled* f(I), *which corresponds to a relative biophasic drug level.*

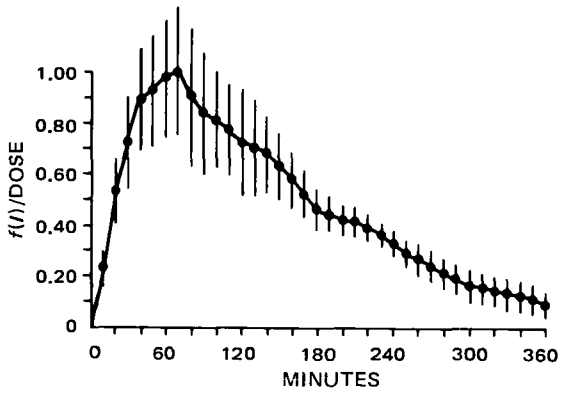

Figure 3—*Pupillary diameter response intensities from Fig. 1 transduced to* f(I) *values (relative biophasic drug levels) using the dose–effect curve as a calibration curve and normalized by dividing by the corresponding dose. The average* f(I)*/dose values are indicated along with the standard error of the mean for each value.*

6. Development of a model transfer function describing the relationship between drug input and response output. The transfer function, $G(s)$, may be defined as the Laplace Transform of the $f(I)/D$ *versus* time function obtained in Step 5. The form of $G(s)$ is given by:

$$G(s) = Lg(t) = L\sum_{i=1}^{N} A_i e^{-m_i t} = \sum_{i=1}^{N} \frac{A_i}{m_i + s} \qquad \text{(Eq. 10)}$$

where $g(t)$ is the "weighting function," and L signifies the Laplace operator.

7. Verification of the transfer function model for use in pharmacokinetic computations by a direct comparison of experimentally known drug inputs obtained by programmed intravenous infusion with results computed from monitoring the pharmacological response intensities during the intravenous administration of the drug. These observed time variations of drug response intensities are converted into their corresponding relative biophasic drug level *versus* time profile using the dose–effect curve. The cumulative amount of drug absorbed (or infused), A_t, is then obtained by deconvolving the biophasic drug levels, Q_B, with the transfer function, $G(s)$, which in the time domain is $g(t)$, in the following manner:

$$A_t = \int_0^t \int_0^t [g(t-\tau)^{-1} Q_B(\tau) d\tau]\, dt \qquad \text{(Eq. 11)}$$

where τ is a dummy variable used to perform the necessary integrations. Good agreement between known and experimentally determined A_t values verifies the mathematical model.

EXPERIMENTAL

Materials—Chlorpromazine hydrochloride[1] was supplied in powder form and used as received without further purification. This drug was freshly solubilized before each experiment using normal saline for injection USP[2]. All syringes used were disposable plastic[3], and the plastic cannulas with needles attached were 23-gauge butterfly infusion sets[2]. Male New Zealand white rabbits[4], 3–4 months old and 2.5–3.5 kg, were used as long as they remained physically healthy. They were not screened in any way prior to experimentation and were utilized as received.

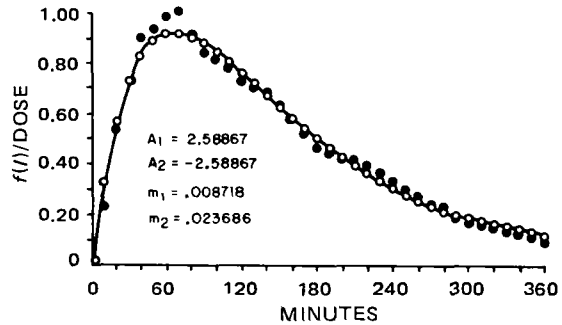

Figure 4—*The* f(I)*/dose values experimentally determined from pupillary diameter decreases* (●) *and computer-predicted values* (○) *based upon a biexponential model obtained by fitting the data via a least-squares procedure. The model has the general form:*

$$y = \sum_{i=1}^{n} A_i e^{-m_i t}$$

where the A_i*'s and* m_i*'s are constants.*

Table III—Intensity of Pupillary Diameter Decrease in a Single Rabbit (No. 17) Measured as $(P_0 - P)/P_0 \times 10^4$ in Response to a Slow Intravenous Infusion of Chlorpromazine, the Infusion Rate Being 0.50 mg/kg/hr

Minutes	I^a	$f(I)$
10	508	0.133
20	740	0.225
30	915	0.300
40	1070	0.383
50	1205	0.470
60	1325	0.555
70	1415	0.635
80	1480	0.705
90	1535	0.768
100	1580	0.825
110	1625	0.885
120	1650	0.923
130	1675	0.962
140	1690	0.993
150	1705	1.000
160	1710	1.013
170	1720	1.025
180	1725	1.037
190	1730	1.050
200	1735	1.067
210	1740	1.075
220	1745	1.081
230	1750	1.088
240	1755	1.096
250	1760	1.105
260	1770	1.121
270	1775	1.138
280	1780	1.150
290	1785	1.159
300	1790	1.168

[a] The intensity values were transduced to $f(I)$ values (relative biophasic drug levels) using the dose–effect curve as a calibration curve.

Methods—The animals were fasted for 24 hr prior to the experiment and were then restrained in an appropriate holder and weighed. A butterfly infusion cannula was inserted in the marginal ear vein, secured with a paper clip, and flushed with normal saline. The restrained animal was placed in a darkened room where the small amount of constant illumination necessary for the experiment was provided by two microscope lamps[5]. The animal was allowed 60 min to acclimate to the darkened room, during which time measurements of pupil diameter were taken for determining baseline values.

Pupil diameters were determined using a television pupillometer[6] equipped with a video camera and a low intensity, red illumination light. The signal from the video camera was relayed to a 63.5-cm (25-in.) professional size television monitor where the pupil size could be measured easily on the screen using compass dividers and a meter stick. Normal baseline pupil diameters measured from 29.0 to 32.0 cm using this method.

Once the animal's pupil diameter reached constant values, normal saline or a dose of chlorpromazine was administered *via* the cannula in the marginal ear vein in approximately 40–60 sec. The chlorpromazine dosage range employed was 0.5–4.0 mg/kg. Following dosing, pupillometry readings were taken for 5–6 hr depending on the animal's rate of recovery. The degree of pupil diameter decrease due to chlorpromazine doses was calculated in the following manner: the initial value at zero time minus the value at time t divided by the initial value.

[2] Abbott Laboratories, Chicago, Ill.
[3] Becton-Dickinson.
[4] Nicely Farms, Greenfield, Ind.
[5] Bausch & Lomb.
[6] Model 800, Whittaker Industries, Space Sciences Division, Waltham, Mass.

Table IV—Intensity of Pupillary Diameter Decrease in a Single Rabbit (No. 20) Measured as $(P_0 - P)/P_0 \times 10^4$ in Response to a Slow Intravenous Infusion of Chlorpromazine, the Infusion Rate Being 0.50 mg/kg/hr

Minutes	I^a	$f(I)$
10	241	0.053
20	655	0.188
30	975	0.333
40	1225	0.485
50	1375	0.603
60	1500	0.728
70	1598	0.850
80	1695	0.993
90	1725	1.035
100	1775	1.138
110	1820	1.223
120	1850	1.275
130	1875	1.338
140	1890	1.373
150	1900	1.388
160	1918	1.418
170	1925	1.448
180	1935	1.463
190	1950	1.495
200	1965	1.525
210	1975	1.550
220	1980	1.563
230	1995	1.605
240	2005	1.625
250	2015	1.650
260	2025	1.675
270	2035	1.705
280	2050	1.738
290	2065	1.765
300	2075	1.800

[a] The intensity values were transduced to $f(I)$ values (relative biophasic drug levels) using the dose–effect curve as a calibration curve.

Table V—Intensity of Pupillary Diameter Decrease in a Single Rabbit (No. 20) Measured as $(P_0 - P)/P_0 \times 10^4$ in Response to a Slow Intravenous Infusion of Chlorpromazine, the Infusion Rate Being 0.75 mg/kg/hr

Minutes	I^a	$f(I)$
10	625	0.175
20	1025	0.363
30	1315	0.550
40	1515	0.740
50	1640	0.900
60	1725	1.037
70	1765	1.120
80	1775	1.138
90	1780	1.150
100	1785	1.159
110	1790	1.168
120	1795	1.175
130	1800	1.183
140	1855	1.198
150	1810	1.205
160	1810	1.205
170	1815	1.213
180	1820	1.223
190	1825	1.238
200	1825	1.238
210	1825	1.238
220	1830	1.250
230	1830	1.250
240	1835	1.255
250	1840	1.263
260	1845	1.268
270	1850	1.275
280	1855	1.288
290	1860	1.300
300	1865	1.305

[a] The intensity values were transduced to $f(I)$ values (relative biophasic drug levels) using the dose–effect curve as a calibration curve.

RESULTS AND DISCUSSION

Time Course of Drug Response—The intensities of pupillary diameter decrease (miosis) in rabbits as a function of five bolus intravenous doses of chlorpromazine are shown in Fig. 1. The average miotic values for each dose of drug are also indicated in Table I. These averages were determined from data from two to four rabbits for each dose; the dosing schedule was randomized according to a Youden square design. A clearly discernible graded response profile is evidenced by Fig. 1. The times of absolute response intensity maximums were not identical here but the times all were within a narrow time band between 40 and 70 min, with no trend observable with increasing dose.

It can be seen from Fig. 1 that the times of maximum response intensity were removed from zero time. Strictly speaking, the times of maximum response must be identical for the system to be considered linear and modeled using linear mathematical techniques. However, one may still choose to model a system that is nonlinear with a linear mathematical model, depending, of course, on the degree of nonlinearity. Thus, one may obtain an accurate representation of the biokinetic behavior of the drug in that system over the range of doses and intensities of response studied. The intensity maximums did not occur at time zero, indicating that the biophase is a compartment kinetically distinct from the systemic circulation where the drug was administered.

Construction of Dose–Effect Curve—The intravenous dose–effect curve for chlorpromazine-induced pupillary diameter decrease in rabbits was constructed from the maximum response intensities shown in Fig. 1 and can be seen as Fig. 2. The shape of the curve appears as expected because chlorpromazine is known to have a very high therapeutic index and the curve plateaus over a wide dose range. The dose–effect curve represents a single-valued functional relationship between dose or relative biophasic drug levels and miotic response intensity (21).

Transduction of I Values to $f(I)$—Pupillary diameter response intensities were transformed to $f(I)$ values (relative biophasic drug levels) using the dose–effect curve as a calibration curve. The individual dose-normalized $f(I)$ values can be seen in Table II, which also lists the average $f(I)$/dose values and the standard deviations of the means. These average values were plotted as a function of time (Fig. 3), and these data represent the unit impulse response output of the system. The MULTIFIT and PLTEST curve-fitting procedures were then employed to fit these data with a mathematical function in the form of a sum of exponentials.

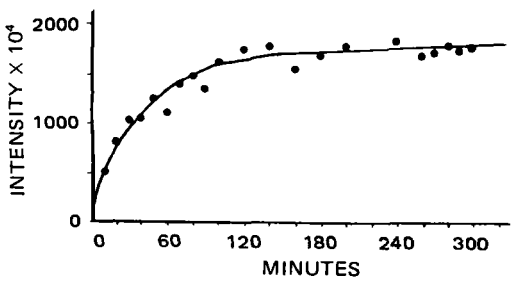

Figure 5—*Intensity of pupillary diameter decrease in a single rabbit (No. 17) in response to a slow intravenous infusion of chlorpromazine, the infusion rate being 0.50 mg/kg/hr.*

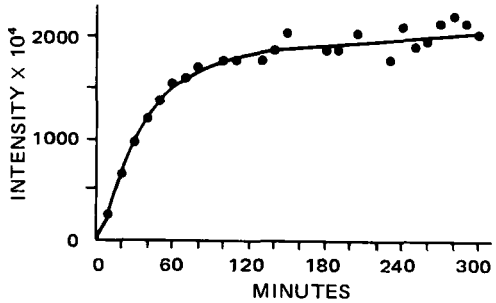

Figure 6—*Intensity of pupillary diameter decrease in a single rabbit (No. 20) in response to a slow intravenous infusion of chlorpromazine, the infusion rate being 0.50 mg/kg/hr.*

Table VI—Amount of Chlorpromazine Infused Determined Experimentally from Pupillary Diameter Decreases in Rabbit 17 *versus* Actual Amount of Chlorpromazine Theoretically Infused at 0.50 mg/kg/hr

$A(t)_{theor}$	$A(t)_{exp}$
0.0833	0.3650
0.1667	0.4030
0.2500	0.4500
0.3333	0.5400
0.4167	0.6450
0.5000	0.7300
0.5833	0.8200
0.6667	0.8900
0.7500	0.9700
0.8333	1.0400
0.9167	1.1380
1.0000	1.1800
1.0333	1.2560
1.1667	1.3125
1.2500	1.3300
1.3333	1.4000
1.4167	1.4628
1.5000	1.5290
1.5833	1.5970
1.6667	1.6725
1.7500	1.7204
1.8333	1.7790
1.9167	1.8433
2.0000	1.9100
2.0833	1.9800
2.1667	2.0650
2.2500	2.1400
2.3333	2.2000
2.4167	2.2600
2.5000	

Model Determination—Figure 4 represents the $f(I)$/dose values experimentally determined from chlorpromazine-induced pupillary diameter decreases plotted with values predicted from computerized MULTIFIT mathematical model parameters. The model so obtained appears to be a reasonable approximation of the true solution as evidenced by the closeness of the fit to the experimental data points.

As noted previously, the PLTEST procedure can be utilized to obtain frequency response information about the system that can lead to the determination of the mathematical model parameters for the system transfer function by an alternative route to be compared with those obtained by the MULTIFIT process. The parameters so obtained were compared with those obtained by the MULTIFIT procedure, and there was no significant difference between the two sets of parameters (22).

Model Verification—The intensities of pupillary diameter decrease in single rabbits in response to various slow intravenous infusions of chlorpromazine can be seen in Tables III–V. The infusion rates employed here *were* 0.50 and 0.75 mg/kg/hr. The purpose of the slow intravenous infusions was to introduce a type of input into the system other than that for which the transfer function had been determined; by using the output from these infusions and this transfer function model, the fidelity of the model was determined by calculating the known input function from pharmacological data alone and comparing experimentally determined values with theoretical values.

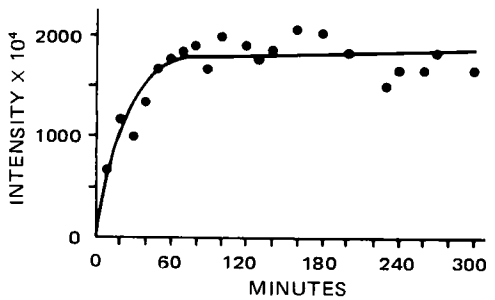

Figure 7—*Intensity of pupillary diameter decrease in a single rabbit (No. 20) in response to a slow intravenous infusion of chlorpromazine, the infusion rate being 0.75 mg/kg/hr.*

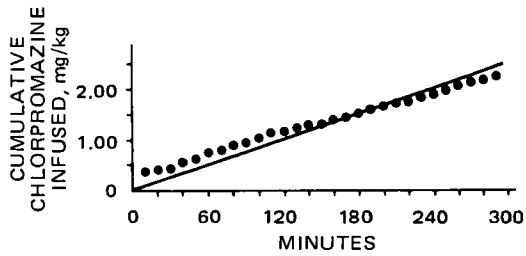

Figure 8—*Experimentally determined amounts of chlorpromazine slowly infused to a single rabbit (No. 17) as a function of time using intensities of pupillary diameter decrease and the transfer function model in a deconvolution procedure. The response intensity values were transduced to* f(I) *values (relative biophasic drug levels) using the dose–effect curve and deconvolved to yield cumulative amounts of chlorpromazine infused. The solid line represents the actual amount of chlorpromazine infused during the experiment, 0.50 mg/kg/hr.*

Figures 5–7 represent graphically the slow intravenous responses from Tables III–V; the curves were smoothed to facilitate their use in the deconvolution procedure.

The response intensity values from Tables III–V were transduced to $f(I)$ values (relative biophasic drug levels) using the dose–effect curve. These $f(I)$ values from the slow intravenous infusions then served as inputs to the deconvolution computer program that calculates cumulative drug input values once the transfer function and the response output from the system are known. Figure 8 indicates the computationally determined amounts of chlorpromazine slowly infused in a single rabbit at 0.50 mg/kg/hr, using intensities of pupillary diameter decrease and the transfer function model in a deconvolution procedure; cumulative amounts of chlorpromazine infused were plotted as a function of time.

Table VI lists the known amounts of chlorpromazine infused for comparison to the amount infused calculated wholly from pharmacological response intensities. The values are plotted in Fig. 9, where the slope of the best straight line forced through the origin is indicated along with the Pearson R correlation coefficient. Table VII summarizes the results from the deconvolution of response intensities from all slow intravenous infusions. It indicates good agreement when the experimentally known amounts of drug slowly infused are compared to the amounts calculated from pharmacological data.

The results indicate excellent agreement, as judged by the linear correlation coefficient and slope of plots of theoretically predicted *versus* experimentally observed values of drug bioavailability inputs of chlorpromazine. The validity of the mathematical model describing drug transfer in this system for the dosage range employed is confirmed.

SUMMARY AND CONCLUSIONS

The principal purpose of the present study was to investigate and confirm a method of performing a pharmacokinetic analysis of drug-responding biological systems using pharmacological response data. The results demonstrate that, through the suitable use of intravenous dose–effect curves to transform observed intensities of pharmacological response into values of relative biophasic drug levels, the postulation of hypothetical models for the drug–receptor interaction is obviated. The following conclusions were also evident:

1. As exemplified for the drug chlorpromazine, the method was confirmed and illustrated using results derived from the drug-induced

Table VII—Summary of the Deconvolution Results of Pupillary Diameter Response Intensities from Slow Intravenous Infusions of Chlorpromazine Where the Amount Theoretically Infused Was Compared with the Amount Calculated Using Pharmacological Data Alone

Rabbit	Infusion Rate, mg/kg/hr	$A(t)$ Slope	Pearson R
17	0.50	1.00405	0.99373
20	0.50	1.42894	0.99650
20	0.75	0.84010	0.98148

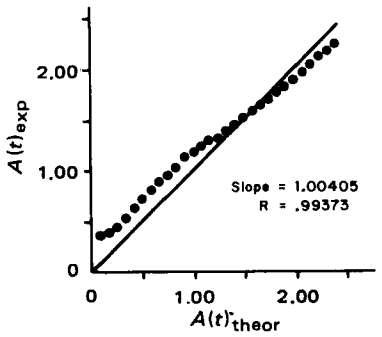

Figure 9—*Amount of chlorpromazine infused determined experimentally from pupillary diameter decreases of a single rabbit (No. 17)* versus *actual amount of chlorpromazine theoretically infused at 0.50 mg/kg/hr. The slope of the best line through the data points was determined using a linear regression technique.*

decrease in pupil diameter in rabbits. Agreement was observed between the quantities of drug known to be infused at any time and the amounts calculated wholly from pharmacological response data.

2. Through the proper selection of linear mathematical equation parameters, a system that is not strictly linear can be modeled in a linear fashion over an appreciable dose range. For chlorpromazine, this dose range was 0.5–4.0 mg/kg iv. The appropriate mathematical model to describe drug-induced changes in pupillary diameter in rabbits was a biexponential equation.

Subsequent reports will demonstrate results for other pharmacological responses to chlorpromazine (*e.g.*, intraocular pressure change and body temperature lowering) similar to those obtained for pupillometry. The use of pharmacological data for bioavailability testing of chlorpromazine in humans was reported elsewhere (22, 23).

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