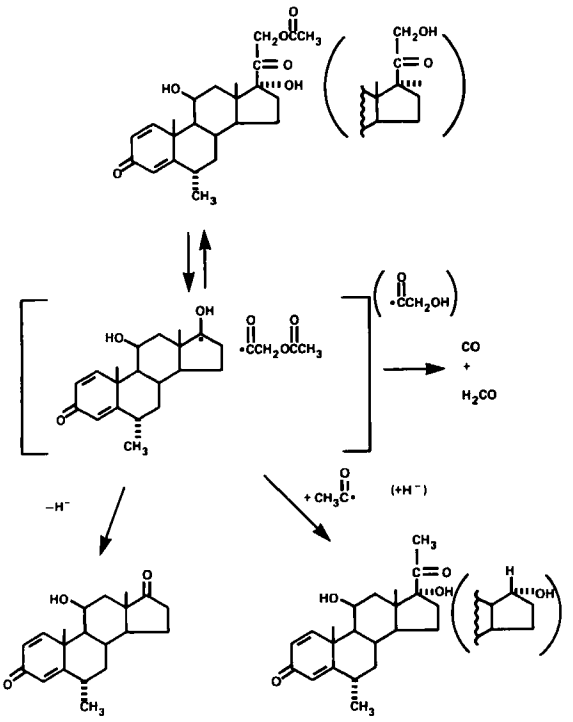

Scheme 13—Postulated mechanism for cobalt 60 radiolytic degradation of C-17 side chain of corticosteroids (example: methylprednisolone and methylprednisolone acetate).

the C-17 side chain of methylprednisolone and methylprednisolone acetate is shown in Scheme XIII. By this mechanism, reaction is initiated by homolytic cleavage between C-17 and C-20. Subsequent loss of a proton from the steroidal fragment would lead to the C-17 ketone. Recombination of the initially formed radicals could also occur. Alternatively, further disintegration of the side chain radical could lead to other radicals. Recombination with any of these radicals could also occur. For example, the C-21 acetate substituted side chain in methylprednisolone acetate could produce carbon monoxide, formaldehyde, and an acetyl radical. Recombination would generate the C-21 desoxy derivative. By a similar process, the side chain in methylprednisolone would produce a radical. Recombination in that case would form the C-21α alcohol. Preservation of the original stereochemistry at C-21 in these degradation products is attributed to the crystal lattice structure which would trap the radical intermediates in close proximity. In addition to formation of carbon monoxide and formaldehyde, other possible products derived from the C-17 side chain include methanol, methyl acetate, acetaldehyde, methane, and hydrogen. Formation of C-11 ketones could proceed by a similar mechanism initiated by loss of the C-11 proton with hydrogen produced as the other product.

Rate of Degradation—The rates of radiolytic degradation for corticosteroids were determined by analyzing irradiated and nonirradiated steroid powders using HPLC methods. The rates of degradation, expressed as percentage per megrad, are shown in Table III. The rates of degradation ranged from 0.2 to 1.4%/Mrad, thus, the majority of corticosteroids are stable to ^{60}Co-irradiation.

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Difficulties in Applying the Scatchard Model of Ligand Binding to Proteins—Proposal of New Mathematical Tools—Application to Salicylates

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Abstract □ Ill-considered use of the Scatchard model often leads to unjustified deductions. Since the main difficulty of this model is its number of parameters, new models are proposed that have only two parameters. After checking the models on simulated data, they were applied to real data on the binding of salicylates to albumin.

Keyphrases □ Scatchard model—difficulties in application to ligand binding to proteins, new mathematical tools, application to salicylates □ Salicylates—application of Scatchard model, difficulties in application to ligand binding to proteins, new mathematical tools □ Binding—ligand to proteins, difficulties in applying the Scatchard model, new mathematical tools, application to salicylates □ Proteins—ligand binding, difficulties in applying the Scatchard model, new mathematical tools, application to salicylates

The binding of ligands to proteins is most often analyzed in terms of the model proposed by Scatchard (1), in which proteins are considered to possess binding sites divided into independent classes according to their affinities. From estimations of the free and bound fractions of the ligand, it is in principle possible to determine the number of

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Table I—Simulations of the Equilibrium Bound Concentration Using the Scatchard Model with Two or Three Classes of Sites

Binding Parameters	Number of Classes (1) $m = 2$	(2) $m = 3$	(3) $m = 3$
K_1	0.0284	0.03954	0.04527
K_2	0.0021	0.00482	0.00959
K_3	—	0.00153	0.00181
n_1	1.46	1.00	0.76
n_2	4.10	2.00	1.30
n_3	—	2.56	3.50
Total Drug μg/ml	Total Drug μmoles/liter	Bound Drug μmoles/liter	Bound Drug μmoles/liter	Bound Drug μmoles/liter
50	362	346	346	346
100	725	680	679	679
200	1449	1290	1291	1292
300	2174	1807	1811	1809
400	2898	2211	2211	2210
500	3623	2494	2490	2489
600	4348	2679	2672	2672
800	5797	2881	2872	2873
1000	7246	2979	2971	2973
2000	14,493	3126	3122	3123
3000	21,739	3164	3161	3162
4000	28,985	3180	3178	3179
5000	36,232	3190	3188	3189
8000	57,971	3204	3203	3203
10,000	72,464	3208	3207	3207

classes, the number of sites in each class, and the affinity constant of each class. Before computers became available, graphical methods of solving the nonlinear equations introduced by this problem were proposed (2–4). Discrepancies in the results could be attributed to the imprecisions of graphical methods, and the emergence of computers should then have caused the results to become more consistent. The results of various graphical methods were compared (4) with the results obtained by computer methods, while taking care not to adopt the algorithm of these graphical methods for the computer. Despite the extremely powerful methods available in large computing centers (5–11) difficulties remained. It was shown (8) that the results for binding of dicumarol to albumin were doubtful when errors became as large as 2%, even when based on 50 experimental measurements, and that the results obtained from 200 experimental measurements with a precision of 5% had no significance. In addition, 50 publications were reviewed (12) that contained various errors of graphical construction. However, many authors continue to use this model and to calculate the classical parameters (universally called n_i, K_i, and m).

This model is habitually used since most scientists accept it as a good representation of the real phenomenon. A study of the discussions of the independence and the initial existence of all the sites (the basic hypothesis of Scatchard), and the introduction of an interaction between sites (13–20) may provide an understanding of how efficiently this model represents reality.

Problems are encountered in the study of this model. If available experimental data do not allow determination of all parameters of the model, it becomes simply a mathematical tool whose parameters have no significance. If the experimental data do not allow an accurate determination of the parameters, then a question arises as to whether the use of other mathematical functions would provide better results.

An attempt was made in this study to solve these problems using the binding of salicylate to albumin. Other models are proposed using simulated and real data.

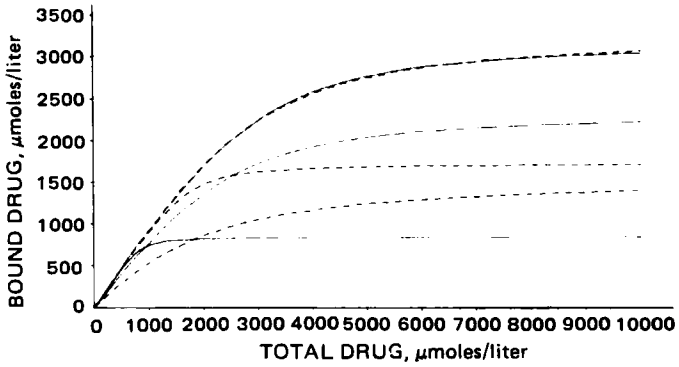

Figure 1—*Scatchard model with two classes of sites: curve of ligand binding to each class and to both classes taken as a whole, with two different combinations of parameters. Key: (—)* $K_1 = 0.00210$ *μmole/liter,* $K_2 = 0.02840$ *μmoles/liter,* $n_1 = 4.10$, $n_2 = 1.46$; *(- - - -)* $K_1 = 0.00109$ *μmoles/liter,* $K_2 = 0.01103$ *μmoles/liter,* $n_1 = 2.68$, $n_2 = 3.00$.

EXPERIMENTAL

To analyze and test different models, simulated and real data are used. Simulated data are the values of a published example on the binding of salicylate to albumin (21, 22). Real data are obtained *in vitro* by equilibrium dialysis on an apparatus[1], equipped with membranes[2] (with a molecular weight cutoff of 14,000), separating two 1.0-ml cells. The dialysis cells were placed in a water bath thermostated at 37°. Salicylate was estimated by spectrofluorimetry (excitation at 300 nm, emission at 410 nm).

Classical Scatchard Model—This model is deduced from the law of mass action and from the probability of binding to a carrier protein (1). It is based on a classification of independent sites of binding to the protein:

$$\frac{B}{P} = \sum_{i=1}^{m} \frac{n_i K_i F}{1 + K_i F} \qquad \text{(Eq. 1)}$$

where B is the concentration of the bound substance, F is the concentration of the unbound substance, P is the concentration of the protein, m is the number of classes of sites, and K_i and n_i are, respectively, the affinity constant and the number of sites of the ith class of sites.

Proposed Models—These are mathematical functions that must have the same shape as the amount of ligand bound at equilibrium expressed as a function of the total ligand concentration present. These functions have only two parameters:

$$\text{trigonometric model: } B = a_1 \arctan(a_2 T) \qquad \text{(Eq. 2)}$$

$$\text{exponential model: } B = b_1[1 - \exp(-b_2 T)] \qquad \text{(Eq. 3)}$$

where T is the total concentration of the ligand present, B is the concentration of the bound fraction at equilibrium, and a_1 and a_2, b_1 and b_2 are the two parameters of the models that are determined from the experimental data. These parameters are not totally meaningless, since in each case a_1 and b_1 are directly related to the saturating values B_∞ of the quantity of ligand bound to the protein:

$$B_\infty = a_1 \frac{\pi}{2} \qquad \text{(Eq. 4)}$$

$$B_\infty = b_1 \qquad \text{(Eq. 5)}$$

The two models proposed make it possible to study the equilibrium binding of the ligand either to binding proteins, where the protein concentration must be specified, or per unit concentration of binding protein:

$$\frac{B}{P} = \frac{a_1}{P} \arctan(a_2 T) \qquad \text{(Eq. 6)}$$

$$\frac{B}{P} = \frac{b_1}{P}[1 - \exp(-b_2 T)] \qquad \text{(Eq. 7)}$$

[1] Dianorm.
[2] Spectrapor 2.

Table II—Comparison of the Results of Simulations Using the Scatchard Model with Two Classes of Sites and Various Combinations of Parameters

		(1)	(2)	(3)	(4)	(5)	(6)	(7)
	K_1	0.00210	0.00210	0.00267	0.00170	0.00109	0.00055	0.00007
	n_1	4.10000	4.10080	4.46524	3.60528	2.68335	1.79370	1.36566
Binding	K_2	0.02840	0.02843	0.04407	0.01863	0.01103	0.00740	0.00494
Parameters	n_2	1.46000	1.45899	1.00000	2.00000	3.00000	4.00000	5.00000

Total Drug		Bound Drug	Bound Drug	Bound Drug	Bound Drug	Bound Drug	Bound Drug	Bound Drug
μg/ml	μmoles/liter	μmoles/liter	μmoles/liter	μmoles/liter	μmoles/liter	μmoles/liter	μmoles/liter	μmoles/liter
50	362	346	346	346	345	343	340	336
100	725	680	680	680	679	677	673	665
200	1449	1290	1290	1288	1295	1300	1299	1289
300	2174	1807	1807	1814	1810	1820	1831	1835
400	2898	2211	2211	2226	2207	2208	2220	2248
500	3623	2494	2494	2508	2486	2478	2479	2514
600	4348	2679	2679	2686	2672	2661	2653	2675
800	5797	2881	2881	2872	2879	2873	2862	2856
1000	7246	2979	2979	2959	2983	2985	2980	2962
1250	9060	3045	3045	3017	3054	3064	3068	3051
W		—	0.109	1788	266	1337	2615	3845
Max. limit of bound drug								
Class 1 + Class 2			3225	3170	3251	3296	3360	3692
Class 1			2379	2590	2091	1556	1040	792
Class 2			846	580	1160	1740	2320	290

In this case the parameters a_1 and b_1 are divided by the protein concentration P, whereas other parameters, a_2 and b_2, are independent of the protein concentration.

The Scatchard model, when limited to a single class of sites, can be used in certain cases and more precisely when the zone being studied is more limited (in the case of therapeutic or toxic doses of salicylates, up to or exceeding 400 μg/ml.)

The model (Eq. 1) is then written:

$$B = \frac{nPKF}{1 + KF} \quad \text{or} \quad B = \frac{nPK(T - F)}{1 + K(T - F)} \qquad \text{(Eq. 8)}$$

This model has only two parameters and occasionally gives good results, but it is disadvantageous because it is also a mere mathematical tool requiring solution of a nonlinear equation.

RESULTS AND DISCUSSION

General Applications of the Model—The usual procedure is to use a computer (or graph paper) to determine the parameters n_i, K_i, and sometimes m starting from the experimental data that has been obtained.

There are, however, problems encountered with this procedure. The study of the binding phenomenon itself, while assuming that the model is a true reflection of reality, is one problem. It must be possible to determine the parameters m, n_i, and K_i, and each must have a unique value. Other problems encountered are the study of the influence of a pathological condition on the quantity of ligand bound (which leads to a comparison of groups) and the study of a particular region of the curve of binding to a protein, such as therapeutic or toxic doses, or the saturation value of binding.

Difficulties Encountered—Difficulties are not related to the theoretical model itself but are related to the number of unknown parameters whose estimation is often incompatible with the quantity and quality of the experimental data (8). The determination of these parameters is considered from simulated data, thus eliminating experimental errors, taking as an example published data on the binding of salicylate (^{14}C-labeled COOH-salicylic acid) to albumin (21, 22).

It is essential to know the number (m) of classes of sites if one wishes the model to reflect the real phenomenon. The number m, determined only from estimations of the concentrations at equilibrium of free and bound ligand as a function of the total ligand concentration, is in reality only the minimum possible number of classes compatible with the experimental data.

As an example, Table I represents the concentrations of salicylate bound at equilibrium throughout the whole concentration range (0–10 mg/ml). The results were obtained by simulation for three cases. The first case (Table I, column 1) assumed two classes of sites (m = 2); using the values of the parameters for the binding of salicylate generally accepted in published sources. The second and third cases assumed three classes

Table III—Simulations Using the Two Proposed Models over the Concentration Range 0 to 1250 μg/ml (0 to 9060 μmole/liter [a])

		Scatchard Model— Two Classes Reference	Trigonometric Model a_1 = 2370.00 a_2 = 0.000449	Exponential Model b_1 = 3210.57 b_2 = 0.000386
Total Drug		Bound Drug	Bound Drug	Bound Drug
μg/ml	μmoles/liter	μmoles/liter	μmoles/liter	μmoles/liter
50	362	346	382	419
100	724	680	746	783
200	1449	1290	1369	1375
300	2174	1807	1835	1823
400	2898	2211	2172	2162
500	3623	2494	2418	2418
600	4348	2679	2602	2611
700	5072	2800	2744	2757
800	5797	2881	2855	2868
900	6522	2938	2944	2951
1000	7246	2979	3017	3015
1250	9060	3045	3152	3113
W		—	42743	44562

[a] Comparison with the reference values derived from the Scatchard model with two classes of sites. W Represents the residual sum of squares.

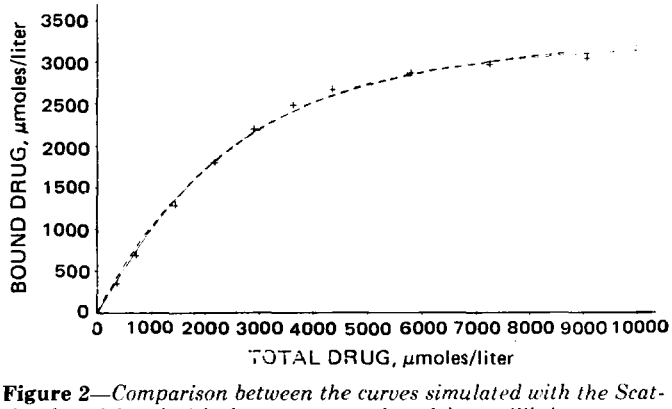

Figure 2—*Comparison between the curves simulated with the Scatchard model and with the two proposed models: equilibrium concentration <1250 μg/ml (9060 μmoles/liter). Key: (—) trigonometric model; (- - - -) exponential model; (+) Scatchard model with two classes of sites.*

Table IV—Therapeutic Concentrations [a]

		Scatchard Model Two Classes Reference	Trigonometric Model $a_1 = 2783.6$ $a_2 = 0.000347$	Exponential Model $b_1 = 3858.4$ $b_2 = 0.000285$	Scatchard Model One Class $K = 0.006972$ $n = 4.3964$
Total Drug		Bound Drug	Bound Drug	Bound Drug	Bound Drug
μg/ml	μmoles/liter	μmoles/liter	μmoles/liter	μmoles/liter	μmoles/liter
25	181	174	175	194	171
50	362	346	348	379	340
75	543	514	519	554	508
100	724	679	686	721	673
125	906	840	848	879	836
150	1087	995	1005	1029	995
175	1268	1145	1155	1172	1150
200	1449	1290	1298	1307	1300
225	1630	1429	1435	1436	1443
250	1811	1561	1563	1558	1579
275	1993	1688	1686	1674	1704
300	2174	1807	1801	1784	1818
325	2355	1919	1909	1888	1918
350	2536	2024	2011	1988	2007
W		—	806	11,544	1445

[a] Simulations using the trigonometric model, the exponential model, and the Scatchard model with one class of binding sites. Comparison with the reference values derived from the Scatchard model with two classes of sites. W represents the residual sum of squares.

of sites ($m = 3$) (Table I, columns 2 and 3), with two combinations of the six parameters n_i and K_i. However, in practice there is an infinite number of these combinations, since they were obtained by simple identification of the parameters K_i. The values of n_i were chosen arbitrarily as:

$$(n_1 + n_2 + n_3)_{m=3} = (n_1 + n_2)_{m=2} \qquad \text{(Eq. 9)}$$

so that the bound fractions reached the same limit at saturation (Eq. 1).

The exact number of classes of sites may be confused with the minimum number compatible with the available experimental results. This does not matter if the model is used simply as a mathematical tool (for example for smoothing), but it becomes a problem if the phenomenon itself is to be used and a real significance is to be given to the parameters.

Suppose that the number of classes of sites is known (or is accepted to be equal to the minimum number defined above). Difficulties have already been reported with the determination of other parameters such as n_i and K_i from the results of equilibrium estimations (4) as shown in Table II. Again the data used are the 12 values of the bound fraction simulated from published parameters (21, 22) for the binding of salicylate to albumin (Table II, column 1). The parameters (n_i and K_i are calculated in several cases: For the four parameters, n_1, n_2, K_1, and K_2, even starting the iterations from remote initial values, the results are not much different from the correct values (Table II, column 2); for the three parameters, n_1, K_1, and K_2, to the fourth parameter (n_2) we attribute the values 1, 2, 3, 4, and 5, successively (Table II, columns 3–7). The results yield combinations for the values of the parameters that are naturally very different; the quantity of ligand bound, however, remains similar from one of these groups of values to another. Figure 1 represents this phenomenon. The curves represented correspond to the two combinations:

$$K_1 = 0.00109\ K_2 = 0.01103\ n_1 = 2.6833\ n_2 = 3.0000$$
$$\text{and } K_1 = 0.0021\ K_2 = 0.0284\ n_1 = 4.1000\ n_2 = 1.4600 \qquad \text{(Eq. 10)}$$

where K_1 and K_2 are given in micromoles per liter.

In both cases the curves drawn represent the quantity bound to each site and the overall quantity for the whole of the two sites, respectively. These two curves, representing the total quantity bound to albumin, are practically identical (Fig. 1), which shows that it cannot be determined how much ligand is bound to each site; that is, the four parameters n_1, n_2, K_1, and K_2 cannot be determined.

Conclusions About Current Application of the Scatchard Model—The difficulties encountered at the various stages of the application of the model to simulated cases have been shown, in the total absence of experimental errors, for the determination of the various parameters of binding to proteins. The problem of the number of classes of sites alone destroys any significance of the parameters.

Therefore, this model should not at present be used except as a mathematical tool. It requires that a nonlinear equation be solved at each point; this drawback, which was the reason for the various graphical methods, makes it difficult to use. The large number of these parameters that cause this practical indetermination that has been reviewed previously prohibits any direct interpretation of the results (such as comparison of the parameters from two groups of patients). One solution is to arbitrarily fix the values of certain parameters (for example n_1 and n_2), as suggested previously (8). Other mathematical models are proposed here as mathematical tools but they are easier to manipulate, and have

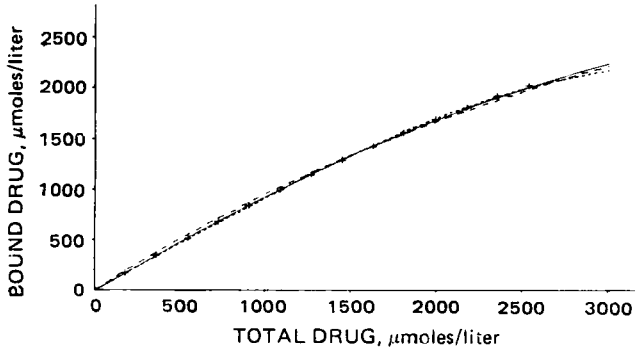

Figure 3—*Comparison of the models over the therapeutic range of concentration (<350 μg/liter, or 2536 μmoles/liter). Key: (—) trigonometric model; (- - - -) exponential model; (- · - · -) Scatchard model with one class of sites; (+) Scatchard model with two classes of sites.*

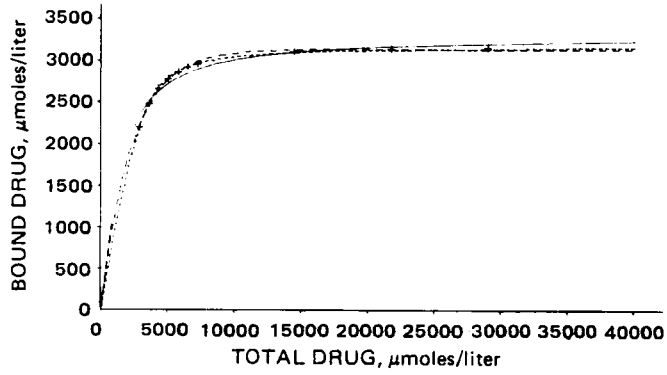

Figure 4—*Comparison of models over the high range of concentrations (>400 μg/ml, or 2900 μmoles/liter). Key: (—) trigonometric model; (- - -) exponential model; (- · - · -) Scatchard model with one class of sites; (+) Scatchard model with two classes of sites.*

Table V—Toxic and High Concentrations [a]

Total Drug		Scatchard Model Two Classes Reference	Scatchard Model One Class $K = 0.003104$ $n = 5.536$	Exponential Model $b_1 = 3161$ $b_2 = 0.000421$	Trigonometric Model $a_1 = 2125$ $a_2 = 0.000682$
μg/ml	μmoles/liter	Bound Drug μmoles/liter	Bound Drug μmoles/liter	Bound Drug μmoles/liter	Bound Drug μmoles/liter
400	2899	2210	2190	2229	2343
500	3623	2494	2497	2474	2521
600	4348	2679	2689	2655	2647
700	5072	2800	2811	2788	2740
800	5797	2881	2891	2886	2811
900	6522	2938	2946	2959	2868
1000	7246	2979	2985	3012	2914
2000	14493	3126	3122	3154	3124
3000	21739	3164	3156	3161	3195
4000	28985	3180	3171	3161	3230
5000	36232	3190	3180	3161	3251
W		—	1091	4974	44,053

[a] Simulation using the trigonometric model, the exponential model, and the Scatchard model with one class of sites; comparison with the reference values of the Scatchard model with two classes of sites. W represents the residual sum of squares.

been used during a study of the variation of the binding of salicylate in rheumatoid arthritis.

Application of the Proposed Models—In the current state of interpretation, no physiological significance should be sought in the calculated parameters. The models proposed are merely mathematical tools. They are the trigonometric model in Eq. 2 and the exponential model in Eq. 3. These equations make it possible to represent the variation in the quantity of ligand bound at equilibrium as a function of a total quantity present, while using the minimum number of parameters. The choice is not exhaustive, but the models have been tested in various circumstances: a broad-range study of the phenomenon, a detailed study of therapeutic doses over a narrowed region of study, and a study of high doses with an attempt to determine the saturating value of the quantity of ligand bound. These models can also be used to compare groups of patients.

Checking Models with Simulated Data—Simulated input data values of the equilibrium fraction of salicylate bound to albumin (Table III), derived from the Scatchard model (Eq. 1) were used for two classes of sites whose parameters have the published values (21, 22):

$$m = 2 \quad n_1 = 1.46 \quad K_1 = 0.0284\ \mu\text{moles/liter}$$
$$n_2 = 4.10 \quad K_2 = 0.0021\ \mu\text{moles/liter} \qquad \text{(Eq. 11)}$$

These data were approached as closely as possible by adjusting the parameters a_1, a_2, b_1, b_2, and perhaps n and K of the various models proposed (Eqs. 2, 3, and 8).

The proposed models were tested at equilibrium in three zones of total concentration of ligand; <1250 μg/ml (9060 μmoles/liter); <350 μg/ml (therapeutic doses); and >400 μg/ml (high concentrations).

Doses <1250 μg/ml (i.e., 9060 μmoles/liter)—This is the concentration generally studied because it covers therapeutic doses, and also approaches the limit of saturation of binding. It is more difficult to approximate a function while minimizing the number of parameters to be adjusted over a wide range of a variable (0–1250 μg/ml) than over a narrow range. However, the differences between the theoretical curve (4-Parameter Scatchard model) and the curves from the proposed models (with two parameters) remain small and less than the experimental errors encountered (Fig. 2, and Table III). The values obtained for the parameters of the two models were:

trigonometric model (Eq. 2): $a_1 = 2370.00$ μmoles/liter and $a_2 = 0.00044979$ liter/μmoles

exponential model (Eq. 3): $b_1 = 3210.57$ μmoles/liter and $b_2 = 0.00038606$ liter/μmoles (Eq. 12)

The last line of Table III represents the sum of squares of the differences between the results from the Scatchard model and from the proposed models:

$$W = \sum_{i=1}^{p} (B1_i - B2_i)^2 \qquad \text{(Eq. 13)}$$

where $B1_i$ and $B2_i$ are the fractions of ligand bound at point i at equilibrium from, respectively, the Scatchard model with two classes of sites (Eqs. 1 and 11, the input data) and one of the proposed models, p is the number of data points.

Comparison of the values obtained for W with each of the two proposed models shows that the trigonometric model gives the better results. The exponential model is a poor fit at low salicylate concentrations (of the order of 50–100 μg/ml)—at the first two points it gives an unacceptable

Table VI–Application of the Models to Real Data at Equilibrium [a]

Experimental Data			Trigonometric Model $a_1 = 2865.7$ $a_2 = 0.00284$	Exponential Model $b_1 = 3909.8$ $b_2 = 0.0002397$
Total Drug		Bound Drug	Bound Drug	Bound Drug
μg/ml	μmoles/liter	μmoles/liter	μmoles/liter	μmoles/liter
46.68	291.7	271	237	264
91.60	572.5	520	462	501
176.20	1101.2	952	869	907
380.08	2375.5	1626	1701	1697
518.01	3237.5	2100	2131	2110
650.94	4068.4	2511	2458	2435
718.99	4493.6	2737	2598	2578
938.08	5863.0	2632	2952	2951
1160.92	7255.7	3261	3206	3223
1273.95	7962.2	3424	3309	3330
W			158,000	150,000

[a] Total concentrations <1250 μg/ml (8000 μmoles/liter). Comparison of the trigonometric and exponential models with the reference Scatchard model with two classes of sites.

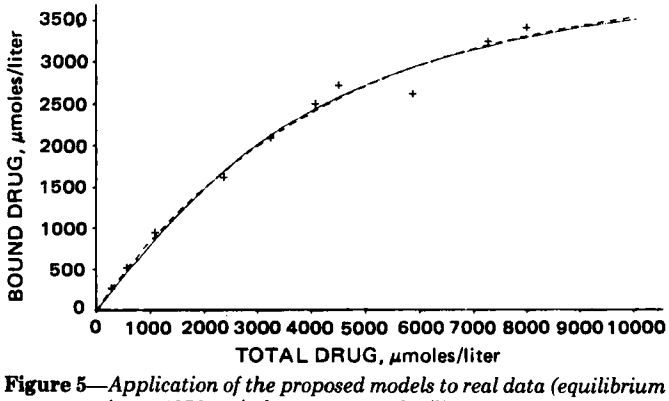

Figure 5—*Application of the proposed models to real data (equilibrium concentration <1250 μg/ml, or 8000 μmoles/liter). Key: (—) trigonometric model; (– – –) exponential model; (+) experimental values.*

Table VII—Application of the Models to Real Data at Therapeutic Concentrations [a]

Experimental Data			Trigonometric Model $a_1 = 1720.68$ $a_2 = 0.000565$	Exponential Model $b_1 = 3142.97$ $b_2 = 0.000317$	Scatchard Model One Class $K = 0.009100$ $n = 3.4784$
Total Drug		Bound Drug	Bound Drug	Bound Drug	Bound Drug
μg/ml	μmoles/liter	μmoles/liter	μmoles/liter	μmoles/liter	μmoles/liter
47.18	294.9	277.2	284	280	274
65.74	410.9	384.2	393	384	380
91.02	568.9	512.7	535	518	522
135.74	848.4	759.2	769	741	763
178.39	1114.9	979.9	968	936	976
219.01	1368.8	1175.1	1133	1106	1154
253.33	1583.3	1291.6	1256	1246	1281
282.59	1766.2	1344.9	1350	1347	1428
307.41	1921.3	1342.6	1422	1433	1543
380.37	2377.3	1629.6	1602	1664	1563
W			11,050	19,121	52,200

[a] Comparison of the trigonometric model, the exponential model, the Scatchard model with one class of sites, and the reference Scatchard model with two classes of sites.

value for the bound fraction, greater than the total concentration. The Scatchard model with a single class of sites is inapplicable over this wide range of variation of total salicylate concentration.

Therapeutic Zone of Concentrations <350 μg/ml (2500 μmoles/liter)—Over this limited zone the approximation is easier. Table IV and the curves in Fig. 3 represent the results obtained from the 14 data points from the first two columns of the table. The values of the parameters obtained with the two proposed models are:

trigonometric model (Eq. 2):

$$a_1 = 2783.6\ \mu\text{moles/liter} \quad a_2 = 0.000347\ \text{liter}/\mu\text{mole}$$

exponential model (Eq. 3):

$$b_1 = 3858.4\ \mu\text{moles/liter} \quad b_2 = 0.000285\ \text{liter}/\mu\text{mole} \quad \text{(Eq. 14)}$$

Here again the trigonometric model gives better results than does the exponential model, which has three unacceptable points where more than the total salicylate concentration is bound. A weighting of the first points in the computation remedied this disadvantage, but the differences then became greater at the points for higher concentrations.

The Scatchard model with one class of sites (Eq. 8) is applicable over this region (Fig. 3 and Table IV) and gave good results with the values $K = 0.006972$ μmoles/liter and $n = 4.3964$.

High Concentrations >400 μg/ml, 2900 μmoles/liter—The 11 points lying between 400 and 5000 μg/ml in Table V were used. The exponential model gave better results than the trigonometric model (Table V and Fig. 4):

trigonometric model (Eq. 2):

$$a_1 = 2124.92\ \mu\text{moles/liter} \quad a_2 = 0.000682\ \text{liter}/\mu\text{mole}$$

exponential model (Eq. 3):

$$b_1 = 3161.30\ \mu\text{moles/liter} \quad b_2 = 0.000421\ \text{liter}/\mu\text{mole} \quad \text{(Eq. 15)}$$

However, for high concentrations the best results were obtained with the Scatchard model limited to a single class of sites (Eq. 8). The corresponding curve was then similar to the curve from the model with two classes of sites used as data (Fig. 4 and Table V). The parameters obtained had the values $n = 5.536$ and $K = 0.003104$ μmole/liter.

Application of the Proposed Models to Real Data—The models were applied to experimental results on the binding of sodium salicylate to albumin and studied over the various zones mentioned.

Total Salicylate Concentration Lying Between 0 and 1200 μg/ml—Table VI and Fig. 5 show the bound fraction at equilibrium obtained with the trigonometric model having parameters a_1 (2865 μmoles/liter) and a_2 (0.000284 liter/μmole) and with the exponential model having parameter b_1 (3910 μmoles/liter) and b_2 (0.000240 liter/μmole).

This first zone is too broad for application of the Scatchard model limited to a single class of sites (Eq. 8).

Note that the two curves are almost identical (Fig. 5). This figure shows also that the saturation limit was still not reached and any extrapolation to estimate the value of this limit would be dangerous, whatever model is used.

Therapeutic Concentrations <350 μg/ml—Table VII and Fig. 6 show the results obtained with the trigonometric model with a_1 (1720.68 μmoles/liter) and a_2 (0.0005654 liter/μmole), and with the exponential model with b_1 (3142.97 μmoles/liter) and b_2 (0.000317 liter/μmole). Over this relatively narrow zone the single-class Scatchard model (Eq. 8) can be applied with n equal to 3.4784 and K equal to 0.00910 μmole/liter. Of the three models, the trigonometric model gave the best results (Table VII).

High Concentrations (from 400–500 μg/ml)—This zone can be of interest for example in studies of toxic doses or to estimate the saturation limit of bound fraction of substance (the plateau of the curve). The coefficients, a_1 and b_1, here have an important role since they are directly related to the value of this limit (Eqs. 4 and 5). But the experimental

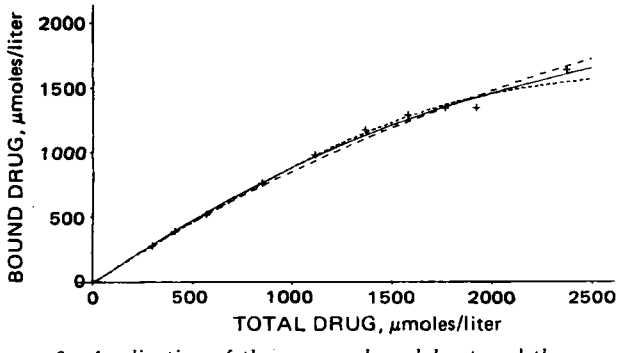

Figure 6—*Application of the proposed models at real therapeutic concentrations (<400 μg/ml, or 2500 μmoles/liter). Key: (—) trigonometric model; (- - - -) exponential model; (- - - - -) Scatchard model with one class of sites; (+) experimental values.*

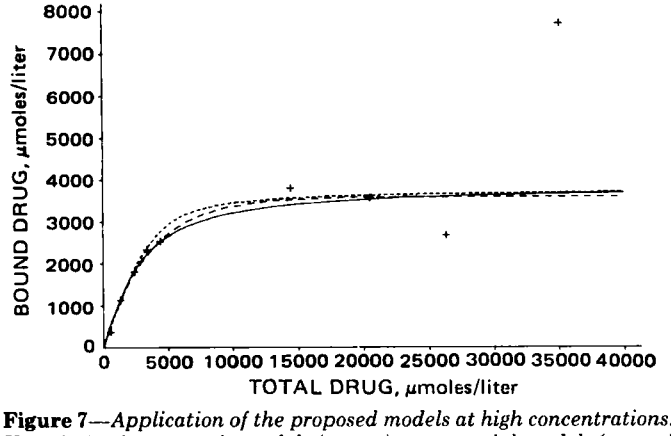

Figure 7—*Application of the proposed models at high concentrations. Key: (—) trigonometric model; (- - - -) exponential model; (- - - - -) Scatchard model with one class of sites; (+) experimental values.*

Table VIII—Application of the Models to Real Data at High Concentrations; Comparison of the Two Models

Experimental Data			Trigonometric Model $a_1 = 2466.2$ $a_2 = 0.000377$	Exponential Model $b_1 = 3618.2$ $b_2 = 0.000279$	Scatchard Model One Model $K = 0.00171$ $n = 6.00$
Total Drug		Bound Drug	Bound Drug	Bound Drug	Bound Drug
μg/ml	μmoles/liter	μmoles/liter	μmoles/liter	μmoles/liter	μmoles/liter
93.98	587	549	537	547	498
216.67	1354	1146	1163	1138	1111
396.30	2477	1829	1851	1805	1892
537.04	3356	2338	2223	2199	2373
703.70	4398	2546	2534	2557	2780
2305.83	14,411	3823	3425	3553	3591
3286.90	20,543	3586	3557	3606	3658
4214.90	26,343	2687	3626	3615	3689
5619.05	35,119	7744	3688	3618	3715
W			17.10^6	18.10^6	17.10^6

difficulties are greater at high concentrations; there are problems of dilution and precision of the measurements. The curves in Fig. 7 represent our first results in this zone (Table VIII). They were obtained with the following parameter values:

trigonometric model:

$$a_1 = 2466.2\ \mu\text{moles/liter} \quad a_2 = 0.000377\ \text{liter}/\mu\text{mole}$$

exponential model:

$$b_1 = 3618.2\ \mu\text{moles/liter} \quad b_2 = 0.000279\ \text{liter}/\mu\text{mole}$$

single-class Scatchard model: $K = 0.00171\ \mu\text{moles/liter} \quad n = 6.0$ (Eq. 16)

The basis of the Scatchard model is not questioned, but rather its quasi-automatic application in studies of the binding of a substrate to a protein. Even if this model is an accurate reflection of a real biochemical phenomenon, there may be insufficient experimental data to determine the values of the parameters unambiguously. The indistinguishability of the true number of binding classes and the minimum number of classes compatible with experimental data is sufficient to show that this model can be used only as a mathematical tool, for example, to interpolate data, or to calculate derivatives of curves or areas under them. This tool is difficult to manipulate, since it requires that a nonlinear equation be solved at each point. In addition, the practical indeterminacy of these parameters prevents them from being used separately as elements of reference for comparing groups of patients. Therefore, other models with only two parameters that have been used to study the influence of a pathological condition on the binding of a substance to a protein have been proposed.

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