sphere almost as much as small changes in solar luminosity and large changes in atmospheric composition. The large changes of composition suggested in (*4*, *14*) and used in (*9*) to prevent an ice-covered early earth may be unnecessary. More modest increases of CO_2 in the early atmosphere than discussed in (*9*) might be sufficient to produce surface temperatures higher than today.

However, we do not know which parameterization of cloud feedback is correct. Our results, when compared with those of other models with and without cloud feedbacks, indicate the very large uncertainties that remain in reconstructing the history of the early earth. They also illustrate the importance of incorporating correct cloud feedbacks in climate models. Until more study of hydrospheric processes, including clouds, can improve the parameterizations used in simple climate models, reconstructions of past climates remain so uncertain that conclusions about the early atmosphere based on such models must remain tentative.

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10. From table 1 in Owen *et al.* (*9*), a 3 percent decrease in solar constant with constant CO_2 abundance produces a 3 K drop in surface temperature. An 8 percent decrease in solar constant is not quite offset by a 90-fold increase in CO_2. At lower temperatures, where water vapor feedback is weaker (*15*), even more CO_2 would be needed to raise the temperature by the same amount. We estimate in this way that, in the model of Owen *et al.* without any cloud feedback, more than 100 times the present amount of atmospheric CO_2 is needed to maintain a surface temperature of 273 K.
11. Warren and Schneider [see (*2*)] illustrate a similar conclusion with regard to parameterized ice albedo feedback and feedbacks on infrared cooling rates at a given surface temperature. Their results illustrate that simple models of the early earth, which are not ice-covered, may be within the range of uncertainty in parameterizing these processes. We show here that the same may be said for cloud feedbacks.
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Numbers of Receptor Sites from Scatchard Graphs: Facts and Fantasies

Abstract. *Data for ligand and receptor binding presented in the format of a Scatchard graph are compared with the same data shown as bound ligand plotted against the logarithm of free ligand. From this comparison it is apparent that extrapolations in the Scatchard graph to yield total number of receptor sites are generally not correct.*

For the validity of their conceptual and experimental methods, most scientists depend on assurances from reputable predecessors in their field. The latter individuals in turn have usually adopted the procedures from some comparable persons who preceded them. If the forerunners in the use of a technique have not recognized its limitations or have obscured them, a tradition of analysis may develop that generates pervasive misinformation in the scientific literature.

Ligand binding by biological receptors is widely summarized and analyzed in a Scatchard graph in which the ordinate shows the moles of effector bound per total moles of receptor divided by the concentration of free ligand (B/F in the endocrine literature) and the abscissa shows the moles of bound effector (B). For the simplest possible situation, one in which it has been established unequivocally by means other than binding measurements that there is only one receptor site, a Scatchard graph is a reliable device for measuring the binding constant.

In biological systems there are usually many receptor sites on a single binding entity, be it a pure protein, cellular constituent or organelle, membrane, or cell. The simplest possible molecular situation is one in which every binding site is identical in nature and has the same affinity for the ligand. In such a case, the Scatchard graph in principle will be linear and the intercept on the abscissa will be the total number of receptor sites, n. In this case also, a graph showing the moles bound, B, plotted against the concentration of free ligand, F, on a logarithmic scale (Fig. 1a) has some characteristic features: (i) an inflection point (+) appears at half-maximum binding, that is, at $n_{1/2}$; (ii) the S-shaped curve is symmetric about the inflection point; and (iii) a plateau at n is reached asymptotically as the concentration of free ligand approaches very large (infinite) values.

In practice, when binding data are plotted on a Scatchard graph there is an enormous temptation to fit them to a straight line, either by eye or by least-squares methods, so that the number of receptor sites and the binding constant can be extracted. In most cases it can be shown readily, by plotting the same data on a semilogarithmic graph (Fig. 1a), that the conclusions derived from the Scatchard graph are completely untenable.

Let me illustrate with an example from the neurobiological literature. The binding of an effector drug by benzodiazepine receptors from preparations of cerebral cortex, under a specified set of conditions, has been presented in a published Scatchard graph (*1*), a portion of which is reproduced in Fig. 1b. Individual points have been fitted by a straight line with a very good correlation coefficient, and the total number of receptor sites (830) computed from the intercept on the ab-

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scissa. The last experimental point (Fig. 1b), at $B \sim 700$ is situated, presumably, about 15 percent before the saturation value. Let us examine the same data as points on a graph of B versus the logarithm of free drug (Fig. 1c). Since the Scatchard graph (Fig. 1b) has been purported to be a straight line, the semilogarithmic plot (Fig. 1c) should be an ideal S-shaped curve. Clearly, actual experimental data are available for less than half of the "S"; the curve has not reached an inflection point, and obviously there is not even a hint of the slope decreasing to approach a plateau value of n. The experimental point at $B \sim 700$ cannot possibly be within 15 percent of the saturation value. If we arbitrarily assume that the highest observed value of B is at the inflection point, then n must be at least double this value, or 1400, and is likely to be even higher. Clearly the value of 830 deduced from the Scatchard plot is nowhere near the actual number of receptor sites.

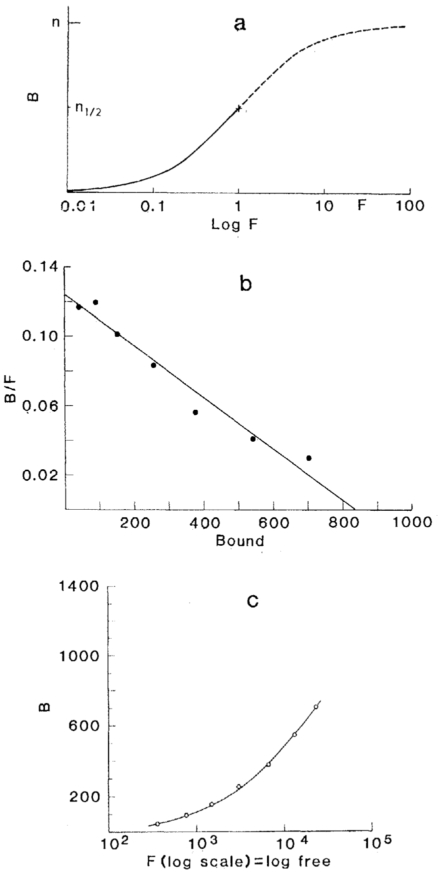

Fig. 1. (a) A semilogarithmic graph in which moles of bound ligand, B, are plotted against the concentration of free ligand, F, on a logarithmic scale. For a receptor with n identical binding sites, the amount bound at the inflection point (+) will be one-half that at saturation. The curve is shown as a broken line in the region where data are usually lacking in most published binding studies. (b) Scatchard graph of data for binding of diazepam to benzodiazepine receptors in membranes of rat cerebral cortex. [Data from (*1*)] (c) The same data as used for (b) now plotted on a semilogarithmic binding graph.

The situation becomes even more treacherous when data plotted on a Scatchard graph are so obviously nonlinear that the authors are forced to fit them to a curve instead of a straight line. In most cases in which a definite intercept on the abscissa is still claimed, one can show readily that the result is a striking example of bending perception to wish.

To illustrate this point, let us examine first the binding by a pure, crystalline protein, where there are no uncertainties as to the receptor preparation. Extensive binding data (*2*) for uptake of a substrate by the pure enzyme aspartate transcarbamylase are assembled in a Scatchard graph (Fig. 2a) *with coordinates blanked out* and *with no curve drawn through the points*. Obviously, with increasing value of B on the abscissa, the data approach the axis horizontally with zero slope; there is no way of telling where the intercept on the x-axis occurs. If, however, one knows (from hydrodynamic and x-ray studies) that aspartate transcarbamylase is constituted of six subunits, and if the axes are labeled with numbers, it is not difficult to persuade oneself, and readers, that the intercept on the abscissa is 6, and to state that binding data show six subunits. In contrast, if the same data are plotted on a semilogarithmic graph (Fig. 2b), it is apparent that the slope of the binding curve has not turned around at an inflection point. Despite the fact that the highest observed value of B in Fig. 2a is about 90 percent of the distance to the intercept drawn by the authors, Fig. 2b shows no hint of a plateau. The total number of receptor sites for this substrate cannot be established from these data.

Published results for the binding of insulin show similar problems. Figure 2c illustrates typical data for the binding of insulin by fibroblasts (*3*), presented on a semilogarithmic graph, in the presence and absence of an effector drug. The original paper (*3*) reported a value for the intercept on the x-axis of a Scatchard graph, and reached specific conclusions as to the effect of the drug on the number of insulin receptor sites. It is obvious from Fig. 2c, however, that there is no way of telling what the number of receptor sites is in either set of experiments, with or without perturbing effector. (The data do support a more limited statement that a particular effector decreases, or increases, binding of agonist over a series of concentrations of insulin.) The example illustrated in Fig. 2c is only one of many that can be cited in the literature on insulin, and on other hormones, in which claims concerning the number of available insulin (or hormone) receptor sites are unjustified.

The central contribution of binding measurements to the interpretation of biological responses is information on the dependence of occupancy of receptor sites on effector concentration and on the effects of various perturbants on such binding. Such information is most explicitly displayed by a graph of moles bound as a function of free concentration of effector, on a logarithmic scale to spread out data uniformly (Fig. 1, a and c, and Fig. 2, b and c). Such a graph provides the essential molecular information for correlation with macromolecular, cellular, or physiological behavior.

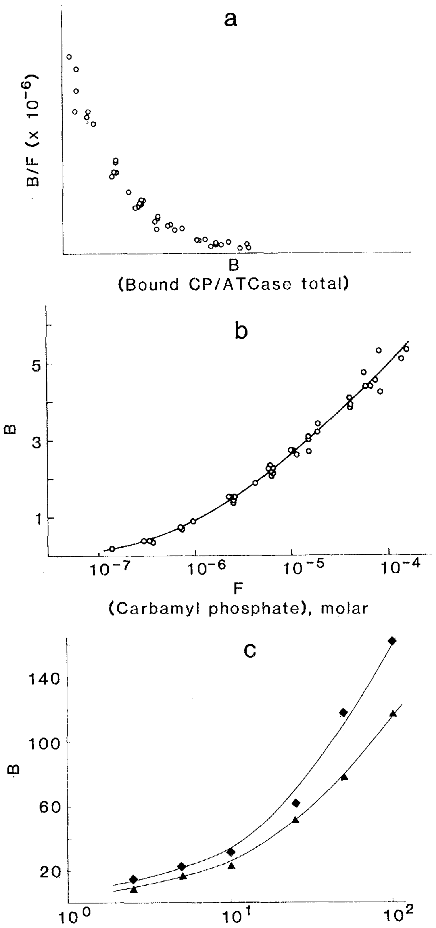

Fig. 2. (a) A Scatchard graph of data for the binding of carbamyl phosphate (*CP*) by aspartate transcarbamylase (*ATCase*). [Data from (*2*)] Numbers have been purposely omitted from coordinate axes. (b) Same data as used for (a) now presented in a semilogarithmic graph. (c) Semilogarithmic presentation of data for binding of insulin by fibroblasts under different environmental conditions (*3*).

For a more quantitative analysis, analytical expressions are available to complement the graphical representations [see (*4*) and (*5*) for definitions and details]. The algebraic equations allow one to extract measures of the affinity of receptors for ligands (*6*–*9*). In almost all circumstances, it is possible, by a simple graphical procedure (*10*), to evaluate the first stoichiometric binding constant (*4*, *5*). With more effort, the amount depending on the quantity and accuracy of the binding data, one can evaluate succeeding stoichiometric binding constants (*6*–*9*). In general it is not possible to obtain site-binding constants (*5*, *8*) unless some model of the interactions between receptor sites is assumed or has been established as realistic. Such a model introduces additional ad hoc assumptions.

How are we going to obtain the total number of receptor sites? We cannot find this parameter unless the experimenter is willing to make, and the system is amenable to the collection of, the necessary extensive measurements. Unless reliable binding data can be obtained at ligand concentrations that place the points unequivocally above the inflection point of Fig. 1a, any estimate of the position of the plateau will be uncertain. It will be even less reliable from a presumed intercept on a Scatchard graph.

We would all do well to recall a perceptive admonition made by T. H. Huxley over a century ago:

> Mathematics may be compared to a mill of exquisite workmanship, which grinds you stuff of any degree of fineness; but, nevertheless, what you get out depends on what you put in; and as the grandest mill in the world will not extract wheat-flour from peascods, so pages of formulae [or graphs] will not get a definite result out of loose data.

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Direct Determination of Ionic Solvation from Neutron Diffraction

Abstract. *Much information on ionic solvation in electrolyte solutions has been inferred from macroscopic thermodynamic and transport properties and from spectroscopy. These ion-water interactions can now be probed directly and unambiguously by neutron diffraction. Such measurements have been done with neodymium trichloride solutions in heavy water that are identical in every respect except the isotopic state of the neodymium ions; these experiments yield in a straightforward manner the distribution of oxygen and deuterium atoms from the water molecules in the first hydration sphere of the neodymium ion. Each ion is surrounded by 8.6 oxygen atoms at a distance of 2.48 angstroms and 16.7 deuterium atoms at 3.13 angstroms indicating a well-defined first hydration sphere of water molecules, the deuterium atoms pointing away from the cation.*

In order to understand and predict the properties of electrolyte solutions it is necessary to know what the entity called an ion is—that is, whether it is the bare ion, or whether it carries with it water molecules sufficiently firmly bound to be regarded as part of the ion and, if so, how many such water molecules are involved. A traditional approach has been to infer these hydration numbers from measurements of bulk thermodynamic and transport properties that are predominantly determined by ion-water interactions. Other techniques, such as nuclear magnetic resonance and Raman and infrared spectroscopy, have also been used in this way. In many instances the numbers thus obtained vary greatly with the experimental approach and with the method used to analyze and interpret the data (*1*).

It has now become clear that these difficulties in probing the ion-water interactions directly at the molecular level can be overcome by diffraction studies. Defining ion-water interactions as the positional and orientational correlations between ion-water pairs in solution, we have a measurable quantity, the diffraction pattern, from which these correlations can be determined. Ionic solutions were among the first liquids to which x-ray diffraction was applied, and a large number of studies have been reported over the years (*2*). However, the interpretation of a single diffraction pattern is always difficult, often ambiguous, and never unique. This ambiguity of interpretation can be eliminated by measuring the neutron diffraction patterns from several ionic solutions that are identical in all respects except the isotopic state of one of the ions. The algebraic difference between any two sets of measurements then yields the ion-water correlations (*2*). In order to obtain sufficiently good statistics for the differences, very large numbers of neutrons have to be counted. Hence, the method is at present limited to the study of relatively concentrated solutions (> 1 molal), and the results may not be representative for the dilute regime.

We measured the neutron diffraction patterns of four neodymium trichloride solutions in D_2O (Table 1). The experi-

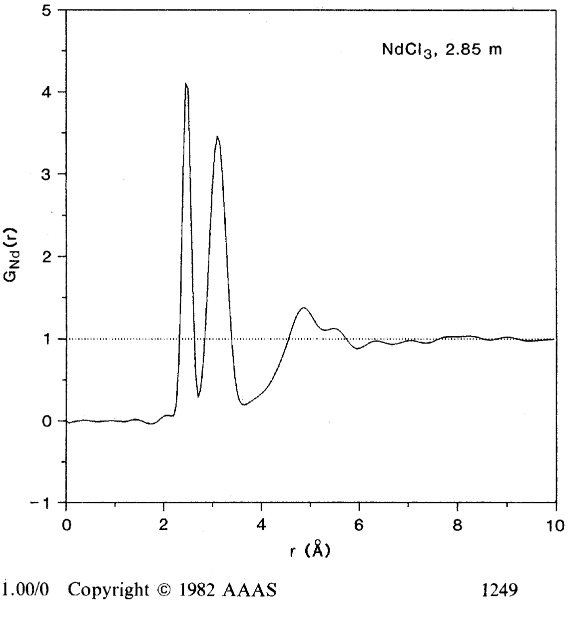

Fig. 1. Radial distribution of water molecules around the Nd^{3+} ion in a 2.85 molal solution of $NdCl_3$ in D_2O. The peaks at 2.48 and 3.13 Å correspond to 8.6 oxygen and 16.7 deuterium atoms from 8.5 ± 0.2 water molecules in the first hydration sphere.