J. Pharm. Pharmacol. 1993, 45: 241

Guest Editorial

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Decisions can be difficult, especially if they involve new developments which you feel you ought to know about but are not actually using yourself. If you say "No", are you insisting that the earth is flat? If you say "Yes", are you being taken for a ride? The stress is further increased when money is involved, as with decisions involving computers and computing. How do you feel about crystallochronography (CCG)? This is an extension of the study of rates, such as is used in pharmacokinetics and in onset and offset processes, to make a global fit to three-dimensional events. Until recently physico-optical methods have been used and these have enjoyed some popularity in skilled hands. Now the process has been computerized and provides a printout instead of analogue output requiring interpretation. Isn't it time you looked into this? What about the System Yielding the Basic Inferred Likelihood (SYBIL)? A package as powerful as this is, of course, expensive. Perhaps the safest decision is to refer the matter to your computer committee but is this any guarantee that a sensible answer will be reached?

The impact of computers is possibly most appreciated by people who can remember the time before they appeared. I still find it intellectually satisfying watching the computer-operated checkout in a supermarket. I can remember that in the 1950s it took three years for PhD students working in X-ray crystallography to complete an analysis: now the structure may be finished overnight. In pharmacology laboratories people like Schild and Stephenson spent much effort on making automated equipment (from telephone relays): now you can buy computer-driven apparatus ready-made and the computer takeover of the laboratory has really only just begun.

But there are snags. The ability of computers to manipulate and organize numbers has been matched by their ability to generate work involving computing (Parkinson's Law; Parkinson 1958). Computers save time but computing can also waste time and in total more may be wasted than saved. For the experimental scientist, computers are a means to an end and time spent struggling with computing is time taken away from work at the bench. Most methods of data analysis can be run on a home computer and learnt in a few minutes, so time spent shopping around is often wasted. What matters is not the computing but the mathematics and the ideas on which the mathematics are based. Although many people find it easier to grasp ideas as pictures than as equations, it is the equations which are rigorous and describe exactly what is to be expected. It is the ability of computers to allow you to fit data to equations other than straight lines which has produced a revolution in data analysis. You can also see when your ideas are wrong and can easily try other equations which may fit better.

In a binding experiment for instance, in which the amount bound is measured for increasing concentrations of ligand, or in an experiment with an enzyme in which the rate is measured for increasing concentrations of substrate, the underlying idea, obtained by applying the law of Mass Action to the binding process, is that amount bound, Y, and the concentration, X, should be related by the equation $Y = MX/(X + K)$, where M is the maximum and K is the value of X for which $Y = M/2$, and should be the dissociation constant for the complex. Values of Y and X can be used to calculate the values of M and K such that the sum of $(Y_{obs} - Y_{calc})^2$ is minimal (the method of "least-squares"): the scatter of the points $(Y_{obs} - Y_{calc})$ about the line should be normally distributed and can be used to calculate the standard error of the estimates of M and K. In a displacement binding experiment, with a fixed amount of labelled ligand in the presence of increasing concentrations of a competing unlabelled ligand, X, the amount bound, $Y = MK/(X + K)$, where M is the amount bound in the absence of the competing ligand and K, the value for which $Y = M/2$, will be approximately the same as the dissociation constant for the complex involving the unlabelled ligand if the labelled ligand occupies only a small proportion of the sites.

These ideas can be tested by fitting the data to the logistic equation, in which X and K are raised to the power, P, thus $Y = MX^P/(X^P + K^P)$. If binding follows the law of Mass Action, the value of P should be 1 in a saturation experiment or -1 in a displacement experiment. If there is more than one binding site P will be numerically less than one and if there is positive cooperativity P will be numerically greater than one (P is the same as the Hill coefficient). With data for agonist dose-response curves fitted to the logistic equation the exponent indicates the amplification of the signal produced by the drug-receptor complex: if the response is directly proportional to the complex $P = 1$ but it will be bigger than this for sensitive preparations which have steep dose-response curves.

You need a computer to fit values of X and Y to these equations but programs for doing this have been available for over 20 years and have progressed beyond the stage of CCG and SYBIL, so you needn't be suspicious of them. The older methods, such as Scatchard plots or Eadie-Hofstee plots, are no longer necessary and there is no more merit in teaching students about them than in teaching about shillings and ounces. The direct fit to equations is preferable because it is closer to the experiment: it does not involve manipulation of the data with the consequent distortion of errors.

In pharmacokinetics the equations involve exponentials describing rates of uptake or removal, rather than equilibrium constants, but the end result is the same, the production of a mathematical statement which describes events in terms of rate or equilibrium constants and which can be tested. A chemical statement of events is provided by the Van't Hoff relation, $-\Delta G$ (the change in free energy) $= RT\ln(K)$, where R is the gas constant and T the absolute temperature, so it is log·K which is the most fundamental measure of the result and the expectation is that the errors in K will be log. normally distributed, as is found for measurements of the pKs of acids or bases or of pA_2 values for antagonists at receptors. In theory, rate constants can be related to equilibrium constants for transition states, so the situation is similar.

The change in free energy, however, depends upon two factors, the change in enthalpy, ΔH, and the change in entropy, ΔS, with $\Delta G = \Delta H - T\Delta S$, where T is the absolute temperature, and it is this which limits the predictive value of the equations and puts me off buying SYBIL and similar fortune-telling packages. Please will someone invent an entropystat to avoid having to make all measurements at the absolute zero (0°K)?

Dr Barlow retired in 1990 after over 40 years research experience in chemistry and pharmacology, but maintains an interest in the application of computers to pharmaceutical science. Copies of his latest book "Foundations of Pharmacology" and accompanying software can be obtained from the author.

References

Parkinson, C. N. (1958) Parkinson's Law or the Pursuit of Progress. John Murray, London p. 69