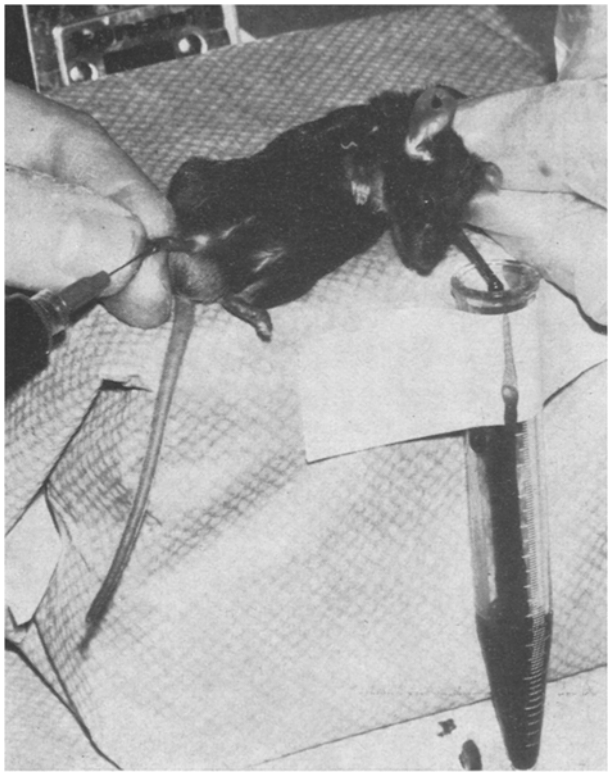

Illustration of blood exchange accomplished by injection via the corpus cavernosum of the penis and bleeding through the orbital sinus of the mouse.

Animals which survived the first 2 h after transfusion (> 90%) showed no observable ill effects during the following 3 months. When normal male C57Bl mice were transfused with 2 volumes of fresh normal heparinized blood, there was a 19% drop in the 1 h post-transfusion hematocrit (Table). This decrease was probably the result of leakage of greater than the 0.2 ml compensated for, and is difficult to avoid. When corrections were made for the change in hematocrit, approximately 14% of the initial ^{59}Fe-labelled red cell counts remained in the animal after transfusion.

A method has been described for transfusing mice by injection of fresh whole blood into the corpus cavernosum of the penis while blood is concurrently removed from the venus plexus of the orbital cavity. Both these routes are easily accessible and when reasonable precautions are taken, they facilitate rapid and relatively safe blood transfusion in small animals. The high survival rate when syngeneic blood is used makes it a useful procedure for longterm studies. Based on a 7% blood volume per weight mouse, only 2 volumes of blood were transfused in this study. However, it seems likely that larger amounts can be transfused, and a concomitant reduction in the endogenous blood obtained. If necessary, the procedure could be repeated at frequent intervals with no damage to the penis. The orbit is more prone to injury; however, as Riley[5] noted 1–2 bleedings of small volumes via this route per week seem to be harmless to the animal and daily bleedings are even possible. Furthermore, although chloral hydrate results in a decreased blood flow to the tail of the mouse, it is possible to transfuse blood via the tail vein. Thus, though more difficult, transfusion is possible in chloral hydrate anesthetized female mice.

A Simple Algorithm for the Solution of the '$n \times m$' Case of a Binding Equilibrium

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Summary. Accurate estimates of the equilibrium concentrations in the non-interactive reaction of several ligands with several classes of binding sites with univalent stoichiometry can be rapidly obtained by a simple method of successive approximations on a programmable desk calculator.

Binding reactions between macromolecules and other compounds (ligands) are common biochemical phenomena. Well-known examples are the formation of enzyme-substrate complexes, the specific and nonspecific binding of hormones to plasma or cell proteins, the antigen-antibody reaction, etc. In the '$n \times m$' case[2], m independent classes of noninteracting, univalent binding sites react with n univalent ligands simultaneously to reach an equilibrium defined by $n \times m$ equations given by

$$B_{ij} = K_{ij} (N_j - C_j) (S_i - D_i) \qquad (1)$$

where B_{ij} is the equilibrium concentration of the ith ligand bound to the jth class of binding sites ($i = 1,2,\ldots, n$; $j = 1,2,\ldots, m$), K_{ij} is the corresponding equilibrium association constant, $C_j = \sum_{i=1}^{n} B_{ij}$, $D_i = \sum_{j=1}^{m} B_{ij}$, and N_j and S_i are the total concentrations for the jth class of binding sites and the ith ligand, respectively. For a given positive integer m, it is possible to estimate the values of K_{ij} and N_j from experimental binding data and known S_i's by the method of least squares as applied to nonlinear regression[3]. Several computer programs are available for this kind of parameter fitting[4–8], including ours that incorporates a goodness-of-fit test to decide (for $n = 1$) which of several values assumed by m best describes the experimental data[9].

Equally important to researchers is the converse problem of calculating the equilibrium concentrations of bound and unbound ligands and of filled and empty bindings sites when the association constants and total concentrations of reactants are known or allowed to assume given values (simulation or modeling). Stucki[10] has described an algorithm for implicitly solving this problem when $n = 1$. Others have used various computer programs based on linearization (Taylor series) or differential equations when $n > 1$[11–13]. The present report describes a simpler method which requires only a programmable desk calculator.

Summation of the $n \times m$ equations given by (1) to obtain the m concentrations of jth ligand bound to all sites and the n concentrations of ith class of binding sites

filled by all ligands results, respectively, in m equations of type (2) and n equations of type (3):

$$C_j = N_j \sum_{i=1}^{n} K_{ij} (S_i - D_i) \Big/ \left[1 + \sum_{i=1}^{n} K_{ij} (S_i - D_i) \right] \quad (2$$

$$D_i = S_i \sum_{j=1}^{m} K_{ij} (N_j - C_j) \Big/ \left[1 + \sum_{j=1}^{m} K_{ij} (N_j - C_j) \right] \quad (3)$$

which constitute a system of $m + n$ nonlinear equations in $m + n$ unknowns. No quantity in equations (2) and (3) can be negative and, since the total concentration of a species exceeds the concentrations of its bound fraction, $N_j > C_j$ and $S_i > D_i$. These consideration and the symmetry of equations (2) and (3) permit the application of the following iterative method by successive approximations: Let X_j be an initial guess or approximation of C_j. Substitution of the m approximations of this type in the n equations (3) yields the n approximations, Y_i, for D_i. Their substitution in the m equations (2) then yields a second set of m approximations for X_j. This process is repeated until the following condition is satisfied:

$$\left| \sum_{i=1}^{n} Y_i - \sum_{j=1}^{m} X_j \right| < \delta \quad (4)$$

where δ, the tolerance, is positive and smaller than the least significant digit of accuracy desired. Substitution of the solutions in (1) gives all individual values for B_{ij}.

A program based on this algorithm was written for use on the Hewlett-Packard model 9810A calculator. The program consists of 1123 steps. The storage capability of this calculator (111 data registers in option 001) places ceilings for m and n which must satisfy the expression $mn + 2(m + n) \leq 95$. A listing of the program and instructions for use will be available from the author on request. The printed solution consists of the concentration of filled binding sites and percent occupancy for each class of sites; the concentration of bound ligand, percent total bound, and the ratio bound/unbound for each ligand; and all the individual values of B_{ij}. The method of iteration is not greatly affected by the values of the initial guesses which in the program are $N_j/2$. Using the association constants and concentrations for the binding of 6 steroid hormones and 3 plasma proteins presented by FELDMAN et al.[11] in their tables 2A and 2B as input, and specifying $\delta = 10\ nM^{-1}$, the program converged to a solution identical to theirs in 28 sec. The iteration time increases as an approximately linear function of $-\log\delta$. Although the iterative technique used in this algorithm is not very fast, it is quite adequate for laboratory use, and it is safe provided that the magnitudes of the concentrations of sites and ligands are not so disparate that the digit-carrying capacity of the calculator is exceeded. In our laboratory it has proved useful in simulating the nonspecific and the specific binding of progesterone and cortisol to the two classes of high-affinity sites thought to be present in rat uterine cytosol[14]. Its application to the validation of a filtration technique for the estimation of these high-affinity sites, a modification of the method of SANTI et al.[15], will be reported separately.

[1] Acknowledgments. I am grateful to Miss P. L. MILLER for her help in editing and testing the program and for typing the manuscript. This work was supported in part by Grant No. 5-TO1-HD00104-10, awarded by the National Institute of Child Health and Human Development, DHEW, and by The Ford Foundation, Grant No. 630-0505B, C.

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