$P_{50}$'s of about 30 specimens of different body weights (45 g–7.5 kg) were compared and there was some suggestion of a higher affinity among smaller specimens, but there was much variability. The presence of such a differential was tested because of indications that 'embryonic' haemoglobins have a higher oxygen affinity within the egg cases of skates[4] and other elasmobranchs.

It is concluded that the data reported here reflect conditions in the intact fish more closely than in previous studies because determinations were carried out within half an hour of blood having been withdrawn from catheters that had remained within the fish for several days. There are indications that changes in the respiratory properties of blood also occur in blood samples taken from other fish.

*Résumé.* Quelques propriétés respiratoires du sang de la raie (*Raia clavata*) ont été déterminées avec des échantillons de cathéter intravasculaire. Chaque échantillon était utilisé seulement pour deux points de la courbe de dissociation $-O_2-$, parce que les propriétés changent après une demie-heure. On a constaté un effet de Bohr ($\Delta$ log $P_{50}/\Delta$ pH $= -0.25 \pm 0.03$) ainsi que celui d'Haldane.

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## A Computer Program for the Calculation of the Free Ligand Concentrations in Cases of Multiple Binding to Proteins

In many experimental situations in vitro or in vivo, a regulatory active compound, such as a drug or a hormone, is bound with considerable affinities by proteins which are not directly involved in the regulatory action exerted by the compound. Such unspecific binding diminishes the free active concentration of the compound and influences its metabolism as well as its regulatory functions. Therefore the half-maximal-effective dose of the compound may be overestimated, and also misinterpretations concerning the mode of action of the compound are possible when the dose response curve is not corrected for such secondary binding[1].

Many laboratories have directed their efforts towards the determination of the binding capacities and affinities of various plasma or tissue proteins for drugs and hormones[2,3,4]. The knowledge of these binding parameters permits the calculation of the active concentrations of these substances[1]. In the case of multiple classes of binding sites for one and the same compound, such calculations proved to be quite tedious. Therefore computer programs for a single[5] and two classes of binding sites[6], which were based on the explicit solution of a binding equation, have been published. The generalization for several binding sites was suggested to me by Dr. P. BALLY. In the present report a computer program which solves a binding equation with an implicit method for any number of classes of binding sites is described.

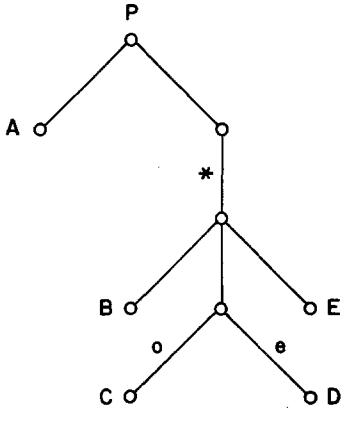


Fig. 1. Set graph of the procedure used for the implicit solution of the binding equation. – The nodes in the set graph designate sets of executable operations. For the meaning of the letters on the nodes and the symbols on the branches see text.

*Algorithm and Computer program.* The binding of a ligand $H$ to multiple classes of binding sites can be described by the following binding equation:

$$H_t = H_f + \sum_{i=1}^{n} \frac{C_i H_f}{A_i + H_f}$$

where $H_t$ designates the total concentration of the ligand, $H_f$ the concentration of the free unbound ligand, $C_i$ and $A_i$ the binding capacities and affinities (expressed as dissociation constants), respectively of the different classes of binding sites and $n$ the number of classes of binding sites.

The logic structure of the algorithm used for the implicit solution of this binding equation is depicted in the set graph in Figure 1. The algorithm starts with an opening set of operations (A) which initialize the procedure (P) by calculating a tolerated absolute error of the solution of the binding equation from a specified relative error and by assuming an initial value for $H_f$. This is done by setting $H_f$ equal to the upper limit $H_t$. The following set of operations (B–E) is executed repeatedly as indicated by the starred symbol in the set graph until either the actual error of the solution is smaller or equal to the tolerated absolute error or the actual number of iterative cycles exceeds a specified upper limit. The binding equation is written in the form:

$$H_t - H_f - \sum_{i=1}^{n} \frac{C_i H_f}{A_i + H_f} = \text{error}$$

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In a first set of operations (B) the error of the solution with the actual value of $H_f$ is calculated. A negative error indicates that the actual value of $H_f$ is too big and $H_f$ is decremented by the fraction $H_f$. con (C) otherwise incremented accordingly (D) as indicated by the symbols *o* and *e* in the set graph. Finally the counter for the number of iterations is incremented by 1 (E).

A convergence of the solution is assured as long as the value of the factor con for decrementing and incrementing the value of $H_f$ is bound within the limits $0 < \text{con} < 1$. The rate of convergence of the solution can be monitored by variation of this factor. It is important to note that the error of the solution does not depend on the number of iterative cycles performed since for every actual value of $H_f$ the whole balance of the ligand is calculated again.

The algorithm described above was coded in the PL/1 language[7] and is presented in Figure 2 in the procedure LIGAND which has the form of a callable subroutine[8].

*Results and discussion.* As an illustrative example, the influence of the binding of chlorpromazine to rat liver microsomes and bovine serum albumin on the free concentration of the drug is shown in Figure 3. In this Figure the total chlorpromazine concentration is plotted versus the concentrations of the unbound chlorpromazine and that bound to the individual binding sites of rat liver microsomes and bovine serum albumin. At concentrations of chlorpromazine lower than $10^{-3}$ $M$ the concentrations of the unbound ligand were about 3 orders of magnitude lower than those of the total. In the region between $10^{-3}$ $M$ and $10^{-1}$ $M$, the different classes of binding sites become saturated with the drug, as is shown in the Figure. At concentrations of chlorpromazine higher than $10^{-1}M$, the free concentration of the drug is about equal to the total. The discussion of the physiological implications of these findings is beyond the scope of this paper and will be published elsewhere[4].

The factor con used for the variation of $H_f$ was set to 0.1 for the calculations shown in Figure 3. The iterative cycles were stopped when the relative error of the solution was smaller or equal to 0.01%. With these parameters, the number of iterative cycles performed was about 1200 for a total drug concentration lower than $10^{-7}$ $M$. Within the concentration range of $10^{-7}$ $M$ to $10^{-1}$ $M$, the number of iterative cycles performed oscillated between 500 and 50 and went up again to 1000 at drug concentrations higher than $10^{-1}$ $M$. No further efforts were made to improve this performance of the computer program, since the central processor unit times used for these calculations were very short.

Previously published computer programs for the calculation of the concentrations of the free unbound ligand were based on a binding equation explicitly

```
LIGAND:
  PROC (HTOT,CAP,AFF,RELERR,CON,IMAX,H,HB,I);
    DCL (CAP(*),AFF(*),HB(*)) DEC FLOAT;
    /* INITIALIZATION */
    ABSERR=RELERR*HTOT;
    ERR=ABSERR+1;
    H=HTOT;
    /* IMPLICIT SOLUTION OF THE BINDING EQUATION */
    DO I=1 TO IMAX;
       HB=CAP*AFF/(AFF+H);
       HBTOT=SUM(HB);
       ERR=HTOT-H-HBTOT;
       IF ABS(ERR)<= ABSERR THEN GOTO E_LOOP;
       IF ERR <0
          THEN H=H-H*CON;
          ELSE H=H+H*CON;
    END; E_LOOP:;
END LIGAND;
```

Fig. 2. Procedure LIGAND for the implicit solution of the binding equation – The input variables of the subroutine are: HTOT, CAP, AFF, RELERR, CON, IMAX and the output variables are: H, HB, I. The parameters CAP, AFF and HB are arrays of the dimension $n$ where $n$ is the number of classes of binding sites. CAP and AFF contain the binding capacities and affinities respectively of the different classes of binding sites and HB contains the concentrations of the ligand bound to the individual classes of binding sites. Other parameters have the following meanings: HTOT, total ligand concentration, CON, factor for the variation of the free ligand concentration, RELERR, relative error of the solution specified as a fraction, IMAX, number of maximally allowed iterative cycles, H, free ligand concentration and I, actual number of iterative cycles performed to obtain the solution of the binding equation. For further details see text. The attributes of all of the variables are assumed by the standard PL/1 defaults[7]. The rules for the invocation of the subroutine as well as for the explicit declarations of the array parameters in the main procedure can be found elsewhere[7,9].

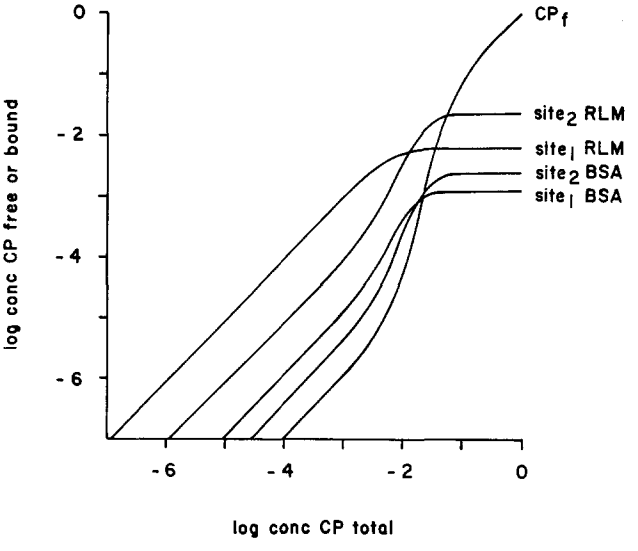


Fig. 3. Influence of the binding of chlorpromazine to bovine serum albumin and rat liver microsomes on the free concentrations of the drug. – The system studied in this calculation had a total of 4 classes of binding sites for chlorpromazine. The following binding capacities (μmol/g protein) and affinities of bovine serum albumin and rat liver microsomes for chlorpromazine were used for the calculations. Bovine serum albumin: $C_1$ 33, $C_2$ 63, $A_1$ 1.1111.$10^{-4}$ $M$, $A_2$ 5.8823.$10^{-4}$ $M$, rat liver microsomes: $C_1$ 161, $C_2$ 544, $A_1$ 7.1428.$10^{-6}$ $M$, $A_2$ 2.5641.$10^{-4}$ $M$. These parameters were obtained from equilibrium dialysis experiments[4]. The affinities were expressed as dissociation constants. A protein concentration of 40 g/l was assumed for both species. The further parameters used for the calculations are given in the text. Abbreviations: CP chlorpromazine, RLM, rat liver microsomes, BSA, bovine serum albumin.

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[8] The same algorithm was programmed for the Hewlett-Packard Mod. 9810A Desk Top Calculator. This version of the program solves the binding equation for up to 10 different classes of binding sites. A listing of the program is available upon request from the author.

expressed for $H_f$[5,6]. This expression takes the form of a polynomial with powers of $H_f$ and is of the $n$ + 1st order when $n$ is the number of classes of binding sites. The value of the concentration of the free ligand $H_f$ was obtained by finding the roots of this polynomial. The structure of the coefficients of this polynomial obeys simple permutation laws, and the coefficients can therefore be obtained by inspection of the powers of $H_f$. However, the expressions for the coefficients rapidly become very cumbersome for higher orders of the polynomial. An algebraic solution for the roots of the polynomial of higher than the 4th order is generally not possible. Therefore it is necessary to find these roots with iterative methods which is, even with the help of high speed digital computers, a time-consuming task.

Furthermore, different programs have to be coded for every number of classes of binding sites, since the coefficients of the corresponding polynomials are different for every order of the polynomial.

These disadvantages can be overcome by solving the binding equation implicitly as described above. The procedure LIGAND can be used without alterations for any possible number of classes of binding sites and is therefore very general. In addition, a solution of the binding equation to any specified possible degree of precision is obtained in much shorter time with the procedure described than with those which solve the binding equation by finding the roots of a polynomial.

The subroutine LIGAND is well suited for implementation in a scientific subroutine package and should be a valuable tool in studies where the total ligand concentration has to be corrected for unspecific binding[10].

*Zusammenfassung.* Es wird ein einfaches numerisches Verfahren beschrieben, mit dessen Hilfe die freien Konzentrationen eines Liganden, der an Blut- und Gewebebestandteile gebunden wird, berechnet werden können.

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*3 December 1973.*

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[10] Acknowledgments. Supported by grants from the Swiss National Science Foundation and the Endowment Fund of IBM. The calculations were done on an IBM 370-158 of the BEDAG, Bern. The author is grateful to Professor M. H. BICKEL for making available unpublished data and to Dr. P. R. BALLY for helpful discussions.

## Myocardial Inactivity of Therapeutic Concentrations of Hydralazine and Diazoxide

Hydralazine and diazoxide are effective vasodilator drugs used in the treatment of systemic hypertension[1-5]. They lower peripheral vascular resistance by a direct relaxing effect on arteriolar smooth muscle but have little action on capacitance vessels[1,3,5-7]. The hypotensive action of hydralazine and diazoxide is accompanied by marked increases in heart rate, left ventricular ejection rate, and cardiac output[1,2,4,5,8,9]. This cardiac hyperactivity is at least in part the result of increased sympathetic stimulation of the heart due to activation of the baroreceptor reflex by the vasodilation-induced hypotension[1,4,5,10-12]. It has been suggested that hydralazine and diazoxide may also exert more proximate positive chronotropic and inotropic effects on the heart itself[5,13,14]. They might produce such effects by releasing norepinephrine from myocardial sympathetic nerve endings, by stimulating cardiac $\beta$-adrenergic receptors, or by a direct myocardial action. We have recently established the serum concentrations of hydralazine[15] and diazoxide[3] that occur during the therapeutic use of these drugs in man. The present study was undertaken to determine if hydralazine or diazoxide exert any direct myocardial actions in such concentrations.

*Materials and methods.* Right ventricular papillary muscles and left atrial strips from kittens (0.4–0.9 kg) and right atrial pacemaker preparations from guinea-pigs (0.3–0.4 kg) were used. To insure adequate oxygenation of the central fibres of papillary muscles, only muscles of less than 0.6 mm² cross-sectional area were chosen[16]. The preparations were fixed between a small plastic electrode block and a stainless-steel wire hook

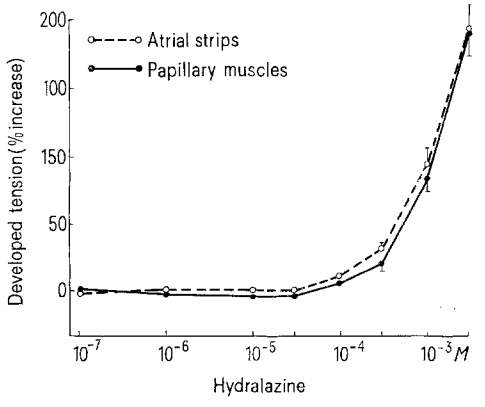


Fig. 1. Effect of hydralazine on isometric tension developed by 12 kitten papillary muscles and 12 kitten atrial strips. Means ± SEM.

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