J. Math. Biol. 43, 313–324 (2001)
Digital Object Identifier (DOI):
10.1007/s002850100086

Mathematical Biology

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Complex ligand-protein systems: a globally convergent iterative method for the $n \times m$ case

Received: 1 November 2000 /
Published online: 21 August 2001 –

Abstract. When n types of univalent ligands are competing for the binding to m types of protein sites, the determination of the system composition at equilibrium reduces to the solving of a non-linear system of n equations in $C = [0; 1]^n$. We present an iterative method to solve such a system. We show that the sequence presented here is always convergent, regardless of the initial value in C. We also prove that the limit of this sequence is the unique solution in C of the non-linear system of equations.

1. Introduction

In biochemical systems, ligands are often found competing for differing protein binding sites. For example, in response to the intrusion of a foreign molecule, the immune system will activate several individual B lymphocytes, each one secreting a particular antibody [26]. The antigen is thus recognized by a population of proteins made of several classes of antibodies with different affinities. Moreover, cross-reactivity is possible; one antibody may bind to two or more antigens. As for the antibody, a given T Cell receptor is able to bind several ligands with different affinities [11], for instance the receptor of immature T lymphocytes is known to interact with multiple peptide ligands in the thymus [27].

Typically, two related aspects of the ligand-binding system are of interest: (i) the determination of the different types of binding sites and their affinity constants, and (ii) the derivation of the system composition at equilibrium.

For (i), in the simplest case of one ligand species and one binding site, the affinity constant is determined via a Scatchard plot [23]. This determination becomes more complex with more than one type of binding site or ligand, and many diverse approaches have been explored [7,8,10,12,13,15–17].

The derivation of the equilibrium composition is the reverse problem; ligand and binding site total concentrations along with association constants are known, and the law of mass action and matter equations are used to establish a relationship between

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Key words or phrases: Protein – Ligand – Binding – Nonlinear system of equations

free ligand concentrations and these known parameters. Under this prescription, the free ligand concentrations are obtained via the solution of a nonlinear system of equations. Such an approach has been utilized in problems as diverse as determining the free and bound concentrations of an hormone in plasma [6], simulating an immunoassay [4], determining the concentrations of different complexes formed between ions and metals in solution [19,20,25], modeling the binding of bivalent antigens to antibodies [9], and the formation of immunoglobulin G polymers [28].

The nonlinear system of equations relating the concentrations of the solution components to the known parameters has in general no analytical solution, and numerical techniques are employed [2,3,19,20,25] to approximate a solution. Although an exhaustive theory of the solving of nonlinear systems by iterative methods has been developed [18], no rigorous treatment of the particular type of nonlinear systems related to complex binding systems has been given. Specifically, there is no assurance that the algorithm will converge, unless the initial guess is close enough to a solution.

In this work, we rigorously investigate the case of univalent ligands competing for several binding sites. Examples of this case are given by the binding of some hormones to serum proteins. For instance, in the blood, several steroids compete for the binding to the sex hormone-binding globulin, the corticosterone-binding globulin and albumin [24]. Likewise, the thyroid hormones T3 (triiodothyronine), rT3 (reverse T3) and T4 (thyroxine) are free in the serum or bound to either Thyroxine Binding Globulin (TBG), albumin, or prealbumin. T3, rT3 and T4 thus compete for the same sites on three proteins. Further, the complexity of this system is increased in experimental situations where an antibody having a very strong affinity for T4 is introduced to determine the concentration of free T4 [4]. In this paper we consider the general case of n univalent ligands and m binding sites. Namely, after deriving the representative system of nonlinear equations, an iterative map is defined to solve it. It is then shown that the generated sequence converges to the unique solution of the system. We finally illustrate the method by applying it to the case of the thyroid hormones.

2. Case of n univalent ligands and m binding sites

We consider n ligands $(L_1, \cdots, L_n)$ and m binding sites $(P_1, \cdots, P_m)$. Each ligand L_i can bind to each site P_j according to a reversible reaction

$$L_i + P_j \rightleftharpoons P_j L_i, \tag{1}$$

whose equilibrium obeys the law of mass-action

$$K_{ij} = \frac{[P_j L_i]}{[P_j][L_i]}, \tag{2}$$

where K_{ij} is the association constant, and the square brackets denote the concentrations. If a ligand i cannot bind to a site j, then $K_{ij} = 0$. The value of K_{ij} corresponds to a specific set of experimental conditions. K_{ij} does not vary only with the temperature but is also sensitive to changes of pH or ion concentrations (see for instance [22]).

The association constants K_{ij} are supposed known, as are the total concentrations of ligands ($[L_i]_t$) and binding sites ($[P_j]_t$):

$$[L_i]_t = [L_i] + \sum_{j=1}^{m} [P_j L_i], \tag{3}$$

$$[P_j]_t = [P_j] + \sum_{k=1}^{n} [P_j L_k], \tag{4}$$

where $[X]_t$ stands for the total concentration of component X. The aim is to determine the detailed composition of the system, that is $[L_i]$, $[P_j]$ and $[P_j L_i]$ for all i and j. To this end, (2) and (3) are combined to obtain

$$[L_i] = [L_i]_t \left(1 + \sum_{j=1}^{m} K_{ij} [P_j] \right)^{-1}. \tag{5}$$

Likewise, (2) and (4) lead to

$$[P_j] = [P_j]_t \left(1 + \sum_{k=1}^{n} K_{kj} [L_k] \right)^{-1}. \tag{6}$$

Combining (5) and (6) gives a relation between the concentrations $[L_i]$ of free ligands and the known parameters $[L_i]_t$, $[P_j]_t$ and K_{ij}

$$[L_i] = [L_i]_t \left(1 + \sum_{j=1}^{m} \frac{K_{ij} [P_j]_t}{1 + \sum_{k=1}^{n} K_{kj} [L_k]} \right)^{-1}. \tag{7}$$

Letting x_i denote the proportion of free ligands ($x_i = [L_i]/[L_i]_t$), we have

$$x_i = \left(1 + \sum_{j=1}^{m} \frac{\alpha_{ij}}{1 + \sum_{k=1}^{n} \beta_{kj} x_k} \right)^{-1} \qquad (i = 1, \cdots, n), \tag{8}$$

where the parameters α and β are defined by

$$\alpha_{ij} = K_{ij} [P_j]_t, \quad \beta_{ij} = K_{ij} [L_i]_t. \tag{9}$$

The solutions x_i determine the concentrations of free ligands, whence (6) give the concentrations of free sites and (2) yield the concentrations of complexes. Thus the problem reduces to the solution of the nonlinear system (8).

3. Background

3.1. General methods

Several general algorithms have been developed to solve nonlinear systems of equations [18]. One of the most commonly employed is the Newton-Raphson Method (NRM). A drawback of this method is its tendency to converge only if the initial guess X_0 is sufficiently close to the solution (local convergence) [1,21]. Theorems concerning the global convergence of the NRM can be found in [18], but their conditions of application are too stringent for the system studied here. Another point is that the NRM requires the computation of a Jacobian matrix at each iteration. This makes convergence costly in the computation of systems considered here where the number of ligands and binding sites can be important.

3.2. Specific methods

One of the first iterative methods used in deriving the composition of a complex ligand binding system was published in 1965 [19,20]. Using the same approach as in [20], the system investigated in the present paper can be rewritten as an $m+n$ dimensional system where the quantities to be determined are the n $[L_i]$ and the m $[P_j]$. To solve this system Perrin *et al.* [20] defined a particular sequence. When this sequence is convergent, its limit is the solution of the nonlinear system. However, no proof of convergence was given. Storer *et al.* [25] used an alternative sequence which is almost identical to the one published by Colas [6] the same year. More recently, Barbet *et al.* [2] have developed a method similar in spirit to that previously proposed by Bellon *et al.* [3]. This technique consists in the optimization of a quantity Z. The optimal values of the $m+n$ variables for which Z is an extremum is the solution of the nonlinear system to be solved.

To our knowledge, no result of global convergence has been given for any of the specific methods mentioned above. Only a result of local convergence was given in [2]. That is, if the initial vector of variables is close enough to the solution, then the optimization algorithm will converge. However, in practice, it might be difficult to give an initial guess close enough to the solution.

In the present paper we solve the system (8) by constructing a sequence similar to that used in reference [25] and we give a global convergence result. Namely, we show that for any initial vector in $[0;1]^n$ the sequence is convergent. In addition, we give the proof that the limit of the sequence is the unique solution in $[0;1]^n$ of system (8).

4. Solution of the $n \times m$ case

We first recast the problem as the solving of the nonlinear system:

$$X = G(X), \quad X \in C, \tag{10}$$

where $X = (x_1, \cdots, x_n)^T$, $G(X) = (g_1(X), \cdots, g_n(X))^T$, $C = [0; 1]^n$, and the functions g_i are given by

$$g_i(X) = \frac{1}{1 + \sum_{j=1}^{m} \frac{\alpha_{ij}}{1 + \sum_{k=1}^{n} \beta_{kj} x_k}}. \tag{11}$$

We next define three order relations.
Let $X = (x_1, \cdots, x_n)^T$ and $Y = (y_1, \cdots, y_n)^T$ be two vectors of $\mathbb{R}^n$, then

$$\begin{array}{ll} X \geq Y & \text{if } x_i \geq y_i, \ \forall i \in \{1, \cdots, n\}, \\ X > Y & \text{if } \quad X \geq Y \ \text{ and } \ X \neq Y, \\ X \gg Y & \text{if } x_i > y_i, \ \forall i \in \{1, \cdots, n\}. \end{array} \tag{12}$$

For the sake of convenience we will use $\bar{0}$ for $(0, \cdots, 0)^T$ and $\bar{1}$ for $(1, \cdots, 1)^T$. The main result of the present paper is summarized by the following theorem.

Theorem 1. *For all initial term X_0 in C, the sequence*

$$X^{l+1} = G(X^l) \ \ (l \in \mathbb{N})$$

is convergent and its limit is the only fixed point X^* of G in C.

This theorem establishes the existence and uniqueness of the solution X^* of the non-linear system $X = G(X)$ in C. Furthermore, it provides the basis of an iterative scheme to solve this system: by choosing any initial vector X^0 in C and by iterating the sequence $X^{l+1} = G(X^l)$, one obtains an estimation of X^*. The advantage of the method presented here is that, unlike in [2], it does not require an initial term X^0 that is close to X^*. The sequence will converge to X^* whatever X^0 is. Of equal importance in the application of iterative schemes is how the estimation error varies in successive iterations. The following result provides an answer to this issue.

Theorem 2. *Let (U^l) and (V^l) be the sequences generated by G with $\bar{0}$ and $\bar{1}$, respectively, as first term. For all rank $l > 0$, we have*

$$\bar{1} \gg V^l \gg V^{l+1} \gg X^* \gg U^{l+1} \gg U^l \gg \bar{0} \tag{13}$$

And, if (X^l) is the sequence generated by G with an arbitrary first term X^0 in C, then, for all rank $l > 0$ and integer $i \leq n$,

$$|x_i^l - x_i^*| < |v_i^{l-1} - u_i^{l-1}|. \tag{14}$$

As can be seen from (13), the estimation error over X^* decreases at each iteration when the iterative method is started with either $\bar{1}$ or $\bar{0}$. The relation in (14) gives a majoration of the estimation error over X^* at rank l when the iterative method is applied with an arbitrary first term X^0 in C.

In summary, in contrast to previous studies, the iterative scheme presented here ensures global convergence. That is, to determine the equilibrium composition of the protein-ligand system, one just needs to pick up an arbitrary vector in C and apply successive transformations by G. This iterative method always converges to the unique solution of the complex binding system. The remainder of this paper is devoted to the proofs of Theorems 1 and 2.

5. Proofs of Theorems 1 and 2

To prove Theorem 1 we shall use the following general result

Lemma 1. *Let* $F = (f_1, f_2, \cdots, f_n)^T$ *be a continuous function with domain* C *and range* $\mathbb{R}^n$. *If the following conditions are fulfilled,*

(H1) $\forall X \in C,\ \forall i,\ \forall j,\ \partial f_i(X)/(\partial x_j) \geq 0$ *and* $\partial f_i(X)/(\partial x_i) > 0$

(H2) $F(\bar{0}) \gg \bar{0}$ *and* $\bar{1} \gg F(\bar{1})$

(H3) $\forall X \in C,\ \forall Y \in C,\ \forall t \in [0; 1]$, *if* $Y \geq X$, *then* $F((1-t)X + tY) \geq (1-t)F(X) + tF(Y)$

then F *has a unique fixed point* X^* *in* C *and for all initial term* X_0 *in* C, *the sequence* $X^{l+1} = F(X^l)$ $(l \in \mathbb{N})$ *converges to* X^*.

We will first prove Lemma 1 and then show that G fulfills the application conditions of this lemma, and, finally, prove Theorem 2.

5.1. Proof of Lemma 1

Let $F = (f_1, f_2, \cdots, f_n)^T$ be a continuous function with domain C and range $\mathbb{R}^n$ that satisfies $(H1)$, $(H2)$ and $(H3)$. First, for all vectors X and Y of C we have the following properties:

$$X \geq Y \Rightarrow F(X) \geq F(Y), \tag{15}$$

$$X \gg Y \Rightarrow F(X) \gg F(Y). \tag{16}$$

Those properties are direct consequences of $(H1)$ and the definition of the order relations. A corollary of (15) is the following property:

$$X \in C \Rightarrow F(X) \in C. \tag{17}$$

Indeed, if $\bar{1} \geq X \geq \bar{0}$, using (15) and $(H2)$ leads to

$$\bar{1} \gg F(\bar{1}) \geq F(X) \geq F(\bar{0}) \gg \bar{0};$$

that is, $F(X)$ belongs to C. Now, as all the functions f_i are continuous, we can apply the *Brouwer theorem* [14] and deduce that F has at least one fixed point in C. The next step is to study the behavior of the sequence

$$U^0 = \bar{0},\quad U^{l+1} = F(U^l),\ (l \in \mathbb{N}). \tag{18}$$

Because of $(H2)$ and (16) this sequence is strictly increasing. Moreover, according to property (17) the sequence (U^l) is bounded. Since (U^l) is strictly increasing and bounded, it is convergent. Let U^* be the limit. We have

$$\begin{aligned} U^* &= \lim_{l \to \infty} U^{l+1} \\ &= \lim_{l \to \infty} F(U^l), \end{aligned}$$

and, since the functions f_i are continuous, $F(U^*) = U^*$. Following the same reasoning one can show that the sequence generated by F with first term $\bar{1}$ is strictly

decreasing and bounded, and thus convergent to a limit V^* such that $V^* = F(V^*)$. We now show that $U^* = V^*$. First, combining $\bar{1} \geq \bar{0}$ and (15) gives $V^* \geq U^*$. We now assume that the two fixed points are distinct ($V^* > U^*$). The set of vectors $\{X(t) = V^* - t(V^* - U^*), t \in \mathbb{R}\}$, is the line passing through U^* and V^*. Let Z be the vector $X(t_k)$ where k is the index minimizing $t_i = v_i^*/(v_i^* - u_i^*)$ (t_i is defined for all index i such that $v_i^* > u_i^*$ and there is at least one such index since we assumed $V^* > U^*$). We have

$$z_k = 0, \tag{19}$$

and all the components z_i of Z are such that

$$\begin{aligned} &z_i = v_i^* - t_k(v_i^* - u_i^*), \\ &z_i \geq v_i^* - t_i(v_i^* - u_i^*) \geq 0 \ \text{ if } \ v_i^* \neq u_i^*, \\ &z_i = v_i^* \geq 0 \ \text{ if } \ v_i^* = u_i^*; \end{aligned}$$

that is, $Z \geq \bar{0}$. Because of property (15) and $(H2)$, we have

$$F(Z) \geq F(\bar{0}) \gg \bar{0}. \tag{20}$$

Now, we set $t = 1/t_k$. Due to the definition of t_k, $0 < t \leq 1$ and $U^* = tZ + (1-t)V^*$. By using $(H3)$ we get $tZ + (1-t)V^* \geq tF(Z) + (1-t)F(V^*)$ and $Z \geq F(Z)$. Combined with (20), this implies

$$Z \gg \bar{0}, \tag{21}$$

which is in contradiction with (19). Therefore, we cannot have $V^* > U^*$, and since $V^* \geq U^*$ we have $U^* = V^*$. Finally, the equality of the two limits U^* and V^* implies the uniqueness of the fixed point in C. Indeed, let Y be a fixed point of F in C. Because of (15) and $(H2)$, we have

$$\lim_{l \to \infty} F^l(\bar{1}) \geq Y \geq \lim_{l \to \infty} F^l(\bar{0});$$

that is, $Y = U^*$.

5.2. Application of Lemma 1 to G

First, since all the coefficients α and β are positive, the functions g_i (11) are continuous in C. For any element X of C, the partial derivatives of g_i are

$$\frac{\partial g_i(X)}{\partial x_h} = g_i^2(X) \sum_{j=1}^{m} \frac{\alpha_{ij}\beta_{hj}}{\left(1 + \sum_{k=1}^{n} \beta_{kj} x_k\right)^2}. \tag{22}$$

Thus, the derivatives are greater than or equal to zero. In addition, a given ligand L_i is assumed to have a non-zero affinity for at least one of the binding sites P_j (if L_i does not bind to any site, then $x_i = 1$ and there is no reason to include L_i in the model). This means that there is at least one index j for which $K_{ij} > 0$. According

to the definition of the coefficients α and β (9), it implies that $\alpha_{ij}\beta_{ij} > 0$ and thus $\partial g_i(X)/(\partial x_i) > 0$ since $g_i(X) > 0$. Hence, $(H1)$ holds for G. Showing that $(H2)$ is also verified is straightforward using the definition of the functions g_i (11). Thus, it just remains to show that $(H3)$ holds. According to the definition of the order relation "$\geq$" (12), inequality $(H3)$ means that, if X and Y are two elements of C such that $Y \geq X$ and $t \in [0; 1]$, then

$$\forall i \in \{1, \cdots, n\},\ g_i((1-t)X + tY) \geq (1-t)g_i(X) + tg_i(Y). \tag{23}$$

To simplify the notations, given two vectors X and Y of C such that $Y \geq X$ and given an index i, we define

$$g(t) = g_i((1-t)X + tY). \tag{24}$$

A proposition equivalent to (23) [5] is

$$g''(t) < 0, \quad \forall t \in [0; 1]. \tag{25}$$

Hence, it only remains to prove that (25) holds. We use the following notations:

$$\begin{aligned} h(t) &= \frac{1}{g(t)}, & h(t) &= 1 + \sum_{j=1}^{m} \frac{\alpha_j}{A_j + B_j t}, \\ A_j &= 1 + \sum_{k=1}^{n} \beta_{kj} x_k, & B_j &= \sum_{k=1}^{n} \beta_{kj}(y_k - x_k), \end{aligned} \tag{26}$$

with $\alpha_j = \alpha_{ij}$. All the coefficients A_j and B_j are positive and we have

$$g''(t) = \frac{2h'^2(t) - h''(t)h(t)}{h^3(t)},$$

$$h'(t) = -\sum_{j=1}^{m} \frac{\alpha_j B_j}{(A_j + B_j t)^2}, \quad h''(t) = 2\sum_{j=1}^{m} \frac{\alpha_j B_j^2}{(A_j + B_j t)^3}.$$

The sign of $g''(t)$ is the sign of $S_m(t) = P_m(t)^2 - Q_m(t)R_m(t)$, where $P(t) = h'(t)$, $Q(t) = h''(t)/2$ and $R(t) = h(t)$. When $m = 1$ the sign of $S(t)$ is easily determined

$$S_1(t) = -\frac{\alpha_1 B_1^2}{(A_1 + B_1 t)^3} < 0.$$

It is now assumed that there is an integer m for which

$$S_m(t) < 0. \tag{27}$$

For the sake of convenience, we drop the indices m and i as well as the parenthesis indicating a function of t. We have

$$P < \sqrt{Q}\sqrt{R}, \tag{28}$$

and

$$S_{m+1}(t) = \left(P + \frac{\alpha B}{(A+Bt)^3}\right)^2 - \left(Q + \frac{\alpha B^2}{(A+Bt)^3}\right)\left(R + \frac{\alpha}{A+Bt}\right),$$
$$S_{m+1}(t) = S_m(t) + \frac{2P\alpha B}{(A+Bt)^2} - \frac{Q\alpha}{A+Bt} - \frac{R\alpha B^2}{(A+Bt)^3}.$$

Using (27) and (28) leads to

$$S_{m+1}(t) < \frac{1}{(A+Bt)^3}\left\{2\sqrt{Q}\sqrt{R}\alpha B(A+Bt) - Q\alpha(A+Bt)^2 - R\alpha B^2\right\}.$$

Now, setting $a = \sqrt{R}\sqrt{\alpha}B$ and $b = \sqrt{Q}\sqrt{\alpha}(A+Bt)$, we obtain

$$S_{m+1}(t) < \frac{-(a-b)^2}{(A+Bt)^3} < 0.$$

Thus, if $S_m(t) < 0$, then $S_{m+1}(t) < 0$. As $S_1(t) < 0$, $S_m(t)$ is negative for all integers m. Consequently, (25) holds and G fulfills $(H3)$. Since $(H1)$ and $(H2)$ have been shown to hold, Lemma 1 can be applied to G and Theorem 1 is proved.

5.3. Proof of theorem 2

Let X^* be the unique fixed point of G in C, and (U^l) and (V^l) the sequences generated by G with first terms $\bar{0}$ and $\bar{1}$. We have $\bar{1} \geq X^* \geq \bar{0}$. Because of $(H2)$ and (16) it implies that $\bar{1} \gg X^* \gg \bar{0}$. Using property (16) l times gives $V^l \gg X^* \gg U^l$. In addition, because G satisfies $(H2)$ we have $\bar{1} \gg G(\bar{1})$ and $G(\bar{0}) \gg \bar{0}$. Again, using property (16) $l+1$ times leads to $\bar{1} \gg V^l \gg V^{l+1}$ and $U^l \gg U^{l+1} \gg \bar{0}$. Thus, (13) holds.

Let X^0 be an element of C, and X^l the term of rank l of the sequence generated by G with X^0 as a first term. We have $\bar{1} \geq X^0 \geq \bar{0}$ and thus, because of $(H2)$ and (16), $\bar{1} \gg X^1 \gg \bar{0}$. Using then (16) gives $V^l \gg X^{l+1} \gg U^l$, which combined to (13) finally leads to (14).

6. Computation of thyroid hormone levels

We used the sequence generated by the function G to determine the concentrations of free thyroid hormones (T4, T3 and rT3) in the human serum. In this case $n = 3$ and $m = 5$. The binding affinities and total concentrations are taken from [4] and presented in Tables 1 and 2. The total concentrations are given for two different types of sera: sera with normal level of TBG and sera of subjects with congenitally low TBG levels. For both sets of parameters, when initializing the sequence with either $X_0 = (0,0,0)^T$ or $X_0 = (1,1,1)^T$, six iterations were sufficient to obtain an estimation of the solution X^* with an error of less than 10^{-6} for each component. The results are presented in Figures 1A and 1B. Although the concentrations of free hormones are very similar for both types of sera, the concentrations of bound hormones are very different. To obtain those concentrations we used the estimated solution X^* and equations (2) and (6). Figures 1C and 1D show that in the normal serum the majority of T4 is bound to TBG; whereas in the low TBG serum, most of T4 is bound to prealbumin.

Table 1. Affinity constants of T4, T3 and rT3 for the serum binding proteins.

Protein	Binding site	T4	T3	rT3
TBG	1	1.0×10^{10}	4.6×10^{8}	3.1×10^{8}
PA	1	2.2×10^{8}	1.8×10^{7}	1.1×10^{7}
	2	3.5×10^{6}	8.8×10^{5}	3.0×10^{5}
Alb	1	7.0×10^{5}	1.2×10^{5}	7.0×10^{5}
	2–6	4.8×10^{4}	8.0×10^{3}	4.8×10^{4}

The values are given in M^{-1}. TBG, thyroxine binding globulin; PA, prealbumin; Alb, serum albumin; T4, thyroxine; T3, triiodothyronine; rT3, reverse T3. Values from Blomberg and Engblom [4].

Table 2. Concentrations of binding proteins, T4, T3 and rT3.

		Normal serum	Low TBG serum
Protein	TBG	340 nM	3.4 nM
	PA	5.0 μM	5.0 μM
	Alb	640 μM	640 μM
Ligand	T4	100 nM	30 nM
	T3	2 nM	1.2 nM
	rT3	500 pM	400 pM

Values from Blomberg and Engblom [4].

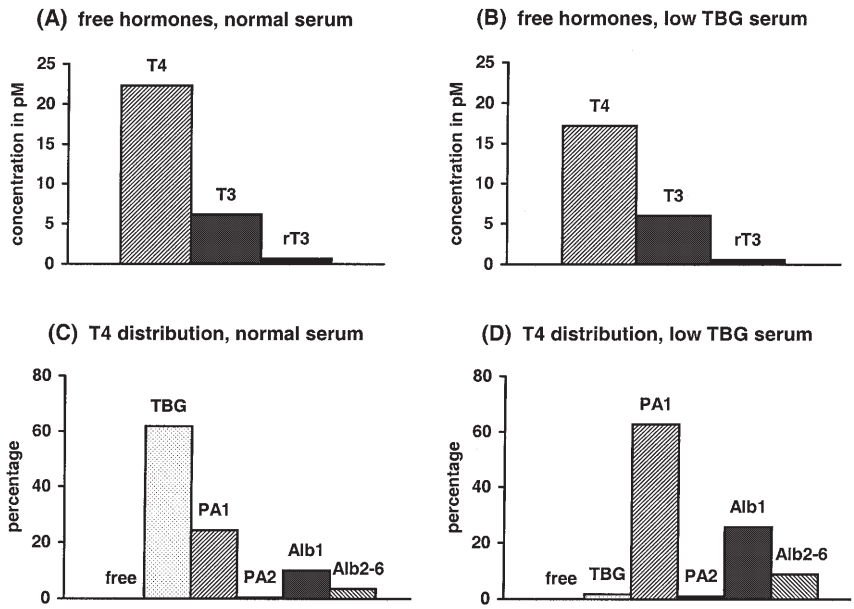

Fig. 1. Concentrations of free thyroid hormones in a normal serum (A) and in a low TBG serum (B). Distribution of free and bound T4 in the case of a normal serum (C) and for a low TBG serum (D).

Acknowledgements. This work was supported by the U.S. Department of Energy. The authors thank Michael Maurice for his comments on the manuscript.

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