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Mathematical Model of Clonal Selection and Antibody Production

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A mathematical model is developed for predicting the cellular proliferation and production of circulating antibodies that takes place when an adult animal is injected with an antigen. A clonal selection theory is used, in which it is assumed that certain target cells are capable of being stimulated by antigen into a proliferating and antibody producing state. The proliferating cells increase in number until the free antigen concentration reaches a low level, whereupon they divide asymmetrically to become plasma cells and memory cells. The plasma cells are terminal antibody producing cells while memory cells become identical to target cells. It is assumed that the target and proliferating cells carry antibody-like surface receptors which are capable of combining reversibly with antigen and that the average number of bound receptors determines the mitotic activity of a cell. The binding of a receptor site to antigen is taken to be characterized by an intrinsic association constant and the state of the binding is calculated, at any time, for univalent antigen interacting with bivalent antibodies and multivalent cells. Heterogeneous antibodies are allowed by having many groups of cells which differ in their affinity for antigen and which produce correspondingly differing antibodies. The rates of change of the various cell populations, antibody and antigen concentrations, are taken to be governed by certain differential equations that embody the biological assumptions of the model. In the model, the level of free antigen selects certain groups of cells, according to their affinity for antigen, for proliferation; the resulting antibody population reflects this proliferation together with the rapid elimination of bound antigen–antibody complexes. A mechanism for inducing high dose tolerance is included in the model and a mechanism for low dose tolerance is suggested, based on depletion of the target cells by repeated production of small clones.

The system of equations is solved numerically by a digital computer program and results have been compared with many experiments in which 2,4 dinitrophenyl-bovine γ-globulin was used as antigen in rabbits. Effective association constants and concentrations of antibodies have been compared with experiments and an encouraging degree of agreement is found. It is indicated that in some experiments, the antibody pro-

ducing system was probably overloaded. Some possible applications of the model to planning and analysis of experiments are discussed. By studying the time dependence of antibody concentration and affinity, following injection of antigen, one can deduce some characteristics of the target cells and their origin. Moreover, if enough is known about the target cells it may be possible to tailor exposure to antigen so as to achieve special effects, such as high dose tolerance, low dose tolerance, or the production of homogeneous antibodies. Experiments which studied quantitatively the interaction of the receptor sites of target or proliferating cells with antigen would be of great help in confirming or denying this model.

1. Introduction

In recent years, considerable progress has been made in understanding the nature of the immune response and in clarifying mechanisms of antibody production (see, for example, Davis, Dulbecco, Eisen, Ginsberg & Wood, 1967; Cold Spring Harbor Symposium, 1967). Although several fundamental questions remain unanswered, such as how many different kinds of cells are required for initiating a primary response (e.g. Gottlieb, Glisin & Doty, 1967; Richter, 1969; Talmage, Radovich & Hemmingsen, 1969 and other references therein), there is nevertheless a fairly widespread acceptance of some general mechanisms of antibody production based on the clonal selection hypothesis of Jerne (1955) and Burnett (1959). The intent of the present paper is to formulate these ideas as a quantitative mathematical model which can be used for prediction and analysis of experiments on the kinetics of antibody production, i.e. the development in time of cell and antibody concentrations following exposure of the system to antigen.

It may be helpful to sketch some of the salient experimental facts which I have in mind in constructing the mathematical model. These have been well summarized in Davis *et al.* (1967). When any one of a vast number of foreign substances, called an *antigen* or immunogen, is injected into an adult mammal (or other vertebrate), the animal may respond by production of antibodies, which are proteins that are capable of specifically combining with the antigen and often of hastening its elimination from the animal. The quantitative response is affected in many ways by the manner in which the antigen is administered. If the antigen is first presented to the animal slowly, for example, when injected together with a substance, called an adjuvant, which slows down the assimilation of the antigen, then the response is usually more vigorous than if the antigen is presented all at once, for example, by intravenous injection. When an antigen is *first* presented to an animal, the (*primary*) response may be rather slow so that a week or more may elapse before the corresponding circulating antibodies can be detected. Upon subsequent exposure of the animal to the same antigen, the (*secondary*)

response is much more rapid and vigorous; this enhanced secondary response forms, of course, the basis for immunization procedures. If the animal is presented with a sudden and very large dose of antigen it may not respond but instead become *tolerant* to the antigen, failing to respond to subsequent exposures to the antigen.

The antibodies formed in response to a particular antigen are exceedingly specific in that they may interact only weakly with an almost identical antigen. Nevertheless, the antibodies formed in response to even a simple antigen are heterogeneous and exhibit a large range of association constants for binding with the antigen. There is a tendency for the antibodies produced after prolonged or secondary exposure to an antigen to have much higher association constants than those produced at first.

Unfortunately, most of the experimental information on antibody production is qualitative rather than quantitative in nature. There are, however, some more or less quantitative experimental data on responses to simple antigens and comparison will be made with some of these in section 4.

The experimental facts may be interpreted according to a clonal selection model (Davis *et al.*, 1967; Jerne, 1955; Burnett, 1959) as follows. It is assumed that there exists in the adult animal a population of target cells which are capable of recognizing antigen and thereby being stimulated to proliferate and produce antibodies. Of the total population of target cells, only a small fraction [perhaps 10^{-5}, Jerne (1967)] may be able to respond to a particular simple antigen. A particular target cell will produce a clone of cells making homogeneous antibody. Thus, the antigen selects certain clones of cells for proliferation and heterogeneity of antibodies reflects the heterogeneity of target cells which are selected for clonal proliferation. Some of the cells resulting from the clonal proliferation are similar to target cells and form an immunological memory, so that upon subsequent exposure of the animal to the antigen, more target cells are available to give a quicker response.

In this paper a mathematical model will be developed for predicting the response of an adult animal to a particular antigen, through the production of humoral or circulating antibodies. I will attempt throughout to keep the model as simple as possible and to use assumptions which are fairly conventional in the clonal selection theory. However, if the model were too simple, it would be difficult to relate to many experiments. Therefore, I have, for example, allowed for a heterogeneity of antibodies and several cell types in the model; these complications are not logically required but seem important for making comparisons with experiment.

The qualitative features of the model are discussed in the following few paragraphs. In section 2, the model is formulated quantitatively for a single

population of target cells producing homogeneous antibody. It will be seen that a number of rather arbitrary assumptions must be made regarding parameters and functions which appear in the model; in principle, they can all be determined by experiment but at present they are estimates at best. In section 3 the straightforward generalizations to heterogeneous target cells are worked out. In section 4 the response of the model is examined to a variety of exposures to antigen and comparisons are made with experiment. In general, qualitative and sometimes quantitative agreement is found between the model and experiment. Finally, in section 5, the results and interpretations are discussed.

The four types of cells which are included in the model are indicated in Fig. 1 (see also Fig. 15–14 of Davis *et al.*, 1967). First of all, there are *target cells*, that is cells which are present before the introduction of antigen

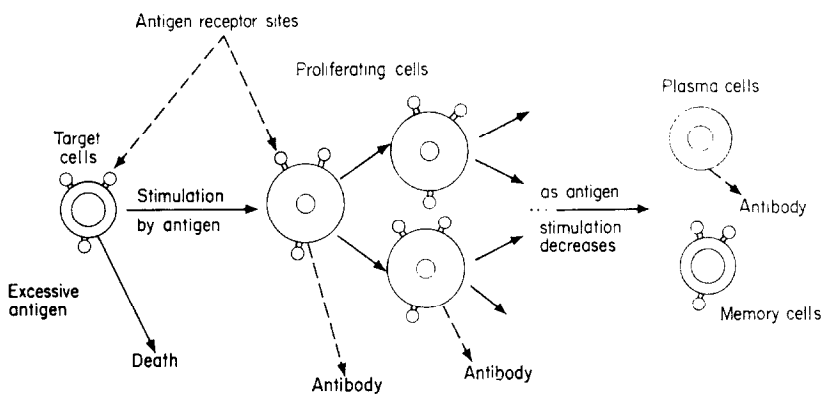

FIG. 1. Cells in the model.

and which are capable of being stimulated by antigen and thus becoming *proliferating cells*. I have in mind that the target cells are probably small lymphocytes that are well known to be transformed into a proliferating state by various chemicals, Ling (1968). It is assumed that the combination of antigen and target cell is sufficient to initiate the proliferation. In fact, there is evidence (e.g. Gottlieb *et al.*, 1967; Richter, 1969; Talmage *et al.*, 1969 and other references therein) that further cell types such as macrophages may sometimes be required to process the antigen or in some way initiate the response. Such complications, which are not clearly understood at present, are not considered in the present model.

I assume that in the presence of sufficient antigen the proliferating cells continue to multiply and to produce antibody. The combination of antibody

with antigen hastens the elimination of antigen and when the antigen concentration has fallen to a low level the cells cease proliferating and divide asymmetrically to become *plasma cells* and *memory cells*. The plasma cells are assumed to be terminal cells which produce antibody but are incapable of further division; they are not very important in the model but apparently exist (Davis *et al.*, 1967). The memory cells are assumed to be similar to target cells, i.e. they produce no antibodies but are capable of being stimulated by antigen. In most of the work to be reported here, memory cells have been assumed to be functionally identical to target cells.

I assume that the target cells, proliferating cells and memory cells carry on their surfaces specific receptors which are similar to antibody molecules and can combine specifically and reversibly with antigen. The number of receptors which are bound to antigen then determines the activity of the cell, in particular whether a target cell will be stimulated to become a proliferating cell and whether a proliferating cell will divide to produce more proliferating cells or to produce memory and plasma cells. Such surface receptors were suggested 70 years ago by Paul Ehrlich (1900). More recently, various experiments (e.g. Mitchison, 1967; Plotz, 1969) have been interpreted as confirming the existence of surface receptors. In the present model, the interaction of surface receptors and antigen plays the essential role whereby cells recognize antigen. The binding of antigen to receptors turns on the immune response while a lack of binding (due to a low level of free antigen) turns it off. It is further postulated that a large amount of antigen, which binds a large fraction of the receptor sites can lead to death (or at least non-functioning) of the target cells thereby producing tolerance to the antigen.

I assume that simple considerations of chemical equilibrium can be used to deduce the extent of binding between antigen molecules and receptors and between antigen molecules and antibody molecules. Moreover, the model is developed only for univalent molecules, i.e. antigen having a single combining site or antigenic determinant per "molecule." In fact, most antigens have many binding sites and a theory of their association with bivalent antibodies and with cells containing many surface receptors would be very complicated. Even a theory of binding between just antibodies and multivalent antigen is complicated, Goldberg (1952). In the present model the association of univalent antigen with bivalent antibodies and multivalent cells is treated. When comparison is made with experiments, an effective number of independent antigenic sites is postulated to describe the experimental antigen. This assumption presumably means that the model will be more directly applicable to small and simple antigens than to large and complicated ones such as foreign cells. For example, a large antigen might combine with several

receptors on the same target cell, an effect not considered in the present model.

In this model, the "animal" or its immunological system is treated as a fixed volume within which all concentrations are uniform. In principle there would be no difficulty in introducing a few compartments and labeling them spleen, blood, lymph, etc., but such complications seem hardly warranted in this initial study.

2. Model for Homogeneous Antibody

In this section I will develop the notation and the equations for a case in which all target cells are taken to be equivalent and in which all antibody molecules are identical. It will be seen in section 3 that the more general and interesting case of heterogeneous target cells and antibodies, can be obtained by simply modifying the notation and a few of the equations of this section.

The four kinds of cells in the model are those indicated in Fig. 1. The target cells, proliferating cells, and memory cells are assumed to have sites on their surfaces (receptor sites) which are capable of combining with antigen. The interaction between antigen and a receptor site is characterized by an intrinsic association constant (or affinity) and the division of a cell is assumed to be governed by the number of sites which are occupied. In particular, a target cell will be transformed into a proliferating cell if one or a few sites are occupied but will be killed if most sites are occupied. A proliferating cell will continue dividing symmetrically until it has no or few receptor sites occupied whereupon it will divide into a plasma cell and a memory cell. Both proliferating and target cells are assumed to produce the same kind of antibody. The interaction between antigen and antibody is also governed by an intrinsic association constant. Equilibrium conditions will be worked out for the interaction of univalent antigen with bivalent antibodies and multivalent cells.

The following notation is employed. The units given are those used in the numerical calculations.

t = time (hr)
$N_1(t)$ = number of target cells per unit volume (M)
$N_2(t)$ = number of proliferating cells per unit volume (M)
$N_3(t)$ = number of plasma cells per unit volume (M)
$N_4(t)$ = number of memory cells per unit volume (M)
$N_5(t) \equiv Ab(t)$ = concentration of bivalent antibodies, including free antibodies and those bound to antigen (M)

$N_6(t) \equiv Ag(t)$ = concentration of univalent antigen (or antigen sites), including free antigen and that bound to antibody and to receptor sites (M)

L = concentration of free antigen ($\leq Ag$)

S = concentration of free sites on antibodies ($\leq 2AB$)

$[SL]$ = concentration of antibody sites bound to antigen

S' = concentration of free receptor sites

$[S'L]$ = concentration of receptor sites bound to antigen

m = number of receptor sites per target, proliferating, or memory cell (10^3)

r = fraction of antibody sites occupied ($= [SL]/2Ab$)

r' = fraction of receptor sites occupied [$= [S'L]/m(N_1+N_2+N_4)$]

R' = average *number* of occupied receptor sites per cell ($= mr'$)

R = concentration of receptor sites [$= m(N_1+N_2\times N_4)$]

k = intrinsic association constant for antigen–antibody interaction (l./mole).

k' = intrinsic association constant for antigen-receptor site interaction (l./mole)

$c2$ = rate of antibody production per proliferating cell (h^{-1})

$c3$ = rate of antibody production per plasma cell (h^{-1})

T_1 = mean time for optimally stimulated target cell to become a proliferating cell (hr)

T_2 = mean time for proliferating cell to divide

T_2' = mean time for removal or death of proliferating cell

T_3 = mean time for removal or death of plasma cell

T_4 = mean time for removal or death of memory cell

T_5 = mean time for removal of antigen combined with antibody

T_5' = mean time for removal of free antibody

T_6 = mean time for removal of free antigen

Allowance is made for possible sources of antigen, antibody and target cells as follows.

source $1(t)$ = source of target cells, if any (for example, due to differentiation of stem cells)

source $5(t)$ = source of injected antibodies, if any (injected antibodies assumed the same as produced antibodies)

source $6(t)$ = source of injected antigen (antigen can be presented to the system as an initial condition or as a source)

At any time, t, it is assumed that there is chemical equilibrium between antigen and antibody and receptor sites. The intrinsic association constants,

k and k', are defined by

$$[SL] = kS \times L$$
$$[S'L] = k'S' \times L$$

In addition

$$2Ab = S + [SL] \quad (= \text{concentration of antibody sites})$$
$$R = S' + [S'L] \quad (= \text{concentration of receptor sites})$$
$$Ag = L + [SL] + [S'L] \quad (= \text{concentration of antigen})$$

These five equations may be solved for L in terms of k, k', Ab, Ag, and R. It is thus found that L is the unique positive root of the equation

$$Ag = L\left(1 + \frac{2kAb}{1+kL} + \frac{k'R}{1+k'L}\right). \tag{1}$$

The existence of such a root follows from the fact that the right-hand side of equation (1), $f(L)$, is a monotone increasing function of L. Also $f(0) = 0 \leq Ag$ while $f(Ag) \geq Ag$ so that the root lies in the range $0 \leq L \leq Ag$, as expected.

Once L has been found, the fractions r and r' of occupied antibody and receptor sites can be found. Thus,

$$r = \frac{kL}{1+kL} \tag{2}$$

$$r' = \frac{k'L}{1+k'L} \qquad R' = mr'. \tag{3}$$

The activities of the various cell types are then governed by r' and R'.

It is assumed that the concentrations of cells, antigen and antibodies are governed by ordinary differential equations. A formulation will first be considered in which memory cells are treated as inert; an appropriate modification to treat them as identical with target cells will be noted shortly. The population of target cells will then be depleted as target cells are stimulated by antigen and transformed into proliferating cells (or killed); target cells may possibly be introduced by the source 1. It is assumed that

$$\frac{dN_1}{dt} = -FN_1/T_1 + \text{source } 1(t) \tag{4}$$

where F is a function of R' such that $F = 0$ when $R' = 0$, i.e. no receptor sites are occupied, and $F \simeq 1{\cdot}0$ when many sites are occupied. A simple choice used in all the following work is $F = R'/(1+R')$. Roughly speaking, this choice implies that when a target cell has one or more receptor sites occupied it has a good chance of being transformed in a time T_1.

The population, N_2, of proliferating cells will increase as target cells are transformed, at a rate GN_1/T_1. The function G is equal to F times the

fraction of cells that are induced to proliferate rather than to die. In order to obtain the possibility of producing tolerance to an antigen, I will assume that most target cells are killed when $r' \simeq 1$, i.e. when nearly all receptor sites are occupied. On the other hand I assume that few of the target cells will be killed if $r' \ll 1$. A simple choice is to let $(1-r')$ be the fraction of target cells which upon transformation, proliferate rather than die. Then $G = (1-r')F$ and this form is assumed in the model.

In addition, the division of proliferating cells will lead to changes in the population. Let the fraction of cells that still proliferate following division be $[1+H(R')]/2$; the fraction that became plasma cells and memory cells is then $[1-H(R')]/2$. Thus, when a proliferating cell divides there results on the average $1+H(R')$ proliferating cells and $1-H(R')$ other cells. The net gain or loss of proliferating cells is $H(R')$. In what follows it is assumed that $H = (R'-1)/(R'+1)$ so that when many sites are occupied and $R' \gg 1$, then $H \simeq 1$ and most divisions lead to proliferating cells, while for $R' \ll 1$, $H \simeq -1$ and most divisions lead to plasma and memory cells. Thus,

$$\frac{dN_2}{dt} = GN_1/T_1 + HN_2/T_2 - N_2/T_2'. \tag{5}$$

The natural lifetime, T_2', of a proliferating cell will be assumed to be long and hence the term N_2/T_2' is of little importance in equation (5). Thus, almost always a proliferating cell divides with the lifetime T_2 to give $1+H$ new proliferating cells.

The division of proliferating cells will serve as the source of plasma and memory cells. If equal numbers of the latter are produced

$$\frac{dN_3}{dt} = \frac{1-H}{2} N_2/T_2 - N_3/T_3 \tag{6}$$

$$\frac{dN_4}{dt} = \frac{1-H}{2} N_2/T_2' - N_4/T_4. \tag{7}$$

In equation (7), memory cells do not interact with antigen. When it is desired to treat them as identical to target cells, this is accomplished by adding the source of memory cells $(1-H)N_2/2T_2$ to the right side of equation (4) thereby putting the memory cells directly into the target cell population. In addition, equation (7) is retained and thereby a record is kept of how many memory cells have been made.

The differential equation for N_5, the concentration of antibodies, will have three source terms, namely the production rate by proliferating cells ($c2N_2$), the production rate by plasma cells ($c3N_3$) and the external source, if any [source $5(t)$]. An antibody which is bound to antigen is assumed to have a lifetime T_5. If r is the fraction of antibody sites that are bound to antigen,

then for bivalent antibody the fraction of antibody molecules that have either one or two independent sites occupied is $2r-r^2$. This may be seen as follows: Let p_i be the probability that i sites are occupied. Then $p_0 = (1-r)^2$, $p_1 = 2r(1-r)$ and $p_2 = r^2$. The probability that either one or two sites are occupied is then $p_1+p_2 = 2r-r^2$. Assuming that this bound fraction of the antibody molecules is eliminated with lifetime T_5 while all antibody molecules† are eliminated with the much longer lifetime T_5', the rate of change of antibody concentration is

$$\frac{dN_5}{dt} = c2N_2+c3N_3+\text{source } 5(t)-[(2r-r^2)/T_5+1/T_5']N_5. \tag{8}$$

The rate of change of the antigen concentration, N_6, will be due to the source $6(t)$ and the assumption that all antigen molecules have a lifetime T_6. In addition, antigen molecules bound to antibody are eliminated with a lifetime T_5. The concentration of such antigen molecules may be written as: concentration of (total antigen − free antigen − antigen bound to cell receptors) $= N_6-L-r'R$, and hence

$$\frac{dN_6}{dt} = \text{source } 6(t)-N_6\left(\frac{1}{T_5}+\frac{1}{T_6}\right)+(L+r'R)/T_5. \tag{9}$$

The set of six differential equations (4) to (9) together with the condition of chemical equilibrium (1) and the equations for r, r', R' [equations (2) and (3)], F, G and H represents the mathematical form of our model. In order to obtain solutions, the initial conditions on N_i, $i = 1, 2, \ldots 6$, source functions and constant parameters must be specified. One may then attempt to solve the resulting initial value problem. Since many of the coefficients in the differential equations are functions of r and r', and hence of L, which is determined from the chemical equilibrium equation (1), the differential equations are quite non-linear. Thus, it appears unlikely that analytic solutions can be found to any problems of interest and numerical solutions have been sought.

A computer program, ABIGAIL 1, has been written to solve the above system of equations on the Los Alamos Computer, "MANIAC II." The differential equations are solved by a second order Runge–Kutta method, Abramowitz & Stegun (1965), while the root of equation (1) is found by a secant method. The results of the calculation, $N_i(t)$, may be regarded as representing the response of the system to a prescribed exposure to antigen.

† This use of the lifetimes T_5 and T_6 [in equation (9)] is not strictly consistent with the earlier definitions, where they were applied only to free antibodies and free antigen, respectively. With the present use, bound antibodies are eliminated with a lifetime $T_5T_5'/(T_5+T_5')$ and bound antigen with a lifetime $T_5T_6/(T_5+T_6)$. However, since $T_5' \gg T_5$ and $T_6 \gg T_5$, the net effect on elimination rates is very slight.

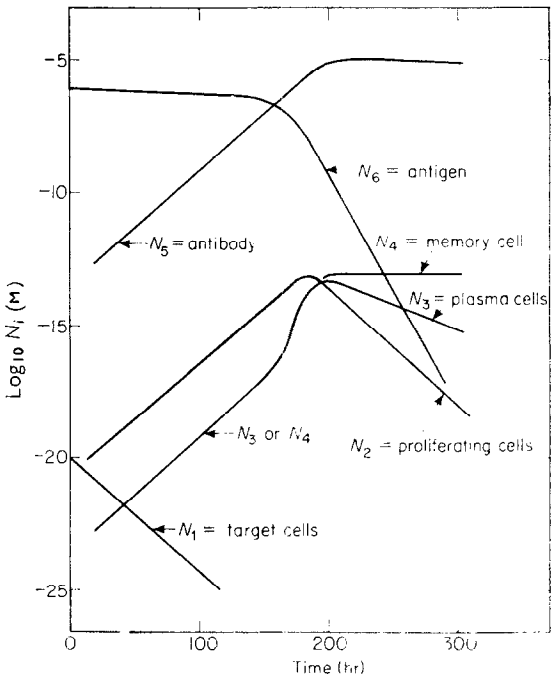

FIG. 2. Response of a system having an initial target cell population of $6000\,l^{-1}$, and an initial antigen concentration of 10^{-6} M. Antibodies are homogeneous and memory cells are inert. See text for further details.

An illustrative example is shown in Fig. 2. In this problem, all sources were set equal to zero and the antigen was presented as an initial condition. The initial concentration of target cells was $6000\,l^{-1}$ and the initial concentration of antigen was $6 \times 10^{17}\,l^{-1}$; hence $N_1(0) = 10^{-20}$ M, $N_6(0) = 10^{-6}$ M. The initial concentrations of proliferating, plasma, and memory cells were zero as was the initial concentration of antibody. Values for the various constants will be discussed in section 4; for this problem the following values were used ($k = k' = 10^6$, $c2 = c3 = 4 \times 10^6$, $m = 10^3$, $T_1 = 10$, $T_2 = 10$, $T_3 = 20$, $T_4 = 10^4$, $T_5 = 5$, $T_6 = 20$, $T_2' = 10^3$, $T_5' = 500$). In this particular example, the memory cells were treated as inert. It is then seen in Fig. 2 that at early times the target cells decrease exponentially with time while the other cell types and antibody concentration increase exponentially. The antigen concentration is unaffected by the antibody until about 150 hr have passed. At that time the antibody concentration is about equal to the antigen concentration. Thereafter, most of the antigen is bound to antibody and rapidly eliminated from the system. Hence, the proliferating cells then divide

to become plasma and memory cells. The final concentration of memory cells is about 10^{-13} or 10^7 times the initial concentration of target cells.

These and other similar results indicate that the response of the system to an injection of antigen is qualitatively reasonable. However, comparisons with experiment will be deferred until section 4 where the more realistic model for heterogeneous antibodies will be used.

3. Model for Heterogeneous Antibodies

In the preceding section it was assumed that all cells had the same association constant or affinity, k', for binding and being stimulated by antigen. Similarly, all antibodies had the same association constant k for binding to antigen sites. The production of heterogeneous antibodies, i.e. antibodies with differing association constants, in response to a single antigen, may be included by dividing the populations of cells and antibodies into a finite number of groups. Let each group have an index $j (j = 1, 2, \ldots J)$ such that all quantities pertaining to that group have the subscript j. Thus, for example, $N_{1,j}$ is the number of target cells per unit volume in group j, etc. It is assumed that the cells in any particular group, j, have the same association constant, k'_j, for binding antigen but that the values of k'_j will differ in the different groups. Similarly, all the antibodies produced by cells in group j have the same association constant, k_j, but the values of k_j will depend on j. It is assumed that a target cell in group j gives rise to other cells and antibodies exclusively in group j. Thus, there is no coupling between the groups *except* through a condition of chemical equilibrium [see equation (10)] whereby the stimulation which any group of cells experiences is affected by all other groups of cells and antibodies. It is also assumed that k'_j is proportional to k_j, in accord with our assumed analogy between receptor sites and antibody molecules.

The equations of section 2 can be readily generalized to allow for a group representation of heterogeneity. First, all cell and antibody populations must be given a subscript j and all quantities depending on binding with antigen must also be subscripted. Thus, for example, r'_j is the fraction of j group cell receptor sites that are bound to antigen. All other quantities, in particular the number of sites per cell, m, the antibody production rates, $c2$ and $c3$, and all lifetimes are, for simplicity, taken independent of the group index j.

The condition of chemical equilibrium, equation (1), is replaced by

$$Ag = L\left[1+2\sum_{j=1}^{J}\frac{k_j N_{5,j}}{1+k_j L}+m\sum_{j=1}^{J}\frac{k'_j(N_{1,j}+N_{2,j})}{1+k'_j L}\right] \tag{10}$$

and L is the unique positive root of this equation ($0 \leq L \leq Ag$). Note that

in this equation, memory cells are not included explicitly in the last sum. Instead they are usually treated as identical to target cells and hence included in $N_{1,j}$, as explained in section 2. Equations (2) and (3) and the assumed forms for F, G and H are generalized by just adding the subscripts j, $(j = 1, 2, \ldots J)$,

$$\begin{aligned} r_j &= k_j L/(1+k_j L) \\ r'_j &= k'_j L/(1+k'_j L) \\ R'_j &= m r'_j \\ F_j &= R'_j/(1+R'_j) \\ G_j &= F_j(1-r'_j) \\ H_j &= (R'_j-1)/(R'_j+1). \end{aligned} \tag{11}$$

Similarly, the differential equations (4) to (9) become, for $j = 1, 2, \ldots J$,

$$\frac{dN_{1,j}}{dt} = -F_j N_{1,j}/T_1 + \text{source } 1_j(t) + \left[\frac{(1-H_j)}{2} N_{2,j}/T_2\right] \tag{12}$$

$$\frac{dN_{2,j}}{dt} = G_j N_{1,j}/T_1 + H_j N_{2,j}/T_2 - N_{2,j}/T'_2 \tag{13}$$

$$\frac{dN_{3,j}}{dt} = \frac{1-H_j}{2} N_{2,j}/T_2 - N_{3,j}/T_3 \tag{14}$$

$$\frac{dN_{4,j}}{dt} = \frac{1-H_j}{2} N_{2,j}/T_2 - N_{4,j}/T_4 \tag{15}$$

$$\frac{dN_{5,j}}{dt} = c2N_{2,j} + c3N_{3,j} + \text{source } 5_j(t) - [(2r_j - r_j^2)/T_5 + 1/T'_5]N_{5,j} \tag{16}$$

$$\frac{dN_6}{dt} = \text{source } 6(t) - N_6/T_6 - \left(N_6 - L - m\sum_{j=1}^{J} r'_j(N_{1,j}+N_{2,j})\right)\Big/ T_5. \tag{17}$$

The last term in equation (12) is an option whereby memory cells are introduced into the target cell population.

These equations have been solved numerically using the second order Runge–Kutta and secant methods mentioned in section 2. The computer program, ABIGAIL 2, does this on the Los Alamos computer, Maniac II. Up to 41 different groups of cells and antibodies are allowed, i.e. $J \leq 41$. For various problems it has been found that the computing time is roughly one minute per 100 time steps and that a few hundred time steps are required to give results accurate to $\sim \pm 20\%$. Such accuracy is regarded as acceptable for these problems and hence the running time is a few minutes per problem.

The range of association constants which are of interest in antigen–antibody interactions is large, from about 10^4 to 10^9 l./mole (Davis *et al.*, 1967, Table 13.1). Therefore, the k_j values are chosen equally spaced on a

logarithmic scale, i.e. k_{j+1} is a constant factor times k_j. In ABIGAIL 2, for $j = 1, 2, \ldots J$,

$$k_j = k1\, b^{[j-(J+1)/2]}$$
$$k'_j = k1'\, b^{[j-(J+1)/2]} \tag{18}$$

where $k1$ and $k1'$ are the association constants for the middle group $[j = (J+1)/2]$ of antibodies and cells, respectively, and b is the constant factor by which successive k values differ. In most problems b has been taken to be 2·0 so that around 17 groups are required to span the range

$$10^4 \leqslant k_j \leqslant 10^9.$$

The basic quantities which are computed and printed by ABIGAIL 2 are the populations of cells, antibodies and antigen. The concentrations of free antigen (i.e. L) and free antibodies are also computed. In addition, some quantities are computed to characterize the average binding of antibodies (and free antibodies) with antigen and of cells with antigen. Since the values of k_j are uniformly spaced on a logarithmic scale, the average logarithm of k_j is first computed,

$$\overline{\log k} = \sum_{j=1}^{J} N_{5,j} \log k_j \Big/ \sum_{j=1}^{J} N_{5,j}$$

and the quantity $\bar{K}$ is defined by

$$\bar{K} = \exp(\overline{\log k}).$$

This value of $\bar{K}$ is very similar to the experimentally measured "average association constant" as determined, for example, from a Sips plot (cf. Davis *et al.*, 1967, Chap. 13; also section 4 of this paper). The analogous quantities $\overline{K \text{ free}}$ and $\bar{K}'$ are determined for the free antibodies and for the proliferating plus target cells. If an average value of k had been directly computed, it would have been unduly affected by those groups having the very highest values of k_j. Finally, variances and other quantities are obtained. For example, "$\sigma^2/2$" is the variance in the antibody $\log k$ distribution, or more precisely,

$$\frac{(\sigma)^2}{2} = \sum_{j=1}^{J} N_{5,j}(\log k_j - \overline{\log k})^2 \Big/ \sum_{j=1}^{J} N_{5,j}.$$

The possibility of achieving tolerance to an antigen is achieved, in this model, by the complete destruction of clones, as follows. If, at any time, the *number* of target cells in a group becomes less than unity, it is set equal to zero. Moreover, if in a group, the number of target cells which would be transformed to proliferating cells during the time T_1 is less than unity, then the source of proliferating cells in that group is set to zero. It is thus possible to eliminate *all* the cells in certain groups and not merely the fraction

$G_j/F_j = 1-r_j$ which would be naturally lost in passing from the target to the proliferating population. If the experimental "animal" has a volume of V liters, then one cell corresponds to a concentration $N_{\min} = (6\times 10^{23}\ V)^{-1}$ M and $N_{1,j}$ is set to zero if $N_{1,j} \leq N_{\min}$.

4. Calculational Results and Comparison with Experiments

Some of the most careful experiments in the immunological literature have been performed by Herman Eisen and colleagues (Eisen & Siskind, 1964; Steiner & Eisen, 1967; Eisen, Little, Steiner, Simms & Gray, 1969). In particular, they have measured antibody–antigen association constants for a fairly simple antigen under a variety of conditions. In this section I will present some results of calculations using the model described in section 3 and the computer program ABIGAIL 2, and, where possible, comparison will be made with these experiments.

The antigen used by Eisen was bovine γ-globulin to which, 2,4-dinitrophenyl (DNP) groups had been attached. There were about 40 DNP groups per bovine γ-globulin molecule and the antibodies directed against these specific groups were detected and analyzed. Thus, we must face at the outset the problem that while our model was developed for univalent antigen, the experimental antigen has up to 40 binding sites per molecule. In what follows, I have somewhat arbitrarily assumed that one antigen molecule is equivalent to 10 DNP sites, with the following arguments in mind. First of all, the concentration of DNP groups will affect the binding of antigen to cell receptor sites and hence the stimulation of the cells. Usually the concentration of DNP groups is much higher than the concentration of receptor sites so that only a small fraction of the DNP sites are bound to cell receptors, and hence the DNP groups may be assumed to act more or less independently in stimulating the cells. However, not all of the DNP groups are equally effective and perhaps 10 active groups per molecule is a reasonable guess. In addition the number of sites per molecule will affect the ratio of antigen molecules to antibody molecules which are removed as bound complexes from the system. With 10 sites per antigen molecule and two per antibody, the ratio of antibody to antigen molecules removed would be roughly five which is in accord with observed precipitation ratios for antigens with similar molecular weight ($\sim 1{\cdot}5\times 10^5$) (Davis *et al.*, 1967, Table 13.4).

The experimental animals were rabbits which are assumed to have a volume of 0·2 l. Hence, if 1 mg of antigen is in the animal, the concentration of sites is

$$N_6 = \frac{10^{-3}\ \text{g} \times 10\ \text{sites/molecule}}{1{\cdot}5\times 10^5\ \text{g/mole} \times 0{\cdot}2\ \text{l.}} = 3\times 10^{-7}\ (\text{M}).$$

Antibody production rates for fully stimulated cells have been estimated to be about 2×10^3 sec^{-1} (Jerne, 1967). Hence, I assume $c2 = c3 = 7 \times 10^6$ hr^{-1}.

The times T_1, the mean life for transformation of a fully stimulated target cell, and T_2, the mean life time for division of a proliferating cell have both been assumed to be 10 hr. Such a value of T_2 can lead to a doubling time for proliferating cells, and antibodies, as small as 7 hr. This seems about as small a doubling time as has been observed for the antibodies produced in response to any antigen (Davis *et al.*, 1967. Chap. 14), and is perhaps too small for some cases (cf. below). The mean life, T_3 of a plasma cell has been taken to be 50 hr (Ehrich, 1965), while the lifetimes T_2' and T_4 have been assumed so long (10^6 hr) as to be unimportant in the problems. The mean life, T_6, of free antigen is taken to be 200 hr (Davis *et al.*, 1967, Table 15.5) and for the bound antigen–antibody combination to be 10 hr (Davis *et al.*, 1967, Fig. 15.9). The mean life, T_5', of unbound antibody is taken to be 200 hr in the rabbit (Davis *et al.*, 1967, p. 462).

The number, m, of receptor sites per target cell is assumed to be 10^3, this arbitrary choice being based on the feeling that the cell surface is somewhat periodic so that what is found once will be found many times.

We have now fixed all the parameters required to specify a problem except for those which determine the antigen dose and target cell population. Various combinations of the latter have been examined. In particular, antigen will be presented to the animal either as an initial condition (as perhaps when injected intravenously) and/or as a source lasting hundreds of hours (as perhaps when given with an adjuvant). The first population of target cells to be considered, called the "standard population", contains 10^3 target cells in 17 groups ($J = 17$) covering the range in k from $k_1 = 3{\cdot}91 \times 10^3$ to $k_{17} = 2{\cdot}56 \times 10^8$ l/mole. The central group has $k_9 = 10^6$, and the population is normally distributed in j such that there are about three cells in each of the two extreme groups. This population was arrived at from the following arguments. From Eisen & Siskind (1964) it is seen that when $\bar{K}$ is of the order of 10^8, there are large fluctuations in results obtained with different rabbits. One explanation would be that there are few target cells having such high association constants; hence, the standard population is cut off around $k = 3 \times 10^8$. In many of the animals, k values around 10^5 to 10^6 were reported; hence, many target cells are included in this range. Finally, the choice of 10^3 target cells is similar to that suggested by several authors (cf. Jerne, 1967). In all calculations it has been assumed that $k_j = k_j'$.

For convenience, parameters used in the following calculations are listed in Table 1.

TABLE 1

Standard parameters used in ABIGAIL 2 calculations

$$c2 = c3 = 7 \times 10^6 \text{ hr}^{-1} \qquad m = 10^3$$
$$T_1 = 10, \quad T_2 = 10, \quad T_3 = 50, \quad T_4 = 10^6, \quad T_5 = 10, \quad T_6 = 100$$
$$T_2' = 10^6, \quad T_5' = 200, \quad N_{\min} = 10^{-23}$$

(A) VARIATION OF RESPONSE WITH ANTIGEN DOSE

A series of problems will now be considered in which the response of a fixed system to varying doses of antigen is examined. In all cases, memory cells are treated as identical to target cells. It will be seen that the response is quite different depending on whether the antigen is given as an initial condition (intravenously) or over a long period of time (with adjuvant). Let us first consider the response to a sudden exposure to antigen.

In a series of eight problems, antigen was imposed as an initial concentration which ranged from 10^{-4} to 10^{-11} moles of sites/l; hence, from 300 mg to 0·03 μg injected dose of antigen. The results are summarized in Table 2(a). In all problems, the production of antibody and the proliferation of cells are stimulated, although in the final problem (dose of 0·03 μg) the response is so weak as to scarcely affect the elimination of antigen. The time dependence of various quantities is shown in Fig. 3 for a typical problem, in which the dose is 3 mg. Throughout, N_i represents a sum of $N_{i,j}$ over all j groups, e.g.

$$N_1 = \sum_{j=1}^{J} N_{1,j}.$$

In all problems, the concentration of antigen decreases continuously with time, slowly at first with the lifetime $T_6 = 100$ hr. Due to the clonal proliferation of cells, the concentration of antibody (and the concentration of free antibody) increases with time more or less exponentially. When the concentration of antibody becomes comparable with that of antigen, i.e. $Ag \simeq Ab$, then antibody begins to bind much of the antigen and hasten its elimination from the system with the lifetime $T_5 = 10$ hr. This will happen unless the concentrations of antigen and antibody are so low that little binding takes place. In the second column of Table 2(a), the time at which $Ab = Ag$ is shown. For the first six problems this time increases with increasing dose simply because it takes longer to make more antibody; the antibody concentration increases (in Fig. 3) about a decade in 23 hr ($23 = T_2 \ln 10$). At the lowest doses, however, as seen in Table 2(a), the time becomes longer again because these low doses only weakly stimulate the proliferation of target cells.

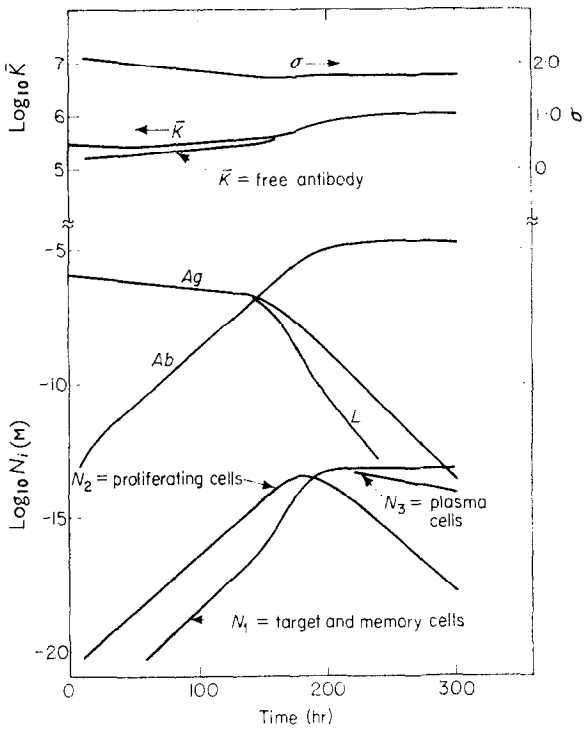

FIG. 3. Calculated concentrations and antibody parameters for a 3 mg dose of antigen presented as an initial condition to the standard target cell distribution. $\bar{K}$ is an effective antigen–antibody association constant for the antibody population and $\sigma^2/2$ is a variance for the antibody population. See text for discussion. Parameters, given in Table 1, have been chosen to represent DNP-bovine γ-globulin as antigen in a rabbit.

In column 3, the value of the effective association constant, $\bar{K}$, when $Ag = Ab$ is shown, and it is seen that the dose of antigen strongly affects the value of $\bar{K}$. The reason is, as will become clear, that the concentration of free antigen selects certain groups of cells for proliferation. A high concentration kills groups of cells having high affinity for antigen and causes those with low affinity to proliferate, while a low concentration selects only cells with high affinity, for proliferation.

A simple estimate can be made of the groups of cells which are selected for rapid proliferation by a free antigen concentration, L. First of all, these will be groups in which a large fraction of the cells are not killed by antigen. Half the cells will be killed when $G_j/F_j = 1/2$, or from equation (11) when $k_j'L = 1$. Hence, in groups for which $k_j'L < 1$, more than half the target cells will survive to possibly become proliferating cells. However, if k_j' is

too small then the rate of proliferation will be slow. From equation (13) it is seen that (for $T_2' \gg T_2$) proliferating cells can increase at the rate H_j/T_2. If it is required that H_j be within about 20% of its maximum value (namely, unity), then from equation (11), $R_j' \geqslant 10$, and $k_j'L \geqslant 10/m = 10^{-2}$. Thus, we may expect that the cells selected for rapid proliferation will have

$$10/m = 10^{-2} \leqslant k_j'L \leqslant 1$$

and hence that $\bar{K}$ will also lie in the range $10^{-2} \leqslant \bar{K}L \leqslant 1$. Referring to Fig. 3 it is seen that L (and Ag) is roughly 4×10^{-7} at early times (before $Ag = Ab$) while $\bar{K}$ is about 3×10^5. Hence $\bar{K}L \approx 10^{-1}$ in agreement with the estimate. For the other problems in Table 2(a), early values of L are proportional to dose and it can be seen that the early values of $\bar{K}$ (e.g. in the third column) satisfy the criterion except for the largest and smallest doses. Thus, for the 0·03 μg dose, there are no groups of cells satisfying the criterion, while for the 0·3 μg and 300 mg doses there are only a few groups. Under these conditions $\bar{K}$ is strongly affected by the availability of target cells as well as the criterion of rapid proliferation.

These problems were terminated at 300 hr, at which time the concentration of free antigen was very low and cellular proliferation had nearly ceased. Final values for antibody concentration, $\bar{K}$, σ and N_1 are indicated in Table 2(a). It should be recalled that in these problems, memory cells are included in N_1 (as functionally identical to target cells); hence, the final cells in N_1 are almost entirely memory cells. They would serve as the target cells in a secondary response. For comparison, note that the initial concentration of target cells was 10^{-20} in all cases. Finally in the last column the number of groups are given in which no cells survive; always the groups with highest k_j' are those in which cells are killed (see Fig. 4).

From Table 2(a), it is seen that the final antibody concentration increases with increasing dose of antigen (though not as fast as linearly) while the final association constant, $\bar{K}$, decreases with increasing dose. The product, $\bar{K} \times Ab$, varies rather slowly with dose. Hence, in making comparison with any experiments on antibody production as a function of antigen dose it is important to know how the antibody assay depends on antibody concentration and how it depends on antibody affinity. I am not aware of quantitative data for the system under present consideration but comparison will be made with measured $\bar{K}$ values in section 4(B). It may be noted that the concentrations and $\bar{K}$ values for free antibody are very similar to those for total antibody; only at earlier times do they differ and then by generally less than a factor of two.

The final values of σ decrease with decreasing dose, except for the lowest dose problem. Since the variance in $\log k$ is $\sigma^2/2$, this indicates that the lower

doses are selecting fewer groups of cells for proliferation (cf. Fig. 4). The reason is that the target cell population contains no cells with $k' > k'_{17} = 2{\cdot}56 \times 10^8$. At the lower doses this restriction of the target cell spectrum cuts off the spectrum of stimulated cells at high affinity and hence reduces σ.

The final concentrations of memory cells are shown in Table 2(a) and their distribution by groups is given in Fig. 4. The killing of high k groups

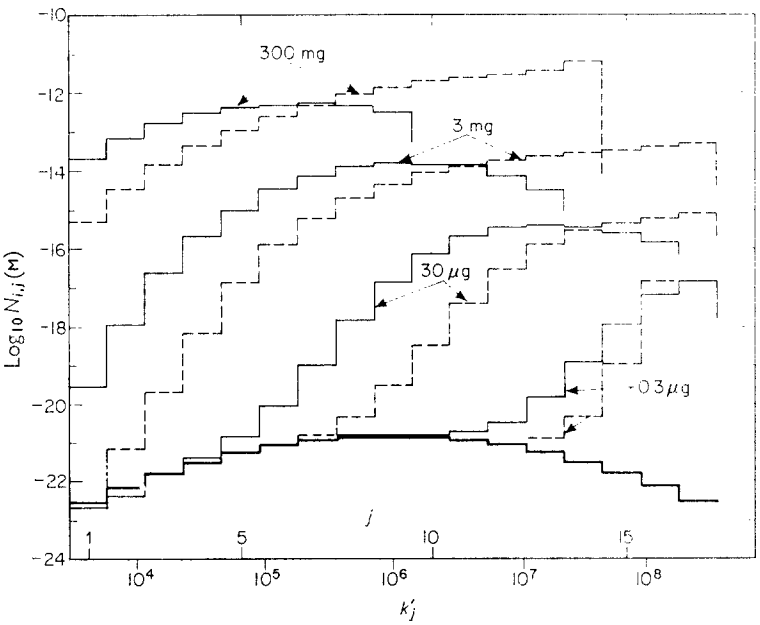

FIG. 4. Affinity spectrum for target and memory cell populations. The concentration of cells per group is shown as a function of the group index, j, and the antigen–receptor association constant or affinity, k'_j. ——, Initial target cell population, same for all curves; ——, final population for rapid presentation of antigen as initial condition; – – –, final population for prolonged (1000 hr) source of antigen.

and selective proliferation of other groups is clearly seen in this figure. In all cases, the final distribution of memory cells is quite different from the initial distribution of target cells. Even for the lowest dose (0·03 μg) a marked distortion of the target cell distribution takes place in that the number of memory cells produced in the highest affinity group exceeds the total initial target cell population.

It is thus seen that in our model the concentration of antigen selects, as intended, certain groups of cells for death or proliferation. When the antigen is presented as an initial condition it is possible to obtain large amounts of

antibody of low affinity (low $\bar{K}$) or small amounts of antibody or high affinity. It will now be seen how, when the antigen is presented as a prolonged source, one can obtain larger amounts of antibody with high affinity.

TABLE 2

Response of standard target cells to various doses of antigen

Dose (μg)	When $Ab = Ag$			Final (300 hr) conditions			No. of cell groups killed
	Time (hr)	$\bar{K}$ (l/mole)	Ab (M)	$\bar{K}$ (l/mole)	σ	N_1 (M)	
			(a) *Antigen as initial condition or sudden injection*				
3×10^{5}	205	$4{\cdot}5\times10^{4}$	$8{\cdot}9\times10^{-4}$	$1{\cdot}4\times10^{5}$	1·9	$2{\cdot}7\times10^{-12}$	8
3×10^{4}	170	$1{\cdot}2\times10^{5}$	$1{\cdot}6\times10^{-4}$	$4{\cdot}0\times10^{5}$	1·9	$4{\cdot}7\times10^{-13}$	6
3×10^{3}	145	$4{\cdot}0\times10^{5}$	$2{\cdot}7\times10^{-5}$	$1{\cdot}3\times10^{6}$	1·8	$7{\cdot}8\times10^{-14}$	4
3×10^{2}	120	$1{\cdot}4\times10^{6}$	$4{\cdot}0\times10^{-6}$	$4{\cdot}8\times10^{6}$	1·7	$1{\cdot}2\times10^{-14}$	2
3×10^{1}	105	$5{\cdot}3\times10^{6}$	$5{\cdot}9\times10^{-7}$	$1{\cdot}8\times10^{7}$	1·6	$1{\cdot}8\times10^{-15}$	1
3×10^{0}	100	$2{\cdot}2\times10^{7}$	$8{\cdot}0\times10^{-8}$	$7{\cdot}2\times10^{7}$	1·3	$2{\cdot}4\times10^{-16}$	0
3×10^{-1}	100	$7{\cdot}5\times10^{7}$	$9{\cdot}1\times10^{-9}$	$1{\cdot}9\times10^{8}$	0·6	$2{\cdot}3\times10^{-17}$	0
3×10^{-2}	140	$8{\cdot}9\times10^{7}$	$1{\cdot}1\times10^{-10}$	$2{\cdot}0\times10^{8}$	1·1	$2{\cdot}4\times10^{-20}$	0
			(b) *Prolonged* (1000 *hr*) *source of antigen, final conditions at* 10^3 *h*				
3×10^{5}	190	$3{\cdot}2\times10^{5}$	$3{\cdot}8\times10^{-3}$	$1{\cdot}9\times10^{7}$	1·3	$2{\cdot}0\times10^{-11}$	3
3×10^{4}	160	$6{\cdot}8\times10^{5}$	$3{\cdot}9\times10^{-4}$	$3{\cdot}8\times10^{7}$	1·2	$2{\cdot}0\times10^{-12}$	2
3×10^{3}	140	$1{\cdot}9\times10^{6}$	$3{\cdot}8\times10^{-5}$	$1{\cdot}4\times10^{8}$	1·3	$2{\cdot}2\times10^{-13}$	0
3×10^{2}	120	$6{\cdot}6\times10^{6}$	$4{\cdot}1\times10^{-6}$	$1{\cdot}6\times10^{8}$	1·1	$2{\cdot}2\times10^{-14}$	0
3×10^{1}	110	$2{\cdot}6\times10^{7}$	$4{\cdot}4\times10^{-7}$	$1{\cdot}7\times10^{8}$	0·9	$2{\cdot}2\times10^{-15}$	0
3×10^{0}	120	$9{\cdot}5\times10^{7}$	$5{\cdot}0\times10^{-8}$	$2{\cdot}1\times10^{8}$	0·5	$2{\cdot}4\times10^{-16}$	0
3×10^{-1}	200	$9{\cdot}3\times10^{7}$	$4{\cdot}2\times10^{-9}$	$1{\cdot}3\times10^{8}$	0·1	$1{\cdot}6\times10^{-17}$	1
3×10^{-2}	$>10^{3}$	—	9×10^{-15}	5×10^{6}	1·8	$7{\cdot}9\times10^{-21}$	3
			(c) *Secondary response. Both primary and secondary sources are prolonged* (1000 *hr*) 3 *mg doses*				
3×10^{3}	<20 hr	$\sim6\times10^{7}$	6×10^{-5}	2×10^{8}	0·8	$4{\cdot}4\times10^{-13}$	0

Cell parameters: as in Table 1, and to simulate DNP-bovine γ-globulin as antigen in a rabbit.

Column 7 represents the final concentration of memory cells plus target cells.

(B) DEPENDENCE OF RESPONSE ON PERSISTENCE OF ANTIGEN (ANTIGEN AS SOURCE VS. ANTIGEN AS INITIAL CONDITION)

When antigen is administered to an animal along with an adjuvant, such as mineral oil, the immune response is usually much enhanced. It is believed (Davis *et al.*, 1967) that an important reason for this enhancement is that the adjuvant retards the absorption of antigen by the animal so that the antigen

is presented to the animal's immunological system over an extended period of time. I have, therefore, examined the effect of a prolonged source of antigen in the present model and compared the antibody production for two equal doses, one as an initial condition, the other as a prolonged source.

In certain experiments, Eisen & Siskind (1964), changes in $\bar{K}$ were measured for times up to eight weeks after injection of rabbits with DNP antigen in Freund's complete adjuvant. I have, therefore, investigated a source lasting a comparable period of time, namely, 1000 hr. The particular source is a linear function of time declining to zero at 1010 hr but it is not believed that the precise time dependence is important.

Results for this source are displayed in Figs 4 and 5 and Table 2(b). The "final" entries in the table refer to conditions at 1000 hr, when the problem is terminated. Upon comparing the final values of $\bar{K}$ with those listed in Table 2(a), it is apparent that, at high doses, the prolonged source of antigen

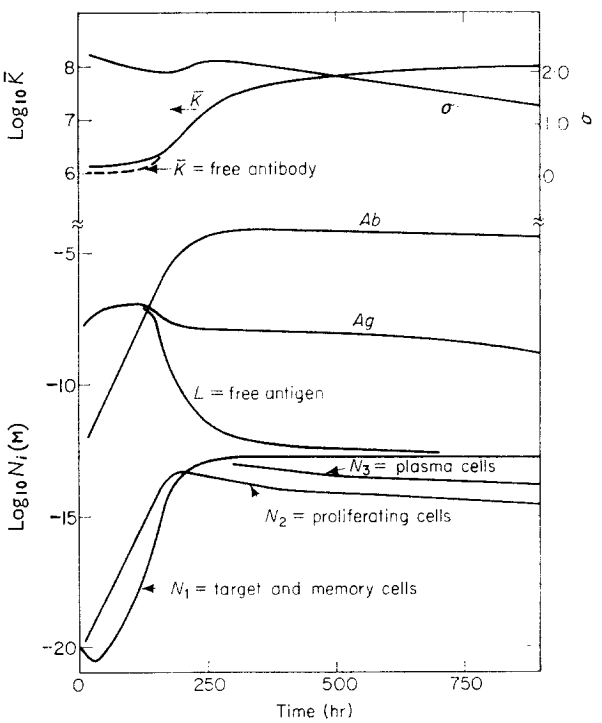

FIG. 5. Calculated concentrations and antibody parameters for a prolonged (1000 hr) source of antigen, containing 3 mg presented to the standard target cells. Parameters have been chosen to represent DNP-bovine γ-globulin as antigen in a rabbit. See text for discussion.

leads to the production of antibodies with much higher binding constants than does the sudden introduction of antigen. There are two important reasons for this. First, when a high dose of antigen is suddenly presented to the system, the concentration of free antigen is such as to kill off many groups of high affinity cells. When the antigen is gradually introduced, fewer groups of cells are killed off. These results are shown in the last columns of Table 2(a) and (b) and in Fig. 4. In addition, when a prolonged source of antigen is present, there will be a low level of free antigen maintained in the system for a long time. During this time, only the cells with high affinity for antigen are stimulated and $\bar{K}$ increases for a long time and by a factor of nearly 100 as seen in Fig. 5. Indeed, $\bar{K}$ would increase even further in Fig. 5 if groups of cells with higher k_j' were present (cf. section 4(D). In Fig. 3 it is seen that when antigen is suddenly introduced, the level of free antigen declines rapidly after $Ag = Ab$. During this decline $\bar{K}$ increases only by about a factor three.

Before comparing these results with experiment, some special features of the results at high and low doses will be noted. In the mathematical model, there is nothing to prevent us from introducing an impossible amount of antigen into the system, and obtaining a mathematical response in which, for example, the mass of cells produced, greatly exceeds the animal's mass. Indeed, the results for the highest doses in Table 2 are not biologically plausible. Consider, in particular, the response of the system to the 300 mg prolonged source in the first line of Table 2(b). The final concentration of memory cells is computed to be 2×10^{-11} M; hence, around 2×10^{12} cells/rabbit. If these are small lymphocytes with a diameter of about 7×10^{-4} cm (Davis *et al.*, 1967, p. 470), and a mass of about 2×10^{-10} g, the computed mass of memory cells is about 400 g. Similarly the final antibody concentration of $3{\cdot}8 \times 10^{-3}$ M corresponds to a concentration of γ-globulin of about 600 g/l. Both these figures are higher than would be expected for a real rabbit, by perhaps two orders of magnitude. It thus appears that such high doses of antigen would overload the antibody producing system (rabbit) and that the computed response, at least at late times, to such high doses could not take place. This would definitely appear to be true for the 30 and 300 mg prolonged sources of antigen [first two lines in Table 2(b)] and for the 300 mg initial dose [first line of Table 2(a)]. Nevertheless, the response to high doses may still be reasonable at early times before antibody concentrations and cell populations have reached impossible levels. For example, the calculation might be reasonable until cell concentrations first reached a critical level $\simeq 10^{-13}$ M (mass $\simeq 2$ gm) after which the actual response would be much less vigorous than the computed response and might be more or less arrested at the critical level.

The response of the system to the lowest doses in Table 2(b) shows an unexpected killing of high k'_j cells. Might this be a manifestation of "low dose tolerance" (e.g. Mitchison, 1964; Thorbecke & Benacerraf, 1967) whereby animals can sometimes be made tolerant by prolonged exposure to exceedingly low doses of antigen? It turns out that in the model this low dose "killing" is due to a detail of the numerical calculation wherein it is assumed that if, in any group, less than one target cell is transformed to the proliferating state in time T_1, then no cells are introduced into the proliferating state in that group. The purpose of this assumption was to ensure that at high antigen concentrations, one would not have fractional numbers of proliferating cells but could instead definitely kill off a group of cells. It is not clear that this numerical assumption corresponds to any biological phenomena at low dose. However, the results suggest a possible mechanism for low dose tolerance in the model, as follows. With a low concentration of free antigen, a particular target cell will have a small probability per unit time of being transformed to the proliferating state. If it is so transformed then the proliferating cell is also unlikely to be stimulated so that the resulting clone will most likely consist of exactly two cells, the result of a single division. If, on the average, there is less than one memory cell in this clone, then the net effect of the stimulation has been to deplete the number of target plus memory cells. Repeated stimulation by low antigen concentrations may then exhaust the supply of target cells. In the model, we assumed that the cells resulting from proliferation are *half* memory cells and *half* plasma cells. If instead we had assumed, for example, that one quarter are memory cells, the results would have been much the same for most of the doses of antigen in Table 2, for which the average number of cells in a clone is very large. However, the results for low antigen concentrations and correspondingly small clones would be much different. Calculations have shown that under these conditions it is possible, by gradually increasing the antigen concentration to completely eliminate all target cells while using a low total dose. The first cells to be eliminated must be those of highest affinity for antigen. It is also clear that the development of small clones may be sensitive to other fine details of the response, such as the relative sensitivity of target cells and proliferating cells to antigen and to stochastic effects that are of much less importance for large clones.

Some of the results in Table 2(b) and Fig. 4 may be compared with experiment. Eisen & Siskind (1964) injected rabbits with DNP-bovine γ-globulin antigen, using doses of 5 to 250 mg in Freund's complete adjuvant, and measured association constants of the anti-DNP antibodies as functions of time. It will be seen below that the measured quantity, K_0, determined from a Sips plot is almost the same as $\bar{K}$ and therefore these two quantities

will be compared. Consider first the 5 mg injection. The measured values of K_0 at early times (2 weeks) are about $1{\cdot}0 \pm 0{\cdot}6 \times 10^6$ l/mole while at eight weeks the values have increased to the neighborhood of 10^8. Upon comparison with the 3 mg calculation in Fig. 4, it is seen that this variation of K_0 with time is very similar to the computed variation of $\bar{K}$. The computed variation of $\bar{K}$ is more rapid than observed, having been largely completed by three weeks, but it could be slowed down in several ways, for example, by lengthening T_2, either in general or only when the cells are weakly stimulated (kL small) or by introducing a time lag between the birth of a memory cell and the time it becomes equivalent to a target cell. Moreover, it is possible that overloading of the rabbit has slowed down the experimental variation of $\bar{K}$ even for the 5 mg dose.

At higher doses of antigen the measured early values of K_0 are smaller, in rough agreement with the values given in column 3 of Table 2(b). In Fig. 6, the values of K_0, at the first time of measurement (2 weeks) are compared with computed values of $\bar{K}$ at the time when $Ag = Ab$, and the values of K_0 and $\bar{K}$ are seen to be in qualitative agreement. The measurements

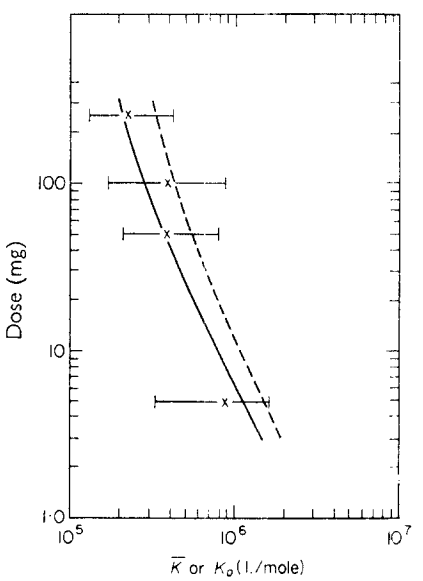

FIG. 6. Early antigen–antibody association constants. K_0 was measured from a Sips plot two weeks after injection of the indicated dose with Freund's complete adjuvant (Eisen & Siskind, 1964). $\bar{K}$ was computed at the time when $Ag = Ab$ and is representative of $\bar{K}$ at earlier times. ——x——, Experimental values of K_0; – – –, computed $\bar{K}$ for total antibody; ——, computed $\bar{K}$ for free antibody only.

of K_0 were actually made on the population of free antibody molecules. At early times, when $Ab \ll Ag$, the effective association constant, $\bar{K}$, for free antibodies is somewhat lower than for all antibodies and is in remarkable agreement with the experimental results, as seen in Fib. 6. Note, however, that the *times* are not the same for computed and experimental points in Fig. 6. The computed values would be much the same for other times before $Ag = Ab$, but would be much higher if taken at two weeks = 336 hr. This is because the computed response is faster than observed. However, we do not know the experimental early time dependence of K_0, since measurements were performed only at two, five and eight weeks. In constructing Fig. 6 we have tacitly assumed that the experimental values of K_0 are not rapidly varying with time around two weeks.

For a 50 mg dose, K_0 increased significantly from two to eight weeks, by around an average factor of 10, while for the higher doses, 100 and 250 mg, no increase was detectable. As noted earlier, the antibody producing system in the model is definitely overloaded at these high doses and hence the computed large increases in $\bar{K}$ are not to be expected. The observed substantial increase for a 50 mg dose may indicate that the rabbit is not quite as overloaded as the model would predict. For example, if we cut off the response to the 30 mg dose when $\sum_{j=1}^{J} N_{2,j} = 10^{13}$, $\bar{K}$ has increased by less than a factor of two above its minimum early value.

Few experiments on the concentrations of antibody have been reported for this experimental system. In Eisen *et al.* (1969) it is stated that between 10 and 100 μg of the antigen are required to evoke a primary response, i.e. to lead to a circulating antibody level of 50 to 75 μg/ml. From Table 2(b) it is seen that 30 μg leads to a final antibody concentration of $4{\cdot}4 \times 10^{-7}$ M or around 70 μg/ml. Hence, the calculated and experimental doses to give this level of response are in good agreement.

It remains to establish that the experimental value, K_0, and the computed value, $\bar{K}$, refer to more or less the same physical quantity. In the experiments of Eisen & Siskind (1964) a population of free antibody molecules is obtained from the blood of a rabbit. Samples are mixed with hapten (DNP) at various concentrations and the fraction, r, of antibody sites that are bound to hapten is determined as a function of the free hapten concentration, c. The results are plotted in a "Sips plot," as $\log [r/(1-r)]$ vs. $\log c$ and K_0 is then determined by fitting the results to a straight line.

$$\log\left(\frac{r}{1-r}\right) = a \log c + a \log K_0$$

where a is a constant. Thus, K_0 is the reciprocal of the concentration at which

half the antibody sites are occupied, i.e. for $r = 1/2$,

$$\log [r/(1-r)] = log\,(1) = 0 \text{ and } K_0 = c^{-1}.$$

A corresponding analysis has been made of some of the numerical data. Thus, at a time when the free antigen concentration of the system is L, the free antibody concentration in group j, Abf_j, is

$$Abf_j = \frac{N_{5,j}}{1+k_jL}.$$

If this free antibody population were exposed to hapten such that the free hapten concentration were c and it is assumed that an antibody molecule binds hapten with the same association constant as for binding with antigen, then the fraction of bound antibody sites is

$$r = \sum_{j=1}^{J} \frac{k_j c Abf_j}{1+k_j c} \Big/ \sum_{j=1}^{J} Abf_j.$$

Sips plots, constructed from this numerical data, are shown in Fig. 7 for the 3 mg extended source calculation, summarized in Fig. 5. It is seen that

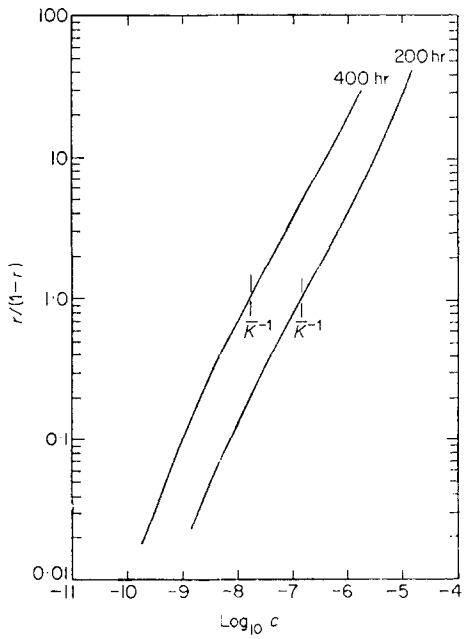

FIG. 7. A comparison of calculated values of K_0 and $\bar{K}$. In this Sips plot, r is the fraction of antibody sites bound to hapten at a free hapten concentration, c M. Free antibody populations were taken from the problem shown in Fig. 5 at 200 and 400 hr. K_0^{-1}, the values of c for which $r = 1/2$ and $r/(1-r) = 1$, is seen to be in good agreement with $\bar{K}^{-1}$.

the values of $\bar{K}^{-1}$, for free antibodies, are close to the values which would be deduced for K_0. Typically the two quantities differ by less than 10%.

Values of a, the heterogeneity index, may also be deduced from Sips plots as in Fig. 7. If a straight line is fitted to the curves of Fig. 7, in the vicinity of $r/(1-r) = 1$, it is found that $a \simeq 0{\cdot}7$ which is typical of the experimental values. It may be noted that for the few experimental populations with highest K_0, a was appreciably smaller and hence the antibodies appeared to be much more heterogeneous. This is in striking contrast to the calculated results wherein high affinity antibody populations, selected by low antigen concentrations, are unusually homogeneous. However, since the experiments are performed over quite a limited range of hapten concentrations, it is possible that the straight line fits to the data and deductions of a may be quite inaccurate in some cases. In particular, there are experimental difficulties in characterizing antibody populations with such high affinities (Eisen & Siskind, 1964).

(C) PRIMARY AND SECONDARY RESPONSES

In this model, the only difference between a primary and secondary response is in the population of target cells that exists when antigen is presented to the system. When the system *first* encounters antigen, the virgin population of target cells is transformed, certain groups of cells being selected by the antigen concentration for proliferation and other groups being perhaps annihilated by high or low antigen concentrations. If it is assumed that the memory cells are equivalent to target cells in their interaction with antigen, then the surviving memory cells will constitute the target cells for the secondary reaction.

As seen in preceding parts of this section, especially Table 2, if the virgin target cell population is not large, then exposure to antigen will lead to considerable cellular proliferation. If, in addition, the lifetime of the memory cell is not short compared to the interval between antigen injections, then the population of target cells will be much larger for the secondary response than for the primary response. It follows that the system will be able to respond much more rapidly to a second exposure to antigen; the antibody concentration will reach a given level much quicker and the antibodies will resemble those made at the *end* of the primary response. In particular, they will have a strong affinity for antigen, i.e. a high value of $\bar{K}$, if the primary response was such that high affinity cells were selected for eventual proliferation, as in Table 2(b). In addition (in the model calculations) more antibodies will be produced in the secondary response than in the primary response.

A secondary response may be simulated with the ABIGAIL 2 computer program by first running a problem with virgin target cells. Assuming that the interval between antigen injections is short compared to the lifetime of a memory or target cell but long compared to lifetimes for proliferating and plasma cell or antibody, then the memory plus target cells from the first problem form the target cells for the second problem and all other populations are set to zero in the second problem. Consider, for example, a 3 mg prolonged source of antigen to elicit both primary and secondary responses. The primary response was indicated in Table 2(b) and Fig. 5 and some corresponding quantities for the secondary response are shown in Table 2(c). The secondary response develops very quickly in the model; within a few hours sufficient antibody has been produced to bind nearly all the antigen and thereafter the level of free antigen is low and little additional stimulation takes place. It is likely that the quickness of the secondary response is exaggerated by the model. From the differential equations (13) and (16) it can be seen that at early times, the concentration of antibody produced in response to a constant source of antigen will be proportional to t^3. Thus, in a secondary response, the model is able to build up antibody very rapidly and hence to quickly neutralize the antigen. In fact, it is more likely that none of the target cells will be able to synthesize antibody at a high rate until a delay time comparable to T_1 has passed. That is, if the target cells are small lymphocytes, then a period comparable to T_1 is required before the cell has manufactured the necessary ribosomes, etc., to be able to synthesize proteins at a high rate, Ling (1968). During this delay time, the level of free antigen may be high enough to stimulate target cells rather effectively, whereas in our model, the antibody quickly combines with the antigen. Thus, it is likely that the model will overestimate the quickness of a secondary response and will underestimate the stimulating effect of antigen and the resulting cellular proliferation and antibody production in the secondary response. This is particularly significant if the dose of antigen in the secondary injection is small compared to that in the primary injection so that only a small fraction of the target cells proliferate. Then, as a result of the primary injection there is a relatively large population of target cells which can readily produce more than enough antibody to combine with all the secondary antigen. A complicating effect in the opposite direction is that when a secondary injection of antigen is made, the level of circulating antibodies from the primary injection may still be high enough to combine with and neutralize much of the secondary antigen.

Ignoring the above complications for the moment, it is calculated that in the secondary response to a 3 mg extended source, the peak antibody concentration is 1·6 times as high as the peak concentration in the primary

response and, at the time of peak concentration, $\bar{K}$ is about 2·5 times larger in the secondary response. In the primary response, $\bar{K}$ increased with time by nearly a factor of 100, while in the secondary response the increase was only by a factor two. In the secondary response, $\bar{K}$ is at all times very high and comparable to the largest values of k_j for groups that contain surviving cells. Such high values of $\bar{K}$ are qualitatively in accord with experiment (Steiner & Eisen, 1967; Eisen *et al.*, 1969) but quantitative values have not been reported. In addition, the experimental antibodies produced *early* in the secondary response have high affinity (Steiner & Eisen, 1967, p. 1190) in agreement with model results. As a result of the second exposure to antigen, the number of target cells is roughly doubled.

Experimental threshold doses of DNP antigen with adjuvant have been reported, which are required in order to "prime for a secondary response" (Steiner & Eisen, 1967) or "evoke a secondary response" (Eisen *et al.*, 1969). In order to "prime for a secondary response" a dose must be large enough so that, although antibodies to the primary injection are not detectable ($Ab \leqslant 50$ μg/ml), when the system is subsequently given a 1 mg injection the early antibodies have significantly higher affinity for antigen than do those in a primary response. A threshold dose for "priming" is stated (Eisen *et al.*, 1969) to lie in the range 1 to 10 μg; however, it is not clear how large a change in $\bar{K}$ is required for a threshold response. In our calculations it appears that a primary dose of 1 μg is clearly adequate while even 0·1 μg will enhance $\bar{K}$ for the early secondary antibodies by about a factor of four. Thus, the calculated priming doses appear to be perhaps an order of magnitude smaller than the experimental values. A possible reason is that in the experiments, the primary dose was given in the rabbits' rear feet while the secondary dose was given in the front feet and the antibodies were obtained from axillary lymph node cells, near the secondary injection. It is thus possible that the primary antigen concentration was unusually low in that region where the secondary reaction was examined.

The threshold dose required to "evoke a secondary response" was experimentally defined (Eisen *et al.*, 1969) as that which would lead to a certain concentration of serum antibody (~ 75 μg/ml) when given 11 months after a 0·1 mg primary injection. The threshold dose was found to be between 0·01 and 0·001 μg. In the model calculations I have found a substantially higher level to be required, between 0·1 and 1 μg. The dose of 0·1 μg leads to a maximum calculated antibody concentration of 20 μg/ml. Two effects may be responsible for this discrepancy. First of all, since small values of the ratio secondary dose: primary dose are now being considered, the delay time discussed above may significantly enhance the cellular stimulation and antibody production. If one delays all antibody production for a

period of the order of 10 hr then the threshold dose is estimated to lie in the range 0·01 to 0·1 μg. If, in addition some of the target cells have higher association constants than $2{\cdot}6 \times 10^8$, the maximum in the calculation, the threshold dose to evoke a secondary response would be lowered further. The possibility of considerably stronger binding is indicated by footnote 5 in Eisen & Siskind (1964), but quantitative data are not available.

(D) OTHER EFFECTS OF THE TARGET CELL POPULATION

In the model, the target cell population significantly affects the response of the system to an antigen. Therefore, if one takes the model seriously one should be able to deduce, from the observed response of an animal to antigen, something about the affinity spectrum of the target cells, i.e. the number of target cells as a function of association constant. Use has already been made of this approach in devising the population of virgin target cells used in the preceding calculations. In particular, the maximum value of k_j' was selected to resemble the highest association constants, k_{max}, reported by Eisen & Siskind (1964), and few such cells were included because of the large observed fluctuations in k_{max} between rabbits.

If there are few target cells then it will take a relatively long time for the antibody concentration to rise to a particular level while with many target cells, the time will be shorter. This was seen above in comparing primary and secondary responses. In addition, it was noted that during the primary response to a prolonged source of antigen, $\bar{K}$ increases strongly while on the other hand, $\bar{K}$ is relatively constant during the secondary response since the selective proliferation of cells with high affinity has already been effected during the primary response. Thus, there are qualitative differences between the response of a virgin target cell population and a secondary target cell population.

A further kind of target cell spectrum has been suggested by studies (Eisen *et. al.*, 1970) of the frequency of mouse myeloma proteins having anti-DNP activity, in which $\sim 1\%$ of the myelomas make protein which binds DNP, mostly with low association constants (10^4 to 10^5 l/mole). If each myeloma arose by a malignant tranformation of one target cell, then the frequency of mouse DNP target cells may be the same as the frequency for mouse myeloma anti-DNP proteins. I have, therefore, concocted a target cell spectrum containing many cells concentrated toward low k and examined the response of such a system to prolonged sources of antigen. Results are summarized in Table 3(a) which may be compared with Table 2(b).

The particular "skew target cell population" has a concentration near 10^{-15} M, hence about 10^8 cells in a 200 cm^3 "rabbit." These 10^8 cells were distributed linearly as a function of j, in 17 groups, such that $k_1 = 3{\cdot}91 \times 10^3$,

$k_{17} = 2{\cdot}56 \times 10^{8}$ and zero cells are in group 17; thus, $N_{1,j} = 1{\cdot}18 \times 10^{-16} - 6{\cdot}92 \times 10^{-18} \times j$. In Table 3(a) it is seen that with these cells, the response occurs quickly but that $\bar{K}$ increases at later times. If, however, $\bar{K}$ were calculated only at later times, say $t \geqslant 10$ days, then the response would resemble a secondary response. At the high doses (30 and 300 mg) in Table 3(a) the system is clearly overloaded as mentioned previously, while for the low doses the target cell population changes only slightly.

It is believed (Eisen, 1970) that target cell spectra of this type may arise if the cells were previously selected for proliferation, not by contact with DNP but by cross reacting antigens. The selected cells would then be numerous but would have, on the average, low affinity for DNP.

The foregoing comparisons of target cell spectra are summarized qualitatively in Table 4. It is assumed that appropriate doses of antigen are given with adjuvant. Of course, if one had abundant experimental data and

TABLE 3

Response to prolonged (1000 hr) antigen sources of two target populations

Dose (μg)	When $Ag = Ab$ Time (hr)	K (l/mole)	Ab (M)	Final conditions (at 10^3 hr) K (l/mole)	σ	N_1 (M)	No. of cell groups killed
			(a) *Large skew target cell population (see text)*†				
3×10^{5}	60	$3{\cdot}8\times10^{4}$	4×10^{-3}	$7{\cdot}4\times10^{7}$	1·4	$2{\cdot}5\times10^{-11}$	0
3×10^{4}	40	$1{\cdot}4\times10^{5}$	4×10^{-4}	$8{\cdot}1\times10^{7}$	1·1	$2{\cdot}4\times10^{-12}$	0
3×10^{3}	20	7×10^{5}	4×10^{-5}	$9{\cdot}1\times10^{7}$	0·9	$2{\cdot}2\times10^{-13}$	0
3×10^{2}	<20	$\sim3\times10^{6}$	4×10^{-6}	$1{\cdot}0\times10^{8}$	0·8	$2{\cdot}3\times10^{-14}$	0
3×10^{1}	<20	$\sim1\times10^{7}$	5×10^{-7}	$1{\cdot}0\times10^{8}$	0·8	$3{\cdot}2\times10^{-15}$	0
3×10^{0}	<20	$\sim2\times10^{7}$	5×10^{-8}	9×10^{7}	1·2	$1{\cdot}1\times10^{-15}$	0
3×10^{-1}	<20	$\sim3\times10^{7}$	9×10^{-9}	4×10^{7}	1·9	$1{\cdot}0\times10^{-15}$	0
3×10^{-2}	<20	$\sim3\times10^{7}$	3×10^{-9}	3×10^{7}	2·0	$9{\cdot}4\times10^{-16}$	0
			(b) *Target cell population with broad affinity range*‡				
3×10^{5}	160	$1{\cdot}0\times10^{5}$	4×10^{-3}	$1{\cdot}5\times10^{8}$	1·5	$2{\cdot}1\times10^{-11}$	7
3×10^{4}	140	$5{\cdot}9\times10^{5}$	$3{\cdot}9\times10^{-4}$	$1{\cdot}1\times10^{9}$	1·6	$2{\cdot}3\times10^{-12}$	4
3×10^{3}	120	$3{\cdot}5\times10^{6}$	$3{\cdot}9\times10^{-5}$	$4{\cdot}9\times10^{9}$	1·5	$2{\cdot}4\times10^{-13}$	2
3×10^{2}	100	$2{\cdot}0\times10^{7}$	$4{\cdot}0\times10^{-6}$	$2{\cdot}0\times10^{10}$	1·4	$2{\cdot}4\times10^{-14}$	0
3×10^{1}	80	$1{\cdot}1\times10^{8}$	$4{\cdot}4\times10^{-7}$	$2{\cdot}3\times10^{10}$	1·0	$2{\cdot}2\times10^{-15}$	0
3×10^{0}	60	$5{\cdot}0\times10^{8}$	$4{\cdot}3\times10^{-8}$	$2{\cdot}5\times10^{10}$	0·8	$2{\cdot}2\times10^{-16}$	0
3×10^{-1}	40	$1{\cdot}4\times10^{9}$	$4{\cdot}5\times10^{-9}$	$2{\cdot}6\times10^{10}$	0·7	$2{\cdot}2\times10^{-17}$	0
3×10^{-2}	20	$3{\cdot}7\times10^{9}$	$4{\cdot}9\times10^{-10}$	$2{\cdot}9\times10^{10}$	0·5	$2{\cdot}4\times10^{-18}$	0

† Initially $N_1 = 10^{-15}$.

‡ 10^4 cells between $k'_1 = 30$ and $k'_{31} = 3{\cdot}3\times10^{10}$ l/mole.

TABLE 4

Responses of various target cells

Response to antigen	Target Cells
Slow $\bar{K}$ increases with time	Virgin target cells, i.e. few cells, wide range of affinity.
Fast $\bar{K}$ constant	Secondary target cells, i.e. many cells, most with high affinity.
Fast $\bar{K}$ increases	Target cells selected by cross-reacting antigen, i.e. many cells, most with low affinity.

took the model literally one should be able to deduce much more detailed information on the target cell spectra.

It has been emphasized repeatedly in this section that the concentration of free antigen selects certain groups of cells for proliferation according to their affinity for antigen. The question remains, to what extent do the results which we compared with experiment depend on the postulated spectrum of virgin target cells and to what extent are they determined by the stimulating effect of antigen? If one wishes to obtain antibody with high k_j one must postulate virgin target cells with correspondingly high k'_j and it is clear that the maximum postulated values of k'_j will importantly affect the response to small doses. However, given a virgin target cell distribution which covers a large range of k_j (fairly uniformly in j), it might be expected that the dose of antigen would largely determine the character of the early antibodies, except for very small doses. To investigate this question I have made some calculations with a very broad target cell population, namely 10^4 target cells in 31 groups between $k'_1 = 30{\cdot}1$ and $k'_{31} = 3{\cdot}28 \times 10^{10}$. These target cells were exposed to prolonged sources of antigen and some results, given in Table 3(b), may be compared with those for the standard target cell population in Table 2(b). It is seen that by far the largest differences between the responses of the two target cell populations arise from the cells of highest affinity for antigen. Thus, in Table 3(b), all the final values of $\bar{K}$ and, for small doses, the early values of $\bar{K}$ are much higher than in Table 2(b), simply because target cells of much higher affinity are present in the problems summarized in Table 3(b). Not only are target cells of higher affinity initially present but also there are more cells in each group so that a particular group has a better chance of surviving high doses of antigen.

Further comparison of Tables 2(b) and 3(b) shows that, except at the lowest dose, nearly the same final concentrations of antibodies and memory cells are found for the two different target cell populations. With the broader distribution of target cells $Ab = Ag$ at a slightly earlier time because more target cells are present and the early values of $\bar{K}$ are somewhat different. These results indicate that the major role in determining the early response to moderate doses of antigen is played by the concentration of free antigen and that a variety of target cell distributions that are fairly broad and do not contain very many cells, give similar responses.

5. Discussion

It may be helpful at this point to review the important features of the model and what has been learned from the calculations and their comparison with experiments.

The model is a quantitative and mathematical clonal selection theory for an adult animal. Four kinds of cells are included in the model: (1) *target cells*, i.e. cells initially present that are capable of being transformed by antigen into active, dividing cells; (2) *proliferating cells*, which result from the stimulation of target cells by antigen, secrete antibody, and in the continued presence of antigen may continue to divide. When no longer stimulated by antigen, they divide unsymmetrically into (3) *plasma cells*, terminal antibody secreting cells, and (4) *memory cells* which have usually, in this paper, been taken to be functionally identical to target cells and which will in any case serve as target cells for subsequent exposures to antigen. All of these cells, save for the plasma cells, are assumed to have antibody-like surface receptors that are capable of combining reversibly with antigen and the mitotic activity of a cell is assumed to be governed by the number or fraction of its surface receptors that are bound to antigen.

The binding of an antigen to an antibody or to a receptor site is characterized by an intrinsic association constant and considerations of chemical equilibrium are used to deduce at any time, the state of the binding and fraction of receptor sites that are bound to antigen. The theory is developed [cf. equation (10)] for binding of univalent antigen to bivalent antibodies and multivalent cells, and an effective number of independent antigen sites must be used for multivalent antigen. All receptor sites on a particular cell have the same association constant for the antigen but various groups of cells are allowed, in the computer program ABIGAIL 2, to have different association constants and to produce correspondingly differing antibodies. Thus, the antibodies produced in response to a given antigen may have a wide range of association constants for binding antigen.

Let J be the number of different groups of receptor association constants. The concentrations of the $4J$ different kinds of cells, J different kinds of antibodies and antigen are assumed to be given by a set of $5J+1$ differential equations, together with source and initial conditions. These are equations (12) to (17). Together with the condition of chemical equilibrium, equation (10), and some auxilliary conditions, equation (11), they constitute a mathematical formulation of the postulated interaction of antigen with cells and with antibody. The response of this system of equations to the introduction of an appropriate amount of antigen gives cellular proliferation and antibody production and, when the antigen concentration drops to a low level the proliferation and antibody production must diminish or cease. These properties have been demonstrated by numerous calculations, and they are perhaps the only properties that are essential for a clonal selection model. There are numerous variations of the present model that would give similar results. Moreover, although the present model may be reasonable in outline, it unquestionably contains some rather arbitrary parameters and functions, for example, m, the number of receptor sites per cell, and the functions F, G and H [equations (11)] that determine in detail how a cell reacts to antigen bound to its sites. Therefore, it seems appropriate to review some of the calculations, with the ABIGAIL 2 computer program, and to note some comparisons with experiment which were made in detail in section 4 which encourage us to believe that the model gives reasonable results. Then, some of the biological assumptions in the model will be discussed together with possibly relevant future experiments and various speculations.

Countless experiments have been reported in the immunological literature on the concentrations and/or affinities of antibodies as functions of time and doses of antigens, the histology of participating cells, induction of tolerance, etc. In principle, many of these experiments could be analyzed by the present model. I have chosen to concentrate on a particular class of experiments (Eisen & Siskind, 1964; Steiner & Eisen, 1967; Eisen *et al.*, 1969), in which a relatively simple antigen was used, bovine γ-globulin with DNP hapten groups, and the association constants of the anti-DNP antibodies were carefully determined. It is to be emphasized that experimental information on antibody production is directly comparable with the calculational results only if both the concentration and affinity of the antibodies are measured or *if* the dependence of the assay on both is known. In the experiments considered, primary emphasis was on the affinity, but some information on concentrations was also reported. Values of the model parameters which were used to represent these experiments were discussed at length in section 4 and summarized in Table 1.

In calculations with several groups of cells (i.e. cells with several values

of k_j', the receptor–antigen association constant) the concentration of free antigen, L, selects certain groups of cells for proliferation. If the product $k_j'L$ is too large, the cells are, by hypothesis, killed so that the possibility of inducing high dose tolerance is built into the model. If the product $k_j'L$ is too low the target cells are not much stimulated. Therefore, groups having intermediate values of $k_j'L(10^{-2} \leqslant k_j'L \leqslant 1)$ are selected for proliferation. The following calculational results, caused by this selective proliferation, may be compared with experiment.

(1) At early times following the typical introduction of antigen, there is a nearly exponential increase in antibody concentration (cf. Figs 3 and 5): this rate of increase diminishes after the antibody concentration exceeds the concentration of antigen sites by an order of magnitude or so. The antibody concentration reaches a maximum value and thereafter gradually declines. These results are in qualitative accord with experiment (Davis *et al.*, 1967, chap. 15) and some details will be discussed below.

(2) The system responds more vigorously to a prolonged exposure to antigen than to a sudden exposure to the same amount of antigen. The main difference is that the effective association constant, $\bar{K}$, becomes much higher (Table 2) in the former case due to the continuing low level of antigen which causes cells with high affinity for antigen to continue proliferating. In addition, the peak antibody concentrations are somewhat higher for prolonged exposure (compare Figs 3 and 5). If one assumes that an important aspect of administering an antigen with an adjuvant is the slowing down of the release of antigen to the animal (Davis *et al.*, 1967, p. 450) then the above effect is in accord with the well-known efficacy of adjuvants.

(3) In the primary response to a prolonged source of antigen, $\bar{K}$ increases with time [cf. Fig. 5 and Table 2(b)] due to the continuing low level of antigen. By a *primary* response I mean one in which the target cells are relatively few in number, have a broad range of affinity for antigen, and are not concentrated toward the largest affinity. For an antigen dose of a few milligrams in rabbits, both experimental and computed values of $\bar{K}$ increase by about a factor of 100. However, the calculated increase in $\bar{K}$ was limited by the assumed maximum value of k_j' which was in turn estimated from the experimental maximum values of $\bar{K}$. Hence the calculated and experimental values at late times are not independent; if higher values of $\bar{K}$ had been observed, I would have postulated some cells with correspondingly high values of k_j' and these would have selectively proliferated at late times.

In addition, special comments are required for both large and small doses. There is nothing in the model to prevent exposing the system to a biologically unreasonable dose of antigen and obtaining an unreasonable response. The calculated responses to 30 and 300 mg doses involve unreasonable numbers

of cells and unreasonably high antibody concentrations; hence, they could not take place. I deduce that such high doses of antigen may, in fact, overload the antibody producing system of a rabbit and that a failure to observe increases of $\bar{K}$ with time for high doses is due to such overloading (Eisen & Siskind, 1964).

For low antigen doses ($\lesssim 1$ μg), the antigen concentration is never high; only cells of high affinity can be stimulated so that at all tomes $\bar{K}$ will be large. Thus $\bar{K}$ does not increase much with time for very low doses [Table 2(b)].

The computed increase in $\bar{K}$ is somewhat more rapid for a few milligram dose than was observed. Possibly there is some overloading of the rabbit even at these doses which contributes to the slower response. Some minor changes in the model which would lead to slower changes were mentioned in section 4.

(4) There is good agreement between the experimental and calculated primary antigen doses that are required to produce a given antibody concentration of 50 to 75 μg/ml. Moreover, the computed effective association constants, $\bar{K}$, for antibodies produced at early times in the primary response are in good agreement with the earliest measured values (at two weeks), as seen in Fig. 5. The computed values of $\bar{K}$ were taken at the time when the antibody concentration equals the antigen site concentration; even for the highest dose (300 mg), overloading of the system has not become manifest at these early times.

(5) Secondary responses were simulated by taking the target cells plus memory cells that remain after the first exposure to antigen to be the target cells for the secondary response. These cells are more numerous than the virgin target cells and are concentrated at high affinity due to the selective proliferation of such cells induced by a prolonged source of antigen (cf. Fig. 4). As a result, the secondary response is much more rapid than the primary response and even the early antibodies have relatively high values of $\bar{K}$ so that the increase of $\bar{K}$ with time is slight.

(6) Computed threshold doses to "prime for a secondary response" and to "evoke a secondary response" are in only fair agreement with the measured values. Possible reasons for this disagreement were noted in section 4(c) and will be referred to in the following discussion.

From the above agreements between calculated and experimental responses to antigen under a variety of circumstances we conclude that the model gives reasonable results and deserves to be considered further. It is, therefore, pertinent to discuss some of the biological assumptions that underlie the model.

First of all, the model is a quantitative clonal selection theory for adult animals. Certain cells are assumed to respond to antigen by proliferation

and antibody production; each clone produces homogeneous antibody and antibody heterogeneity arises from the heterogeneity of target cells and hence clones. The model is restricted to adult animals because target cells are postulated to exist from the beginning. However, this restriction could be readily removed by assuming that cells which are destined to become target cells pass through a sensitive stage during their maturation and are then readily killed by contact with self-antigens.

It is assumed in the model that the presence of target cell plus antigen is sufficient to initiate the immune response. This may be a considerable over-simplification since it appears that other kinds of cells may sometimes be involved in triggering the response (e.g. Gottlieb *et al.*, 1967; Richter, 1969; Talmage *et al.*, 1969, and other references therein). In addition, in the model, a brief exposure to antigen is sufficient to lead to some cell proliferation and antibody production; continued exposure is only required to maintain the proliferation and amplify the antibody production. If, for example, the target cells were exposed to antigen for one hour, 10% could be transformed so that one plasma cell and one memory cell would be produced for every ten target cells. In the model, there is no clear delay between stimulation of a target cell and antibody production. This is probably an unrealistic feature that could be removed in an *ad hoc* manner and, as argued in section 4, it is probably responsible for underestimating the vigor of a secondary response to very small doses of antigen. That is, in the actual secondary response there is probably a delay of 10 hr or so between the introduction of antigen and significant antibody production, during which time target cells may be stimulated. In the model, antibody production begins right away, quickly reducing the free antigen concentration and cell stimulation and thus terminating the response prematurely.

It is postulated that the target and the proliferating cells bear on their surfaces antibody-like receptor sites that can reversibly bind antigen and that the state of binding determines the activity of the cell. The assumption of such receptors is both venerable (Ehrlich, 1900), and indicated by recent experiments (e.g. Mitchison, 1967; Plotz, 1969). I will not attempt to justify it here. In the numerical calculations I have assumed that each cell has 1000 sites and the sense of the F and H functions is that if one or a few sites on a target cell are bound to antigen then that cell will be stimulated with good probability within 10 hr to become a proliferating and antibody producing cell, and that the proliferating cell is likely to continue proliferating only so long as one or more of its sites are bound on the average. The numerical results would remain much the same if the numbers of sites in the above sentence were multiplied by any not too large factor, e.g. 10, giving 10^4 total sites with binding of 10 sites required for stimulation, etc.

In addition, a possibility of high dose tolerance was assumed such that if most of a target cell's sites are bound, the cell is likely to be killed. All of these assumptions can, in principle, be tested by measurements of the binding of antigen to cells and the effects thereby produced on the cells. Such experiments would be most relevant for confirming or altering the basic assumptions of this model. Some progress has already been reported in separating populations of cells that specifically bind to antigen (Wigzell & Andersson, 1969; Abdou & Richter, 1969) and a careful characterization of this binding would be of great interest. Other techniques for obtaining such cells can be readily envisioned, such as using fluorescent antibodies for target cell identification and then physically separating such cells (e.g. Fulwyler, 1965) for study.

The present model is presumably most applicable to the immune response to small antigens, ideally univalent antigens or those with only a single hapten group. Large antigens, with many sites might, for example, bind simultaneously to several receptor sites giving a very tight and almost irreversible binding that would importantly affect the immune response.

It is assumed that both proliferating and plasma cells make antibodies. This assumption is not necessary, but if proliferating cells did not make antibody, it would be necessary to somewhat modify the model in order to get the response to shut off at a reasonable level. It is tempting to speculate that the proliferating cells produce heavy (19 s) antibodies while the plasma cells produce light (7 s) antibodies. This speculation is based on the observation that in the model primary responses, most of the antibodies present at early times were produced by proliferating cells while those present at late times were made by plasma cells. This is similar to the time history of 19 s and 7 s antibodies (Davis *et al.*, 1969, p. 462). During the secondary response, plasma cell antibodies predominate at nearly all times, as do 7 s antibodies. It has recently been shown (Koshland, Davis & Fujita, 1969) that heavy and light antibodies have some of the same genetic markers in their variable regions, which also makes a clonal relationship of the producing cells attractive.

In the model, it is assumed that a continuing presence of antigen is required in order to sustain cellular proliferation and this assumption makes the response self limiting, i.e. when enough antibody has been made to bind the antigen, the proliferation stops. However, the presence of antigen is not assumed to be required to maintain the population of memory cells which are postulated to have a long lifetime.

Equal numbers of plasma and memory cells were assumed to be produced, but precise equality is by no means required in the model. It was noted in section 4 that if memory cells are produced less frequently than plasma cells,

this leads to the following mechanism for inducing low dose tolerance in the model. If a target cell is exposed to a low concentration of antigen it (or the resulting proliferating cell) is likely to divide, if at all, only once. The resulting clone has only two cells and if the probability is less than half that one is a memory cell, then on the average, the number of target plus memory cells is depleted by stimulation. Continued stimulation may thus reduce the population of target plus memory cells to zero, and one could use the model to devise a concentration of antigen as a function of time which would reduce any given initial population of target cells to zero.

In most of the calculations with ABIGAIL 2, memory cells have been assumed to be functionally identical to target cells, the only difference being that memory cells are made after antigen induced proliferation rather than being initially present. If memory cells are to serve as the mechanism for enhancing the secondary response then they must become similar to target cells in time, but they need not be born that way. It was noted in comparison with experiments that the selective proliferation of cells with high binding for antigen was responsible for the increase of $\bar{K}$ with time. It is, thus, of interest to consider whether the participation of memory cells is important in such selective proliferation. Two problems with 1 mg prolonged sources and virgin target cells have been compared; in (a) memory cells are inert while in (b) they are the same as target cells. In case (a) the target cells are quickly depleted to zero but $\bar{K}$ continues increasing for about 400 hr reaching a value of 5×10^7 as compared to a value of $1{\cdot}5 \times 10^8$ reached in problem (b). It appears that the participation of memory cells is not obligatory in the selective proliferation which increases $\bar{K}$, but it is helpful.

In conclusion, it may be of interest to note a few experimental implications of the present model. First of all, there is the general point that in planning and interpreting experiments that involve the production of antibodies in response to antigen, it is fruitful to keep in mind both the concentrations and association constants of the various quantities. From observations of the concentration and association constants of antibodies as a function of time after antigen injection, it should be possible to deduce some properties of the affinity spectrum of target cells, and hence to perhaps infer their origin (as in Table 4). A given concentration of free antigen will select certain clones for proliferation and by an appropriate choice of dose it may be possible to obtain special results. For example, a continuing low concentration of antigen will promote the selective proliferation of cells with high affinity for antigen; under favorable conditions, notably with few target cells, it should then be possible to selectively stimulate a single clone and hence obtain almost homogeneous antibody.

Possible mechanisms for the induction of tolerance by exposing a system

to antigen could be investigated with the present model. For example, it is predicted that the sudden exposure of a system to a high antigen concentration should result in the destruction of target cells with high affinity for antigen; thereafter the system can not produce antibodies of high affinity. It would be expected to be easier to establish tolerance to an antigen for which the range of association constants of the target cells is narrow rather than to one for which the range is broad. In the latter case, a concentration likely to kill off certain cells might stimulate others, though in either case a high enough concentration of antigen would kill off all target cells (and perhaps the animal). If the conjectured mechanism of low dose tolerance is correct, then for any distribution of target cells it should be possible to gradually increase antigen concentration with time in such a way as to kill off all the initial target cells with high probability.

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