J. theor. Biol. (1992) **159**, 215–240

Analysis of a Cellular Model to Account for the Natural History of Infection by the Hepatitis B Virus and its Role in the Development of Primary Hepatocellular Carcinoma

ROBERT J. H. PAYNE†, MARTIN A. NOWAK‡
AND BARUCH S. BLUMBERG§

† *Centre for Mathematical Biology, Mathematical Institute, University of Oxford, Oxford OX1 3LB,* ‡ *Department of Zoology, University of Oxford, OX1 3PS, and* § *Balliol College, Oxford OX1 3BJ, U.K.*

(*Received on* 5 *December* 1991, *Accepted on* 9 *May* 1992)

Infection with the hepatitis B virus (HBV) can have many different outcomes. Transient infection may result in acute hepatitis or may remain subclinical. Persistent infection may also be subclinical, or may involve chronic active hepatitis, and can finally lead to the development of primary hepatocellular carcinoma. A mathematical model is given to account for the many different outcomes of HBV pathogenesis. The model is based on the assumption that the liver contains two cell populations with differing abilities to support active HBV replication and/or viral integration into the genome. The model helps account for the relationship of the different clinical courses of HBV infection to the age when the disease is acquired, together with the state of the immune system of the patient.

1. Introduction

The hepatitis B virus (HBV) is estimated to be carried by more than 300 million people worldwide, of whom 25–50% may develop primary hepatocellular carcinoma (PHC) (Beasley *et al.*, 1981). HBV is therefore one of the most common endemic viral pathogens and one of the most common causes of death (Maynard, 1990), its importance as a known human carcinogen coming second only to tobacco. The discovery of the virus in 1967 and the subsequent development of a protective vaccine by the early 1980s gave rise to the hope of controlling, and possibly eradicating, the virus by mass vaccination (Blumberg, 1989). Currently available vaccines are protective against new infection, but no appropriate treatment is as yet available for people who have already acquired the virus (Hollinger, 1989). Alpha-interferons offer a 50% chance of long-term inhibition of HBV replication in patients who acquire the infection as an adult, but appear to be ineffective when the infection is acquired very young (Alexander *et al.*, 1987; Jacyna & Thomas, 1990). Although the main target cells of HBV are hepatocytes the integrated virus DNA can be found not only in tumour and non-tumour cells of the liver, but has also been observed in

‡ Author to whom correspondence should be addressed.

0022-5193/92/220215+26 $08.00/0

the kidney, spleen, pancreas, skin, bone marrow and circulating blood cells (Tiollais & Buendia, 1991). In addition there are several hepatitis-like viruses, known as hepadnaviruses (Pugh & Bassendine, 1990), that are found in a range of animals, including the woodchuck, the ground squirrel and the Peking duck, which have been used to provide useful "models" for the infection of humans by HBV (Summers *et al.*, 1978; Marion *et al.*, 1980).

The consequences of HBV infection may vary considerably between different individuals, ranging from a subclinical and anicteric course, through acute clinical hepatitis, to chronic hepatitis, cirrhosis and PHC. Acute hepatitis develops after a rather variable incubation period (McIntyre, 1990), with an average of 70–80 days, after which the patient will normally be expected to recover completely. This is the most usual observed outcome of infection in adults, although the actual most common outcome is probably transient infection with no perceptible disease, with subsequent development of detectable antibodies against the surface antigen of HBV. In rare cases fulminant hepatitis can occur, a situation that is often fatal (Fagan & Williams, 1990). In some cases—frequently in infants, occasionally in children, but less commonly in adults—viral antigen will not have cleared from the blood within 6 months and the patient will become an infectious carrier (Dudley *et al.*, 1972). Such cases of chronic infection are often asymptomatic, and the patient may appear healthy and often will even be unaware of having been infected. Chronic carriers may develop chronic active hepatitis, leading to cirrhosis and to PHC (Beasley *et al.*, 1981; Viola *et al.*, 1981; Dusheiko, 1990), although the cancer will not usually manifest until after a 30–50 year latency period. The mechanism for the pathogenesis of PHC is as yet poorly understood, the molecular biology of the process being the subject of much recent research (de The *et al.*, 1987; Hsu *et al.*, 1988; Chisari *et al.*, 1989; Fourel *et al.*, 1990).

The virus itself, which has a genome of only about 3200 base pairs, is the smallest known DNA virus that is a human pathogen. The virus is a 42 nm spherical particle with a host-cell derived membrane made up of "small", "medium" and "large" proteins, which together constitute the viral surface antigen, HBsAg (Ganem & Varmus, 1987; Gerlich *et al.*, 1990). The viral core antigens, HBcAg and HBeAg, form a nucleocapsid including the double-stranded viral DNA and the viral DNA polymerase, an enzyme that also has reverse transcriptase and RNAse H activity. The virus genome contains four open reading frames (Tiollais *et al.*, 1981) encoding for the envelope and nucleocapsid proteins, the DNA polymerase, and a fourth protein, called the HBx protein, that acts as a transcriptional control element to upregulate the expression of other viral genes (Seto *et al.*, 1990). The presence of the *HBx* gene has been associated with the induction of liver cancer in transgenic mice (Kim *et al.*, 1991).

In some ways the structure and the life cycle of HBV are remarkably similar to those of retroviruses, such as HIV for example (Tiollais *et al.*, 1985; Tiollais & Buendia, 1991). After fusion with the cell membrane the envelope and core proteins are removed and the circular double-stranded DNA appears in the cell nucleus. This DNA can be integrated into the host cell genome (Dejean *et al.*, 1984) where it serves as a template for the production of viral messenger RNAs (which lead to viral protein

synthesis), and for the full RNA pre-genome, which is reversely transcribed into new HBV DNA by the virus encoded polymerase (Ganem & Varmus, 1987). The new virus particles are then assembled in the cytoplasm and released from the host cell. This process appears to be of only weak cytotoxicity and so it is assumed that the increased cell death of infected hepatocytes is due mainly to cytotoxic T cell responses, and possibly other immune effects, directed against infected cells (Alexander, 1990). A detailed mathematical model of the initial immune response to HBV infection has recently been published by Marchuk *et al.* (1991*a*, *b*). It has been suggested that the rate of virus replication may depend on the differentiation state of the host cell, and it is a model based on this assumption that we shall be considering in this paper.

The first viral antigen to be detected in the blood is usually HBsAg (1–10 weeks after infection) which is found to be produced by virus-infected cells even in the absence of detectable virus replication. HBxAg, the product of the "X" reading frame, may sometimes be detected before HBsAg. Testing for HBxAg is not, however, routine. The appearance of HBsAg may be followed a few days later by that of HBeAg, whose presence of the serum is indicative of active virus replication. The HBc antigen can be detected in the liver but is not found free in sera.

The first measurable antibody response (anti-HBc) is directed against the HBc antigen (Krugman *et al.*, 1974), although occasionally anti-*pol*, the antibody against the *POL* gene product, may be found prior to anti-HBc (anti-*pol* is not a routine test). It is followed next by anti-HBe and then by anti-HBs. The antibodies directed against the viral surface antigen are neutralizing and protective. Approximately 1–10% of infected adults, 20–30% of young children, and 90% of neonates vertically infected by a carrier mother will not develop anti-HBs. The failure to mount an immune response against the viral surface protein appears to increase the probability of developing chronic hepatitis infection. Men have a greater likelihood of becoming carriers than women and, in addition, immunosuppressed and immunodeficient patients are more likely to develop chronicity. The chronic carrier state can be asymptomatic (without active viral replication, no HBeAg, low infectivity) or symptomatic [virus replication, HBeAg present, anti-HBe undetectable, high infectivity (Realdi *et al.*, 1980; Hoofnagle *et al.*, 1981)].

One of the most important questions is why some people do not clear the virus. It seems that in some cases HBV infection may cause the host to become tolerant to one or more of the viral antigens: infection can lead to the production of large amounts of HBsAg, which is found in non-infectious particles that have no internal structure and occur as small spheres or long filaments of 22 nm radius, and are over a thousand times more abundant in the serum of carriers than the virus itself (Ganem & Varmus, 1987). It may be that this prevents an effective immune response by anti-HBs against the whole virus particles. However, it still seems difficult to understand why carriers are negative for anti-HBs despite the large amounts of surface antigen present. The release of HBeAg by virus replicating cells has been discussed as a strategy for tolerizing circulating T cells that would normally recognize epitopes in the viral core proteins and thereby mount an immune response against infected cells, the suggestion being that when T cells become exposed to high concentrations of

antigens, rather than the small amounts offered to them by antigen-presenting cells, they may lose their ability to respond to their specific antigen. It is possible, therefore, that tolerization could be the strategy employed by HBV to establish a persistent infection.

Nonetheless this still would not account for the considerable variability in the outcome of infection in different individuals, and it is with this variability in mind that we present our analysis of a cellular model, first put forward by London & Blumberg in 1982. In our analysis we show that it is only necessary to have two generalized populations of cells with differing behaviour, assumed to be undifferentiated and differentiated hepatocytes, in order to account for many of the different outcomes of infection by HBV. In addition we relate our model to the role of HBV in the pathogenesis of PHC. Recent experiments with transgenic mice (Kim *et al.*, 1991) have shown that the viral *HBx* gene can induce liver cancer. These transgenic animals contain the *HBx* gene and its regulatory elements in the germline, with the HBx protein being observed in liver, kidney and testis. Interestingly, and consistent with the London–Blumberg model, the *HBx* gene is expressed only in a subset of hepatocytes that are suggested by Kim *et al.* to be related to a specific differentiation state, the histological changes in those hepatocytes being shown to be associated with the *HBx* gene expression.

2. The Model

According to the London & Blumberg (1982) model the liver is considered to contain two populations of cells, which were designated R cells (for resistant to replication) and S cells (for susceptible to replication). The R cells are assumed to be less mature, undifferentiated cells, whereas the S cells are more mature and differentiated hepatocytes. Thus it is taken that the R cells can divide and differentiate further, into either R cells or S cells, but the S cells, being more mature, can only divide one or two times, and only into S cells. The important point to note in this model is the different response of the two cell types upon infection by the hepatitis B virus: it was proposed that R cells are resistant to productive HBV infection, while S cells can allow active virus replication to take place. Thus it is assumed that the S cells will have a greatly reduced survival probability, not only because they may incur a stronger cytotoxic T cell response but also because virus production may compromise the host-cell metabolism. This destruction of S cells provides a stimulus for further cell division, mostly by R cells. Integration of HBV DNA can occur in both R cells and S cells, but because of the reduced life-expectancy of S cells it is of greater importance in R cells, where it may be of tumourigenic significance. The implication is that the increased proliferation of the R cells, stimulated by the death of infected S cells, may allow the integrated DNA to increase the expression of a gene that creates a long-lived infected cell with a large capacity to divide. Secondarily, rapid division of the cells with integrated HBV genomes may increase the probability of other mutational events. The integrated viral DNA may at some later stage then lead to a transformation of some R cells into tumour cells. In addition we assume

that phenotypic transformation of an infected R cell will prevent it from thereafter differentiating into an S cell.

Because of the different nature of the two cell types, we expect that the liver in the fetus and the newborn will consist mostly of R cells, and thus would not support active replication of HBV. Cell division and differentiation in children leads to more and more S cells, until in the adult the liver will be composed mostly of S cells that would allow active HBV replication to take place. In this paper we show that this assumption may explain the different outcome of HBV infection in children and in adults.

3. Mathematical Analysis

3.1. NOTATION

t, time.
R, concentration of uninfected R cells.
S, concentration of uninfected S cells.
R', concentration of infected R cells.
S', concentration of infected S cells.
$N=R+S+R'+S'$, total concentration of cells.
V, concentration of free virus.
$F(N)$, natural growth rate of R cells.
$G(N)$, natural growth of S cells.
E, as subscript denotes equilibrium state.
$F_E=F(N_E)$.
$G_E=G(N_E)$.
$F'_E=\mathrm{d}F/\mathrm{d}N|_{N=N_E}$.
$G'_E=\mathrm{d}G/\mathrm{d}N|_{N=N_E}$.
μ_1, natural mortality rate of R cells.
μ_2, natural mortality rate of S cells.
b, transmission coefficient (probability of cell infection by the free virus).
m_1, additional death rate of infected R cells caused by immune responses.
m_2, additional death rate of infected S cells caused by immune responses.
h_1, replication rate of virus in infected R cells.
h_2, replication rate of virus in infected S cells.
n, natural degeneration rate of the free virus.
S_0, initial concentration of uninfected S cells.
S_C, critical value of S_0, determining initial immunity.
S_A, critical value of S_0, determining occurrence of an acute attack.

3.2. THE EQUATIONS AND RELEVANT CONDITIONS

Our model consists of five ordinary differential equations describing the rates of change at time t of the five variables $R=R(t)$, $S=S(t)$, $R'=R'(t)$, $S'=S'(t)$ and $V=V(t)$, representing the concentration of uninfected R cells, uninfected S cells, infected

R cells, infected S cells and free virus, respectively:

$$\frac{dR}{dt} = (F(N) - \mu_1)R - bVR, \tag{1}$$

$$\frac{dS}{dt} = (G(N) - \mu_2)S - bVS, \tag{2}$$

$$\frac{dR'}{dt} = bVR + (F(N) - \mu_1)R' - m_1R', \tag{3}$$

$$\frac{dS'}{dt} = bVS + (G(N) - \mu_2)S' - m_2S', \tag{4}$$

$$\frac{dV}{dt} = h_1R' + h_2S' - bV(R+S) - nV. \tag{5}$$

The natural growth rates (cell division) of the R and S cells are represented by $F(N)$ and $G(N)$, respectively, and their natural mortality rates by the coefficients μ_1 and μ_2. The cells cannot reproduce unboundedly, so in order to make their growth rates density limited we choose F and G to be monotonically decreasing functions of $N = N(t)$, where $N = R + S + R' + S'$ is the total cell density at time t. R cells are infected by the virus at a rate bVR, and S cells at a rate bVS, where the transmission coefficient b is taken to be the same for both cell types. The shortened survival probability of infected cells is represented by the terms with coefficients m_1 and m_2, for the infected R and infected S cells, respectively, and the replicative ability of the virus in each cell type by the terms with coefficients h_1 and h_2, respectively. The natural degeneration of the virus when outside the cells is represented by the term with coefficient n. The parameters μ_1, μ_2, m_1, m_2, h_1, h_2, b and n are all positive.

Without loss of generality we take the cell and virus concentrations to be scaled such that in the uninfected system the total cell concentration is $N = 1$. This can be achieved by setting

$$F(1) = \mu_1, \tag{6}$$

$$G(1) = \mu_2. \tag{7}$$

This also gives us our initial conditions:

$$R(0) + S(0) = 1, \tag{8}$$

$$R'(0) = 0 = S'(0), \tag{9}$$

$$V(0) = V_0. \tag{10}$$

We will denote the initial concentration of S cells by $S(0) = S_0$.

In our model we are assuming that the survival expectancy of the infected S cells is greatly reduced compared to that of infected R cells, and also that S cells allow a

far higher rate of active replication of the virus to occur than in R cells. Thus we have

$$m_1 \ll m_2, \tag{11}$$

$$h_1 \ll h_2. \tag{12}$$

In addition we are assuming that S cells are more mature than R cells, being more fully differentiated hepatocytes, and thus that the capacity for S cells to divide further is limited compared to that of R cells. This means that in our model we take

$$G(N) \ll F(N). \tag{13}$$

3.3. EQUILIBRIUM STATES

Let $N=(R, S, R', S')$ be the vector of cell concentrations, thus enabling the five dynamic variables to be represented by $(N; V)$. The possible steady states are as follows:

(i) $(N; V)=(R_E, S_E, 0, 0; 0)$
(ii) $(N; V)=(0, 0, R'_E, 0; V_E)$
(iii) $(N; V)=(R_E, 0, R'_E, 0; V_E)$
(iv) $(N; V)=(0, 0, 0, S'_E; V_E)$
(v) $(N; V)=(0, S_E, 0, S'_E; V_E)$
(vi) $(N; V)=(R_E, 0, R'_E, S'_E; V_E)$
(vii) $(N; V)=(0, S_E, R'_E, S'_E; V_E)$
(viii) $(N; V)=(R_E, S_E, R'_E, S'_E; V_E)$,

where the subscript E denotes the equilibrium state, and a zero indicates that particular cell type is not present. We will now show that the majority of these states can be discounted on grounds of either instability or dynamical inaccessibility.

First note that since $F(N)$ and $G(N)$ are monotonically decreasing functions and because of the conditions (6) and (7) we have

$$\left.\begin{aligned} F(N)-\mu_1>0 \\ G(N)-\mu_2>0 \end{aligned}\right\} N<1,$$
$$\left.\begin{aligned} F(N)-\mu_1<0 \\ G(N)-\mu_2<0 \end{aligned}\right\} N>1. \tag{14}$$

Consider the total number of cells

$$\frac{dN}{dt}=\frac{dR}{dt}+\frac{dS}{dt}+\frac{dR'}{dt}+\frac{dS'}{dt}$$
$$=(F(N)-\mu_1)(R+R')+(G(N)-\mu_2)(S+S')-m_1R'-m_2S'. \tag{15}$$

Using eqn (14), and remembering that initially $N=1$, we can see that for all the time while the system is infected we must have $N<1$. We assume that the net reproductive rate for R cells is faster than for S cells, that is

$$G(N)-\mu_2<F(N)-\mu_1.$$

But we have just shown that as long as the system is infected $N<1$, and thus for all time while the system is infected we know that

$$0<G(N)-\mu_2<F(N)-\mu_1. \tag{16}$$

It is now clear that as long as the virus is present

$$\frac{1}{S}\frac{dS}{dt}<\frac{1}{R}\frac{dR}{dt}. \tag{17}$$

This means that given our initial conditions it is not possible for us to have an equilibrium in which $S>0$ and $V>0$. This removes (v), (vii) and (viii) from our list of possible steady states.

Now compare the dynamics of $S'$and R'. Taking $S\to 0$ as just discussed we have

$$\begin{aligned}\frac{1}{S'}\frac{dS'}{dt}&=(G(N)-\mu_2)-m_2\\ &<(F(N)-\mu_1)-m_2 \qquad \text{by (16)}\\ &<(F(N)-\mu_1)-m_1 \qquad \text{by (11)}\\ &<(F(N)-\mu_1)-m_1+bV\frac{R}{R'}\\ &=\frac{1}{R'}\frac{dR'}{dt}.\end{aligned}$$

Thus, once S has become small, we have

$$\frac{1}{S'}\frac{dS'}{dt}<\frac{1}{R'}\frac{dR'}{dt}, \tag{18}$$

and therefore it is not possible for us to have an equilibrium in which $S'>0$. This excludes (iv) and (vi) from being allowable steady states, leaving us only with the following cases:

(i) $(N; V)=(R_E, S_E, 0, 0; 0)$
(ii) $(N; V)=(0, 0, R'_E, 0; V_E)$
(iii) $(N; V)=(R_E, 0, R'_E, 0; V_E)$.

In the next section we shall investigate the existence and stability of these remaining states.

3.4. LINEAR STABILITY ANALYSIS

(i) $(N; V)=(R_E, S_E, 0, 0; 0)$,

where

$$R_E+S_E=1. \tag{19}$$

We investigate the linear stability by considering small perturbations to the system in the vicinity of the steady state. Let

$$\begin{aligned} R &= R_E + r, \\ S &= S_E + s, \\ R' &= R'_E + r', \\ S' &= S'_E + s', \\ V &= V_E + v. \end{aligned} \tag{20}$$

where r, s, r', s' and v are small. Substituting into eqns (1–5) and retaining only linear terms gives us the linearized system

$$\frac{dr}{dt} = (F_E - \mu_1)r + F'_E R_E(r+s+r'+s') - bV_E r - bR_E v, \tag{21}$$

$$\frac{ds}{dt} = (G_E - \mu_2)s + G'_E S_E(r+s+r'+s') - bV_E s - bS_E v, \tag{22}$$

$$\frac{dr'}{dt} = (F_E - \mu_1)r' + F'_E R'_E(r+s+r'+s') + bV_E r + bR_E v - m_1 r', \tag{23}$$

$$\frac{ds'}{dt} = (G_E - \mu_2)s' + G'_E S'_E(r+s+r'+s') + bV_E s + bS_E v - m_2 s', \tag{24}$$

$$\frac{dv}{dt} = h_1 r' + h_2 s' - bV_E(r+s) - b(R_E + S_E)v - nv, \tag{25}$$

where

$$F_E = F(N_E); \qquad G_E = G(N_E),$$

$$F'_E = \left.\frac{dF}{dN}\right|_{N=N_E}; \qquad G'_E = \left.\frac{dG}{dN}\right|_{N=N_E}.$$

In our current case we can use the fact that $R' = S' = V = 0$, and the conditions (6), (7) and (19), to reduce the system to

$$\frac{dr}{dt} = F'_E R_E(r+s+r'+s') - bR_E v, \tag{26}$$

$$\frac{ds}{dt} = G'_E S_E(r+s+r'+s') - bS_E v, \tag{27}$$

$$\frac{dr'}{dt} = bR_E v - m_1 r', \tag{28}$$

$$\frac{ds'}{dt} = bS_E v - m_2 s', \tag{29}$$

$$\frac{dv}{dt} = h_1 r' + h_2 s' - (b+n)v. \tag{30}$$

Note that the last three equations decouple from the other two: for the moment we will look at these three equations on their own. We look for solutions of the form

$$(r', s', v) \propto e^{\lambda t}. \tag{31}$$

Hence

$$\begin{pmatrix} \lambda + m_1 & 0 & -bR_E \\ 0 & \lambda + m_2 & -bS_E \\ -h_1 & -h_2 & \lambda + b + n \end{pmatrix} \begin{pmatrix} r' \\ s' \\ v \end{pmatrix} = \begin{pmatrix} 0 \\ 0 \\ 0 \end{pmatrix}. \tag{32}$$

The solvability condition for (32) is

$$\begin{vmatrix} \lambda + m_1 & 0 & -bR_E \\ 0 & \lambda + m_2 & -bS_E \\ -h_1 & -h_2 & \lambda + b + n \end{vmatrix} = 0, \tag{33}$$

which can be written as the cubic

$$\lambda^3 + a\lambda^3 + c\lambda + d = 0, \tag{34}$$

where

$$a = m_1 + m_2 + b + n,$$

$$c = (m_1 + m_2)(b+n) - b(h_1 + S_E(h_2 - h_1)) + m_1 m_2,$$

$$d = m_1 m_2 (b+n) - b(h_1 m_2 + S_E(h_2 m_1 - h_1 m_2)).$$

For the case $d < 0$ there will always be instability with respect to perturbations in R', S' and V. For the case $d > 0$ it is easy to show that we will also have $c > 0$, and that $ac > d$, and hence that there are no roots with positive real part. Thus we have that if $d > 0$ there will be stability with respect to perturbations in R', S' and V.

Now we look at eqns (26) and (27). The only case of interest is when the perturbations in R', S' and V are stable, and thus we have the system

$$\frac{dr}{dt} = F_E' R_E (r+s),$$

$$\frac{ds}{dt} = G_E' S_E (r+s).$$

Note that

$$\frac{d(r+s)}{dt} = (F_E' R_E + G_E' S_E)(r+s),$$

and thus, since $F'_E<0$ and $G'_E<0$, that $r+s\to 0$. We therefore have neutral stability in the $R+S=1$ plane. Hence overall we have that if

$$1+\frac{n}{b}>\frac{h_1}{m_1}+S_E\left(\frac{h_2}{m_2}-\frac{h_1}{m_1}\right) \tag{35}$$

then the system is stable with respect to the $R+S=1$ plane (i.e. the virus will be wiped out), and if

$$1+\frac{n}{b}<\frac{h_1}{m_1}+S_E\left(\frac{h_2}{m_2}-\frac{h_1}{m_1}\right) \tag{36}$$

then the system is unstable with respect to the $R+S=1$ plane (i.e. the virus is able to invade).

(ii) $(N; V)=(0, 0\ R'_E, 0; V_E)$,

where

$$V_E=\frac{h_1}{n}R'_E,$$

$$F_E=\mu_1+m_1. \tag{37}$$

We have previously established that for any equilibrium when the system is infected the variables S and S' will both converge to zero, thus in this case the linearized system (21–25) reduces to

$$\frac{\mathrm{d}r}{\mathrm{d}t}=m_1 r-bV_E r, \tag{38}$$

$$\frac{\mathrm{d}r'}{\mathrm{d}t}=bV_E r+F'_E R'_E(r+r'), \tag{39}$$

$$\frac{\mathrm{d}v}{\mathrm{d}t}=h_1 r'-bV_E r-nv. \tag{40}$$

Note that if $r=0$ then, since $F'_E<0$, $r'\to 0$, and thus that $v\to 0$ also. Hence the stability depends only on the $\mathrm{d}r/\mathrm{d}t$ equation: the state will be stable if $m_1<bV_E$, and unstable if $m_1>bV_E$. We expect that $R'_E=N_E\approx 1$, and so we know that the steady state in which only infected R cells and virus are present will be

$$\text{stable if}\quad \frac{n}{b}<\frac{h_1}{m_1},$$

$$\text{unstable if}\quad \frac{n}{b}>\frac{h_1}{m_1}. \tag{41}$$

(iii) $(N; V)=(R_E, 0, R'_E, 0; V_E)$,

where we need to satisfy

$$0 = F_E - \mu_1 - bV_E, \tag{42}$$

$$0 = bV_E R_E + (F_E - \mu_1)R'_E - m_1 R'_E, \tag{43}$$

$$0 = h_1 R'_E - (bR_E + n)V_E. \tag{44}$$

Substituting (42) into (43) gives

$$bV_E(R_E + R'_E) = m_1 R'_E.$$

We expect that $R_E + R'_E = N_E \approx 1$, and so we have

$$V_E \approx \frac{m_1}{b} R'_E. \tag{45}$$

Upon substituting this into (44) we obtain

$$R_E \approx \frac{h_1}{m_1} - \frac{n}{b}, \tag{46}$$

and hence that

$$R'_E \approx 1 + \frac{n}{b} - \frac{h_1}{m_1}, \tag{47}$$

and

$$V_E \approx \frac{m_1}{b}\left(1 + \frac{n}{b} - \frac{h_1}{m_1}\right). \tag{48}$$

Since we require that $0 < R_E < 1$ this equilibrium state will only exist if

$$\frac{n}{b} < \frac{h_1}{m_1} < 1 + \frac{n}{b}. \tag{49}$$

We will now demonstrate that this particular equilibrium is always unstable.

As before we know that the S and S' variables will always converge to zero, and so in this case the linearized system (21–25) reduces to

$$\frac{dr}{dt} = F'_E R_E(r + r') - bR_E v, \tag{50}$$

$$\frac{dr'}{dt} = bR_E v + bV_E r + (bV_E - m_1)r' + F'_E R'_E(r + r'), \tag{51}$$

$$\frac{dv}{dt} = h_1 r' - bV_E r - (bR_E + n)v. \tag{52}$$

Again we look for solutions of the form

$$(r', s', v) \propto e^{\lambda t}. \tag{53}$$

Hence

$$\begin{pmatrix} \lambda - R_E F_E' & -R_E F_E' & bR_E \\ -bV_E - R_E' F_E' & \lambda + m_1 - bV_E - R_E' F_E' & -bR_E \\ bV_E & -h_1 & \lambda + bR_E + n \end{pmatrix} \begin{pmatrix} r \\ r' \\ v \end{pmatrix} = \begin{pmatrix} 0 \\ 0 \\ 0 \end{pmatrix}. \tag{54}$$

The solvability for (54) is

$$\begin{vmatrix} \lambda - R_E F_E' & -R_E F_E' & bR_E \\ -bV_E - R_E' F_E' & \lambda + m_1 - bV_E - R_E' F_E' & -bR_E \\ bV_E & -h_1 & \lambda + bR_E + nn \end{vmatrix} = 0. \tag{55}$$

This expression can be written in the form of the cubic

$$\lambda^3 + a\lambda^2 + c\lambda + d = 0, \tag{56}$$

where, after a little algebra [using eqns (45) and (47), and the approximation $N_E \approx 1$],

$$a = n + (b + m_1)R_E - N_E F_E',$$

$$c = (n + bR_E)(m_1 R_E - N_E F_E') - m_1 R_E F_E' - bR_E(h_1 + bV_E),$$

$$d = m_1 bR_E(nV_E + R_E' F_E').$$

Note that, since $F_E' < 0$, $a > 0$. Also we know that the concentration of the virus is usually far smaller than that of the cells, and so we may take that $d < 0$. Thus this equilibrium state will always be unstable.

3.5. PARAMETER REGIMES

Equation (36), the condition for the initial growth of the infection, can be used to define an initial concentration of S cells,

$$S_C = \left(1 + \frac{n}{b} - \frac{h_1}{m_1}\right) \Big/ \left(\frac{h_2}{m_2} - \frac{h_1}{m_1}\right), \tag{57}$$

which is critical to the outcome of the disease. Using this together with the results so far obtained we can predict the system behaviour for various parameter values.

(i) $h_1/m_1 > h_2/m_2$.

(a) $S_C < 0$:
the virus cannot invade.

(b) $0 < S_C < 1$:
if $S_0 < S_C$ the virus will invade, and because of the condition (17) the system will always end up with only infected R cells present; if $S_0 > S_C$ the virus will not invade.

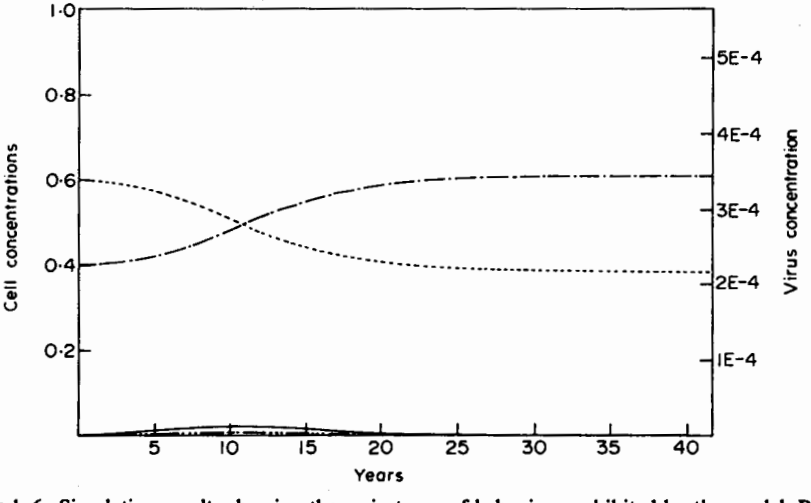
(c) $1 < S_C$:
the virus will always invade, and the system will always end up with only infected R cells present.

(ii) $h_1/m_1 < h_2/m_2$.

(a) $S_C < 0$:
the virus can always invade and the system will always end up with only infected R cells present.

(b) $0 < S_C < 1$ *and* $n/b < h_1/m_1$:
if $S_0 < S_C$ the virus will not invade;
if $S_0 > S_C$ then the equilibrium when the system is infected and the equilibrium when the system is uninfected are both stable. Which of the two the system will end up in cannot be predicted with certainty, but we expect that as $h_1/m_1 \to n/b$ it is more likely to rid itself of the virus, and as $h_1/m_1 \to 1 + n/b$ it is more likely to become persistently infected. Note that this is the regime where the unstable steady state (iii) exists. This state is a form of saddle point and so it is possible that the system may under certain circumstances spend some time in the vicinity of this intermediary state.

(c) $0 < S_C < 1$ *and* $n/b > h_1/m_1$:
if $S_0 < S_C$ the virus will not invade;
if $S_0 > S_C$ the virus can invade, but because of the condition (41) the



FIGS 1–6. Simulation results showing the main types of behaviour exhibited by the model. Parameter values used: $n = 0{\cdot}03$, $b = 0{\cdot}02$, $h_1 = 3 \times 10^{-5}$, $m_1 = M \times 10^{-5}$, $h_2 = 5 \times 10^{-3}$, $m_2 = M \times 10^{-3}$, $F(N) = 0{\cdot}1/(1{\cdot}0 + N^2)$, $G(N) = 10^{-3}/(1{\cdot}0 + N^2)$, $\mu_1 = 5 \times 10^{-2}$, $\mu_2 = 5 \times 10^{-4}$. FIG. 1. Subclinical recovery. $M = 1{\cdot}6$, $S_0 = 0{\cdot}6$. The virus initially invades but is then successfully eliminated by the immune system. The maximum virus concentration during the course of infection is not enough to cause clinical symptoms. After having recovered the patient is then immune to further infection. (——), free virus; (· · · · ·), S cells; (— · —), R cells; (- - -), infected S; (— · · · —) infected R.

system will always eventually rid itself of the infection. Note that when this happens it leaves the system in an immune state—the virus cannot reinvade.

(d) $1 < S_C$:
the virus cannot invade.

3.6. THE ACUTE CASE

Since our model was designed in part to explain the increased incidence of acute hepatitis in older subjects it is worth considering whether we, by using our model, can predict the occurrence of an acute attack.

Acute cases always occur soon after infection, compared to the incubation period for symptomatic chronic disease. This suggests that we may make the following approximations: $R \approx 1 - S_0$; $S \approx S_0$; $N \approx 1$. In addition we assume that R' and S' are of the same magnitude and thus, because of the condition (12), that $h_1 R' \ll h_2 S'$. Hence we consider the system

$$\frac{dR}{dt} = -b(1 - S_0)V, \tag{58}$$

$$\frac{dS}{dt} = -bS_0 V, \tag{59}$$

$$\frac{dR'}{dt} = b(1 - S_0)V - m_1 R', \tag{60}$$

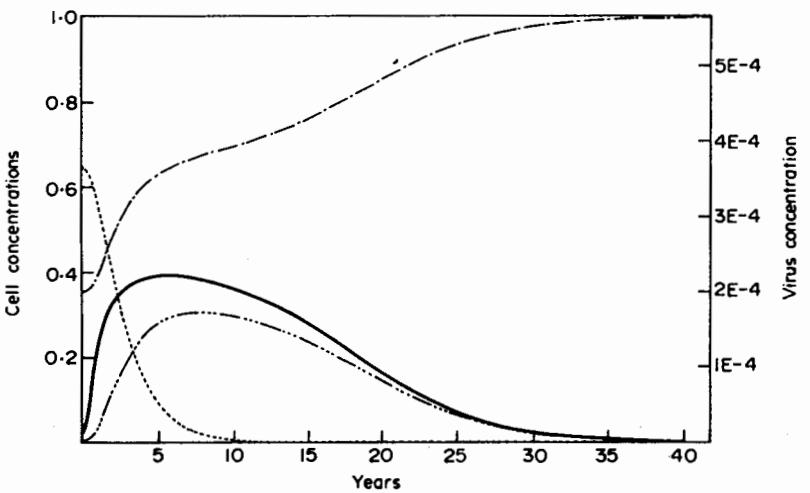


FIG. 2. Recovery preceded by relapse. $M = 1{\cdot}4$, $S_0 = 0{\cdot}65$. The virus initially invades but is then successfully eliminated by the immune system. The maximum virus concentration is higher than in Fig. 1, possibly being large enough to cause clinical symptoms. See Fig. 1 legend for key.

$$\frac{dS'}{dt} = bS_0V - m_2S', \tag{61}$$

$$\frac{dV}{dt} = h_2S' - (b+n)V. \tag{62}$$

Note that the last two equations decouple, and will determine the dynamics of the system.

In order to have the positive feedback that will give the explosion in virus concentration that is characteristic of an acute attack of HBV we require that $dS'/dt > 0$ and $dV/dt > 0$. This will be the case if

$$\frac{S'}{V} < \frac{b}{m_1} S_0$$

and

$$\frac{n+b}{h_2} < \frac{S'}{V}.$$

Combining these we obtain the criterion that:

if $S_0 < S_A$ the disease will not have an acute phase;

if $S_0 > S_A$ the disease will become acute soon after infection,

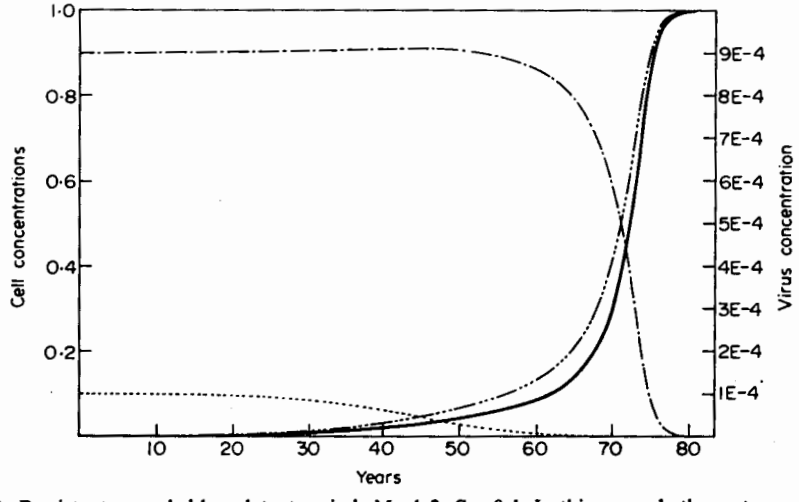


FIG. 3. Persistent preceded by a latent period. $M = 1{\cdot}2$, $S_0 = 0{\cdot}1$. In this example the system eventually consists of nothing but infected R cells. This is the situation with which we associate the occurrence of PHC. Before this occurs there is a long period of time during which there is negligible noticeable activity, so that the patient may well be unaware of having the virus prior to the onset of PHC. See Fig. 1 legend for key.

where

$$S_A = \frac{m_2}{h_2}\left(1+\frac{n}{b}\right). \tag{63}$$

4. Numerical Results

The model eqns (1–5) were solved numerically using a simple Euler scheme with initially no infected cells present. For the examples given here the forms used for $F(N)$ and $G(N)$ were $F(N)=f_1/(f_2+N^2)$ and $G(N)=g_1/(g_2+N^2)$. Other qualitatively similar forms were also tried, the same results being obtained. The simulation was started by adding a small concentration of virus into the system, so that

$$V(0)=10^{-3},$$
$$R(0)=1-S_0,$$
$$S(0)=S_0,$$
$$R'(0)=0,$$
$$S'(0)=0,$$

where, since we are assuming that the initial proportion of infected S cells is not fixed, S_0 was allowed to vary between 0 and 1. All the example plots shown here

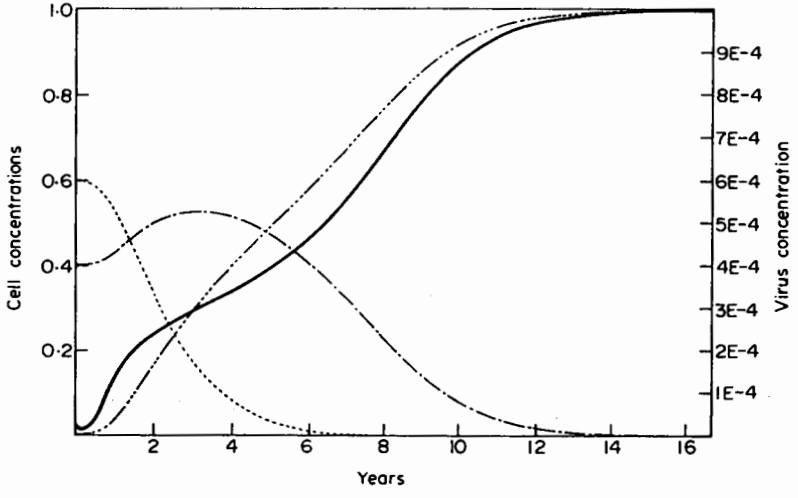


FIG. 4. Persistent without a latent period. $M=1{\cdot}3$, $S_0=0{\cdot}6$. The system eventually becomes overwhelmed by infected R cells. However, unlike in Fig. 3, the saturation with infected R cells occurs relatively quickly, without being preceded by a long latent period. See Fig. 1 legend for key.

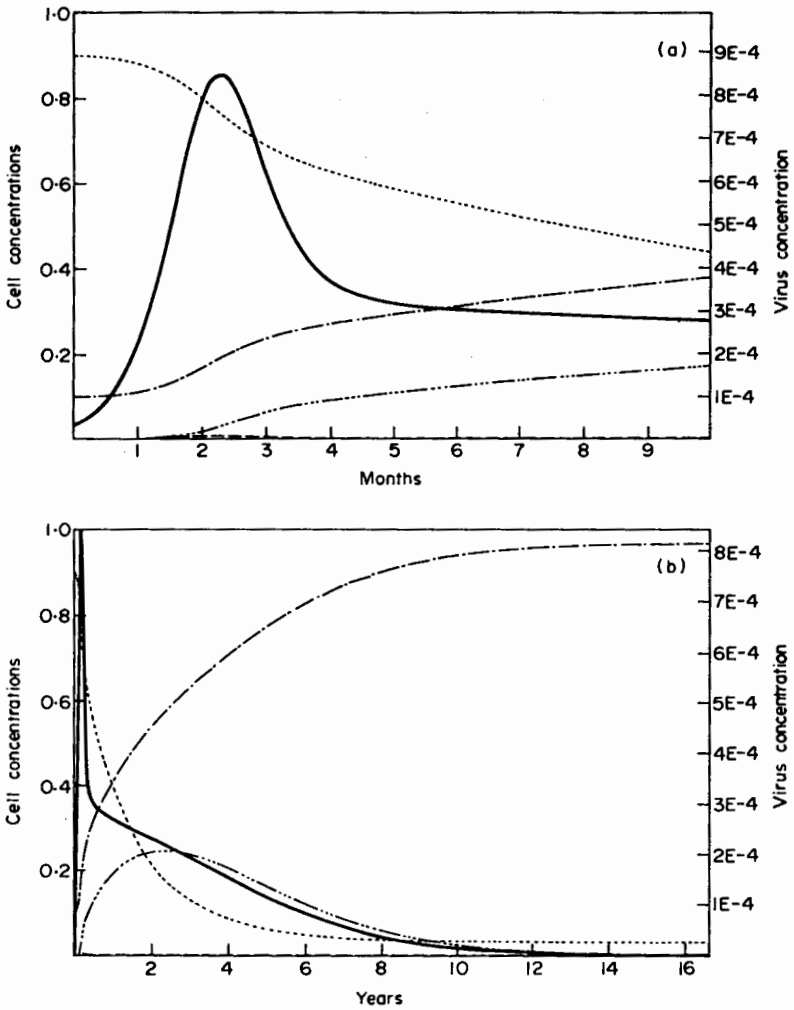


FIG. 5. Acute followed by recovery. (a) Initial behaviour. (b) Overall behaviour. $M=1{\cdot}6$, $S_0=0{\cdot}9$. Initially there is a sudden sharp increase in the virus concentration, which peaks and then drops again. This occurs on a time scale of a few months, as compared to the tens of years necessary for the onset of cancer shown in Figs 3 and 4. After this clinically detectable attack the virus is then eliminated as in Fig. 2, leaving the patient immune to further infection. See Fig. 1 legend for key.

were produced using the following parameter values:

$$n=0{\cdot}03; \quad h_1=3\times10^{-5}; \quad h_2=5\times10^{-3};$$
$$b=0{\cdot}02; \quad m_1=M\times10^{-5}; \quad m_2=M\times10^{-3};$$
$$f_1=0{\cdot}1; \quad f_2=1{\cdot}0; \quad \mu_1=5\times10^{-2};$$
$$g_1=10^{-3}; \quad g_2=1{\cdot}0; \quad \mu_2=5\times10^{-4}.$$

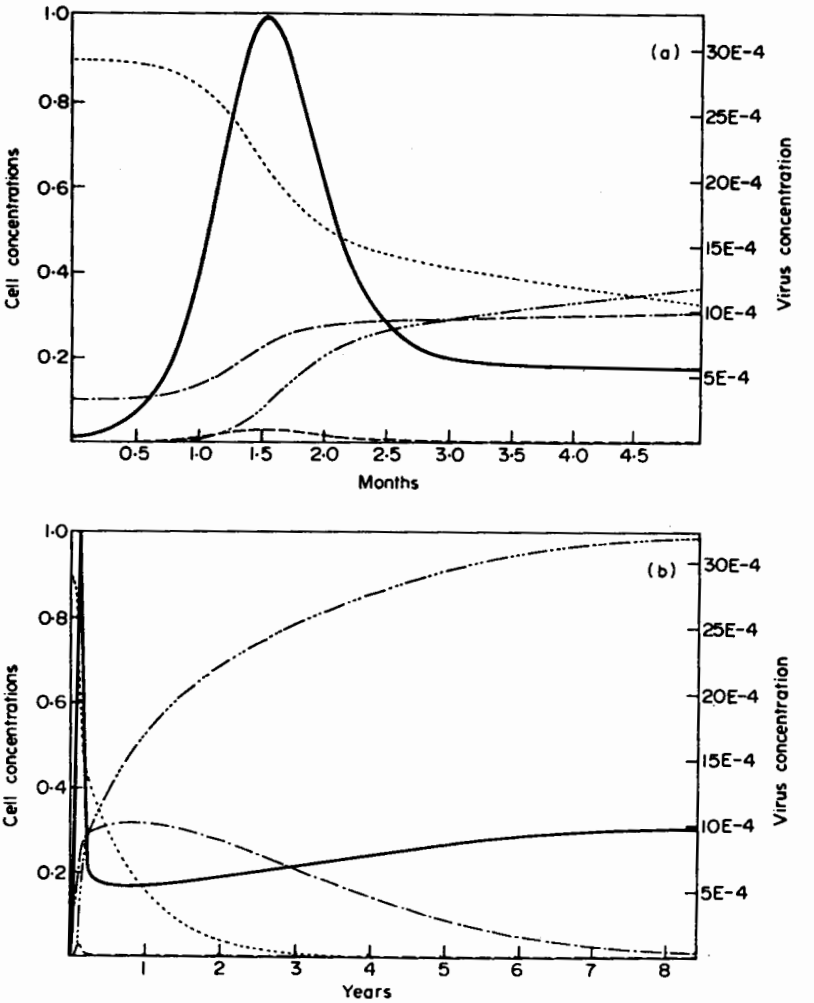


FIG. 6. Acute followed by persistent. (a) Initial behaviour. (b) Overall behaviour. $M = 1{\cdot}4$, $S_0 = 0{\cdot}9$. Initially there is a sudden sharp increase in the virus concentration, which peaks and then drops again. However, unlike in Fig. 5, after the initial attack the system then becomes saturated by infected R cells as in Fig. 4. A severe attack is never followed by a long latent period of the sort shown in Fig. 3. See Fig. 1 legend for key.

The parameter M is used as a means of varying the immune response, the assumption being that in younger subjects the immune system is less fully developed so that the magnitude of both m_1 and m_2 will be less than in fully grown adults.

The different behaviour exhibited by the simulation under different parameter regimes [Figs 1–6] was as predicted by our analysis, and may be summarized by two phase diagrams, one for $h_1/m_1 < h_2/m_2$ and the other for $h_1/m_1 > h_2/m_2$. These are

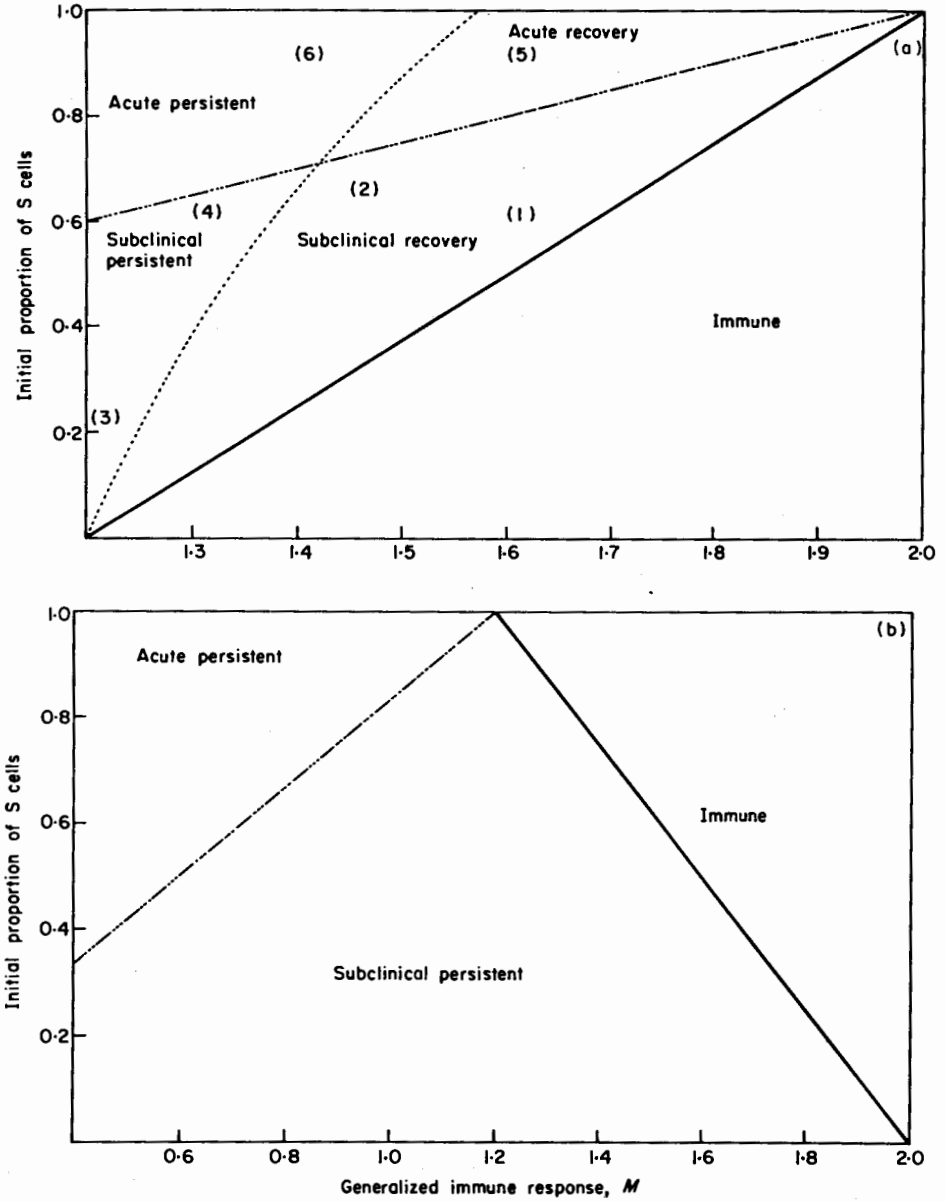

1·0
0·8
0·6
0·4
0·2
Initial proportion of S cells
(6)
Acute recovery
(5)
(a)
Acute persistent
(2)
(4)
(1)
Subclinical persistent
Subclinical recovery
Immune
(3)
1·3
1·4
1·5
1·6
1·7
1·8
1·9
2·0
(b)
Acute persistent
Immune
Subclinical persistent
0·6
0·8
1·0
1·2
1·4
1·6
1·8
2·0
Generalized immune response, M

FIG. 7

shown in Figs 7(a) and (b). Note however that these phase diagrams are two-dimensional representations of a three-dimensional phase space, the immune responses m_1 and m_2 having been tied together in the variable M. In the full three-dimensional diagram the persistent/transient dividing line will depend only on h_1/m_1, and the acute/non-acute dividing line will depend only on h_2/m_2. The independence of these two effects is demonstrated in the next phase diagrams, given in Figs 8(a) and (b), where the behaviour of the system as a function of the immune response against the R and S cells is shown for different fixed values of S_0. Note that the word persistent is being used in this context to describe those cases when the final state of the system is the infected equilibrium, that is, those cases when the system will end up with only infected R cells present. The different regions marked on the phase diagrams are described in the captions to Figs 1–6. In Figs 1–6 note that the magnitude of the cell concentrations are given on the left-hand axis, and that the concentration of the free virus is given on a different scale on the right-hand axis.

5. Discussion

The mathematical analysis presented in this paper helps to explain how the London–Blumberg model can be used to explain the many different outcomes of infection with HBV. Central to the model is the assumption that the liver contains two cell types with differing abilities to support HBV replication, the undifferentiated R cells being less permissive for active virus replication than the more fully differentiated S cells. The virus itself is said to be only weakly cytopathic, and thus most of the damage to the liver is mediated by cytotoxic T lymphocytes directed at infected S cells. The extensive death of S cells then allows for the proliferation of the immature R cells. Virus integration can occur in both R and S cells, but may be relevant only in those R cells that have a longer life span and a higher potential to divide, and it therefore seems likely that transformation of the integrated genome occurs in R cells.

FIG. 7. Diagrams showing the expected outcome of infection for differing initial concentrations of S cells, and differing strengths of the overall immune response. In this case the immune responses against the infected R and S cells have been taken to be proportional: explicitly, $m_1 = M \times 10^{-5}$ and $m_2 = M \times 10^{-3}$. (a) Phase diagram for $h_1/m_1 < h_2/m_2$. The numbered areas on the diagrams refer to Figs 1–6. The assumption of our model is that the more fully differentiated S cells will be scarce in the newborn and in infants. Together with a weak immune system (low M) this places them on the lower left-hand area of the diagram, where the expected outcome of infection is a subclinical chronic carrier state that will eventually develop into PHC. As the age of first infection increases the proportion of S cells increases, and the immune response becomes more effective. This leads us across the diagram moving to the right and upwards, until for adults we are in the area on the top right-hand side (almost all S cells and a strong immune response) for which the most likely outcome is an acute attack followed by complete recovery. During infection the number of infected S cells drops, so that if recovery occurs the patient is then left in the area of the diagram characterized by immunity to further infection. (Parameter values used are as for Figs 1–6.) (b) Phase diagram for $h_1/m_1 > h_2/m_2$. In this parameter region recovery from infection is impossible. The probability that the virus establishes an infection decreases as the initial proportion of S cells increases. This happens because $h_1/m_1 > h_2/m_2$ implies that more free virus particles are released from R cells than from S cells. This seems biologically unlikely, since we expected h_1 to be very small. The diagram is only included for the sake of completeness. (Parameter values used are as for Figs 1–6, except that $h_1 = 5 \times 10^{-5}$ and $h_2 = 3 \times 10^{-3}$.)

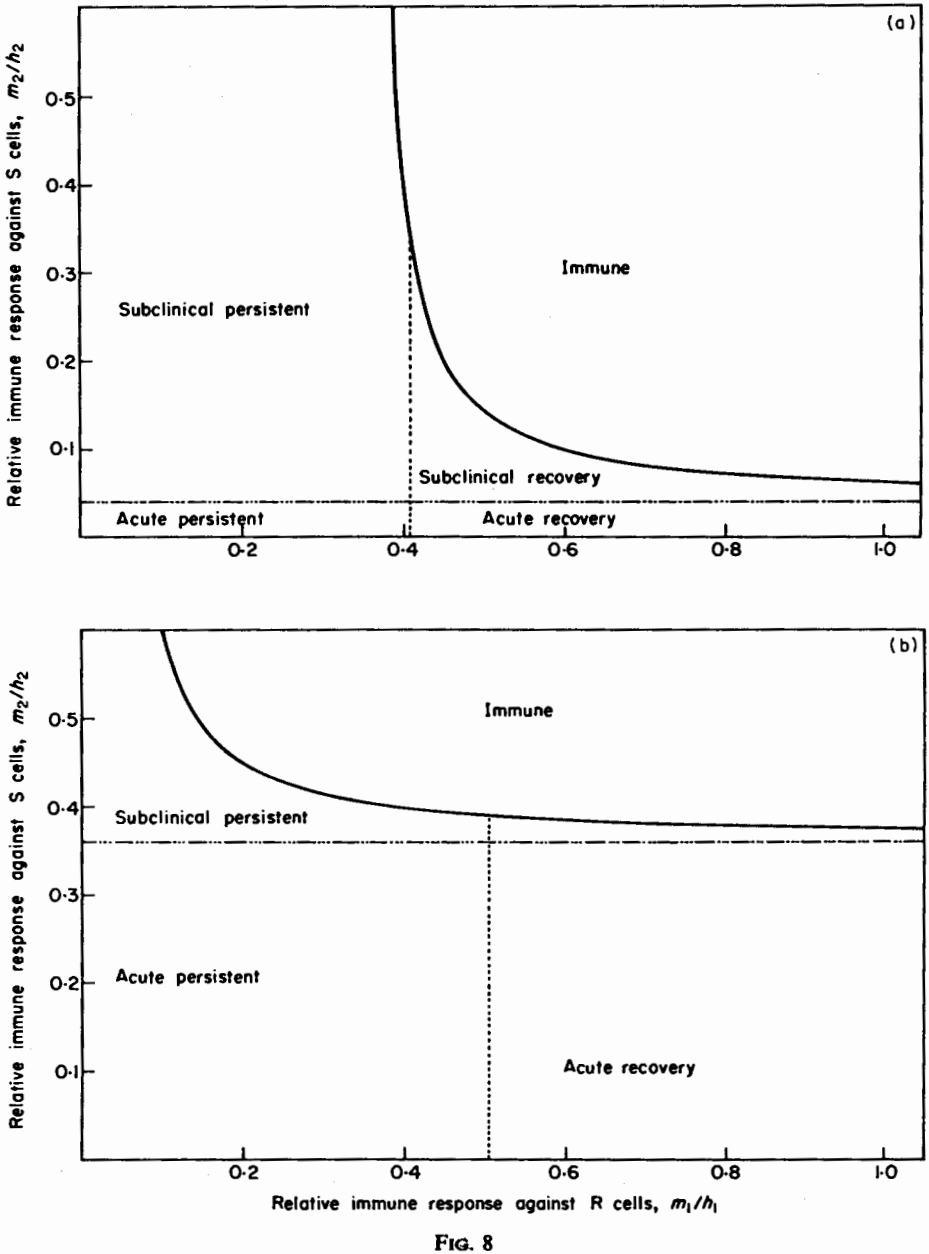

(a)
Relative immune response against S cells, m_2/h_2
0·5
0·4
0·3
0·2
0·1
Immune
Subclinical persistent
Subclinical recovery
Acute persistent
Acute recovery
0·2
0·4
0·6
0·8
1·0
(b)
Relative immune response against S cells, m_2/h_2
Immune
Subclinical persistent
Acute persistent
Acute recovery
Relative immune response against R cells, m_1/h_1

FIG. 8

The phase diagram shown in Fig. 7(a) illustrates the many different outcomes of HBV infection depending on the initial ratio of R and S cells, and the general strength of the immune response to HBV. We expect both of these factors to be dependent on the age of the patient when the infection is acquired: according to the London–Blumberg model the proportion of S cells increases with increasing age, levelling out sometime late in childhood, and in general the ability to mount immune responses will be a one-humped pattern as a function of age, being lower for the young and the elderly.

The fetus and newborn may have a liver that contains only undifferentiated R cells, and this liver cannot support any HBV replication. With increasing age the proportion of S cells increases and we enter the parameter space that leads to a subclinical infection followed by a chronic carrier state. In Asian populations this appears to be at about 3 months, but it may be different in other populations. This outcome is frequent in infected children. Increasing age further leads again to an increased proportion of S cells, and also to an increasingly effective immune response. This places us either in the parameter regime characterized by a subclinical course followed by recovery and immunity, or, for higher proportions of S cells and weaker immune responses (possibly due to some immunodeficiency), in the region of acute hepatitis followed by chronic disease. Most adults however will be able to mount effective immune responses and will have a very high proportion of S cells, which puts them in the region characterized by an acute attack with subsequent recovery. For this case we find that during the course of the disease the reserves of uninfected S cells become depleted, so that after the virus has been eliminated the patient is then left in the parameter space for which there is immunity to further infection. Such immunity may only be temporary, although this is not included explicitly in our current mathematical formulation. This is because after recovery we expect the proportion of S cells to start increasing again, which may possibly lead to the patient moving back into the non-immune parameter regime. In addition the worse the initial attack the greater the immunity conferred upon the patient afterwards. Thus it can be seen that our model has the potential to account for the age dependence of the main different clinical pictures of HBV replication. In addition, both transient and persistent HBV infection may be subclinical, or may show clinical symptoms.

FIG. 8. Diagrams showing the expected outcome of infection for specific initial concentrations of S cells and for differing immune responses against the infected R and the infected S cells. The parameter values used are as for Fig. 7(a), except that m_1 and m_2 are allowed to be independent variables. (a) Phase diagram for $S_0 = 0{\cdot}1$. This is the situation for infants, or for a patient who has recovered from a previous attack. For infants the immune responses against both R cells and S cells are not well developed. This places us in the lower left-hand corner of the diagram, for which the most likely outcome of the disease is to be subclinically but persistently infected. For older patients who have recovered from a previous infection their proportion of S cells becomes reduced to the level normally found in children, while still maintaining an effective immune system, and are thus placed in the region for which immunity is conferred. (b) Phase diagram for $S_0 = 0{\cdot}9$. This is the expected situation for previously uninfected adults. In this case the immune responses are more effective so that both m_1/h_1 and m_2/h_2 will be larger than for infants, and thus the most likely outcomes are either immunity, or a subclinical infection followed by recovery, or an initially acute course followed by recovery.

This is reflected in our model by the different levels of virus concentration and of infected cells during infection.

The phase diagrams plotted for constant S_0 [Figs 8(a) and (b)] show the dependency of the model behaviour on the relative immune responses against the infected R and S cells for subjects of a specific age. Figure 8(a) shows the situation for a patient with a low initial concentration of uninfected S cells. This will be the case both for infants, in which case the immune responses will not be well developed and the most likely outcome will be a subclinical course followed either by recovery or by persistent infection, and for older patients who have recovered from a previous attack, in which case the immune system will remain effective and the subject will be unable to support any further invasion by the virus. As previously mentioned, such immunity may only be temporary. It is interesting to note that this immunity is not a result of any specific change in the immunological status of the patient, but is a "dynamical immunity": the situation is no different to that prior to infection except that the reduction in the proportion of uninfected S cells places the subject in a different area of the phase diagram.

Figure 8(b) shows the situation for a patient with a high initial concentration of uninfected S cells. This will be the case for previously uninfected adults. Since the immune system will be more fully developed the most likely outcomes are either immunity, or a subclinical infection followed by recovery, or an initially acute course followed by recovery. An immunodeficient adult will most likely have an acute attack followed by the onset of persistent infection. Figures 8(a) and (b) also demonstrate clearly the independence of the two main effects: whether the infection is transient or persistent depends only on the behaviour of the R cells; whether the initial attack is acute or not depends only on the behaviour of the S cells. This may have implications in the targeting of treatment. In particular it is worth noting that the increase in the proportion of S cells with age does not increase the probability of recovery (in fact the opposite)—it is only the increase in the immune response against the R cells that makes the difference.

According to our model, primary hepatocellular carcinoma occurs by transformation of R cells, and potentially by the viral *HBx* gene. Therefore we assume that the probability of a patient developing liver cancer will be proportional to the total amount of infected R cells. In our model the concentration of infected R cells increases with time in those patients with chronic disease (Figs 3, 4 and 6). In some cases (Fig. 3) the increase in infected R cells may only be very slow, which may also help to contribute to the long "incubation" period between the initial infection and the development of PHC. An additional prediction of the model is that patients who have acquired infection in adulthood will display a shorter incubation period for PHC.

In addition our model allows for complete elimination of the virus after a period of chronic HBV infection (Fig. 2). It may be that this could explain those cases where PHC develops in people who have been infected, but are found to have little or no replicating virus at the time when liver cancer is diagnosed. It could also explain how evidence of the replicating virus and of the HBs antigen can disappear many years after first infection, as occurs in about 10–30% of chronic carrier cases.

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