Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 6542–6546, June 1996
Medical Sciences

The dynamics of hepatitis B virus infection

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Contributed by Baruch S. Blumberg, February 12, 1996

ABSTRACT **We consider a cellular model of infection by the hepatitis B virus and describe how it may be used to account for two important features of the disease, namely (*i*) the wide variety of manifestations of infection and the age dependence thereof, and (*ii*) the typically long delay before the development of virus-induced liver cancer (primary hepatocellular carcinoma). The model is based on the assumption that the liver is comprised of both immature and mature hepatocytes, with these two subpopulations of cells responding contrastingly upon infection by the virus.**

Although replication of the hepatitis B virus (HBV) is only weakly cytopathic toward the host cell, HBV infection is nonetheless the major cause of liver disease in man (1). The extent of the suffering caused by the virus is a consequence not just of its high prevalence, there being an estimated 300 million carriers worldwide, but also of the variety of different clinical manifestations that can result from infection. Disease in adults usually leads to recovery and immunity, most commonly subsequent to acute hepatitis, but also sometimes following subclinical infection (2); whereas if the hepatitis B virus surface antigen (HBsAg), which can be detected even in the absence of active replication, has not been cleared within 6 months, the disease is termed chronic and infection is likely to persist. The progression to a chronic carrier state is age-dependent, being common in infants, occasional in children, and rare in adults (3, 4). Persistent infection may be either asymptomatic or symptomatic—the mean age of asymptomatic carriers is lower than that of symptomatic carriers (5)—and can lead to various outcomes, including chronic persistent hepatitis, chronic active hepatitis, and postnecrotic cirrhosis (6). Some 25–50% of carriers go on to develop primary hepatocellular carcinoma (PHC), typically around 30–50 years after initial infection (7, 8). The destruction of infected hepatocytes is believed to be a consequence of the host's immune responses, and primarily results from the action of cytotoxic T cells (9, 10).

In this report we describe how a simple cellular model for the pathogenesis of HBV may be used to account for the variety of manifestations of infection, the outcome depending only on the age and immunological status of the patient. In addition, the model provides an explanation for various features of the relationship between HBV and PHC, including a possible dynamical explanation for the characteristic long delay before the onset of virally-induced neoplasms.

The London–Blumberg Model

London and Blumberg (11) assumed that the liver is comprised of two subsets of hepatocytes that respond contrastingly to infection by the virus and that are in different stages of maturation, the less differentiated cells being referred to as R cells ("resistent" to viral replication) and the more differentiated as S cells ("susceptible" to viral replication). The R cells have the capacity to divide, being able to differentiate into either R cells or S cells, but the S cells do not undergo significant further division; the proportion of S cells in an uninfected liver therefore increases with increasing age. It is supposed that the integration of HBV DNA into the genome of both cell types can alter gene expression that, in combination with increased cell proliferation in chronic hepatitis, thereby increasing the probability of mutational events, may increase the probability of carcinogenesis. In addition, the phenotypic transformation of infected R cells is hypothesised to prevent any further differentiation into S cells, thus providing them with a selective advantage. A number of conditions specify the behavior of the two cell types in response to infection by the virus. London and Blumberg suggested that the rate of viral replication must be greater in the S cells than in the R cells, and that the rate of cell death of infected S cells should likewise be much greater than that of the infected R cells. We show that the S cells must also be required to have a higher infectivity than the R cells. A mathematical formulation of the London–Blumberg model is given in Payne *et al.* (12).

There is observational and experimental evidence for the basic scenario of the model. Kim *et al*. (13) showed that the presence of the *HBx* gene [the HBx antigen uses a tumor promoter pathway (14)] is associated with the development of liver cancer in transgenic mice, and found the gene to be expressed in only a subset of hepatocytes, thought to relate to a specific state of differentiation. Thung *et al.* (15), who investigated the presence of antigens in hepatocellular carcinoma, concluded that although tumor cells are histologically monomorphic they are functionally heterogeneous. Brechot *et al.* (16) suggested that viral replication may involve only the more differentiated tumor cells. The existence of stem cells in the liver is controversial (17, 18), but recent work suggests that "oval" cells, which are thought to be progeny of hepatic stem cells, can act as a precursor to liver tumors. These oval cells can proliferate and are capable of differentiating *in vivo* into both mature hepatocytes and bile epithelium cells (19), but when transplanted subcutaneously can produce aggressively growing tumors. Interestingly, the tumorigenic potential of transformed cells transferred intrahepatically is dependent on the microenvironment, being suppressed in situations where hepatocytic differentiation is induced (20). Oval cells therefore seem to correspond to the R cells of our model, with S cells being equivalent to fully mature hepatocytes.

Explaining the Variety in Outcomes of HBV Infection

Computer simulations of the model exhibit dynamics reflecting the main observed outcomes of infection. In this section we use five different examples to demonstrate the variety of possible behaviors. The parameter values used in the simulations (R.J.H.P., unpublished data) were estimated using a combination of analytical predictions and clinical data. Note that all of the figures given here represent possible outcomes in a single patient, varying only in the age at first infection (only

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Abbreviations: HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; PHC, primary hepatocellular carcinoma; HBeAg, hepatitis B e core antigen.

the strength of the immune responses and the initial proportion of S cells are varied). Fig. 1*a–e* are displayed in order of decreasing age at infection. Let X represent the proportion of uninfected S cells in the liver. It can be shown that there is a critical level in the proportion of uninfected S cells, X_A say, for which if $X_A < X$ then the overall concentration of free virus will increase, and if $X < X_A$ then it will decrease. The critical concentration X_A is defined by the strength of the immune response against the infected S cells, but is effectively independent of the immune response against the infected R cells, the rate of viral replication in the R cells being too slow to be significant in this context (for "immune response" we should more properly say "elevated cell death rate resulting from infection," this including not just the immune response itself but also any additional cell death, for example from interference with the host cell metabolism, lysis of infected cells by mechanisms other than the immune system, etc.).

Initial Phase: Acute or Subclinical? If $X_A < X$ at the time of infection, then the virus will prosper and X decreases as susceptible cells become infected. At the same time the infected cells will be dying, mostly as a result of attack by cytotoxic T cells. Importantly, however, the dying S cells are replaced not by more S cells, but by the less differentiated, faster-proliferating R cells. Consequently the proportion of uninfected S cells continues to decrease until such time as $X < X_A$, whereupon the level of active viral replication starts to fall. Such behavior always occurs at the start of infection, but its magnitude may vary widely. The severity of the damage sustained by the liver during this initial phase is controlled by two opposing factors: the number of uninfected S cells that

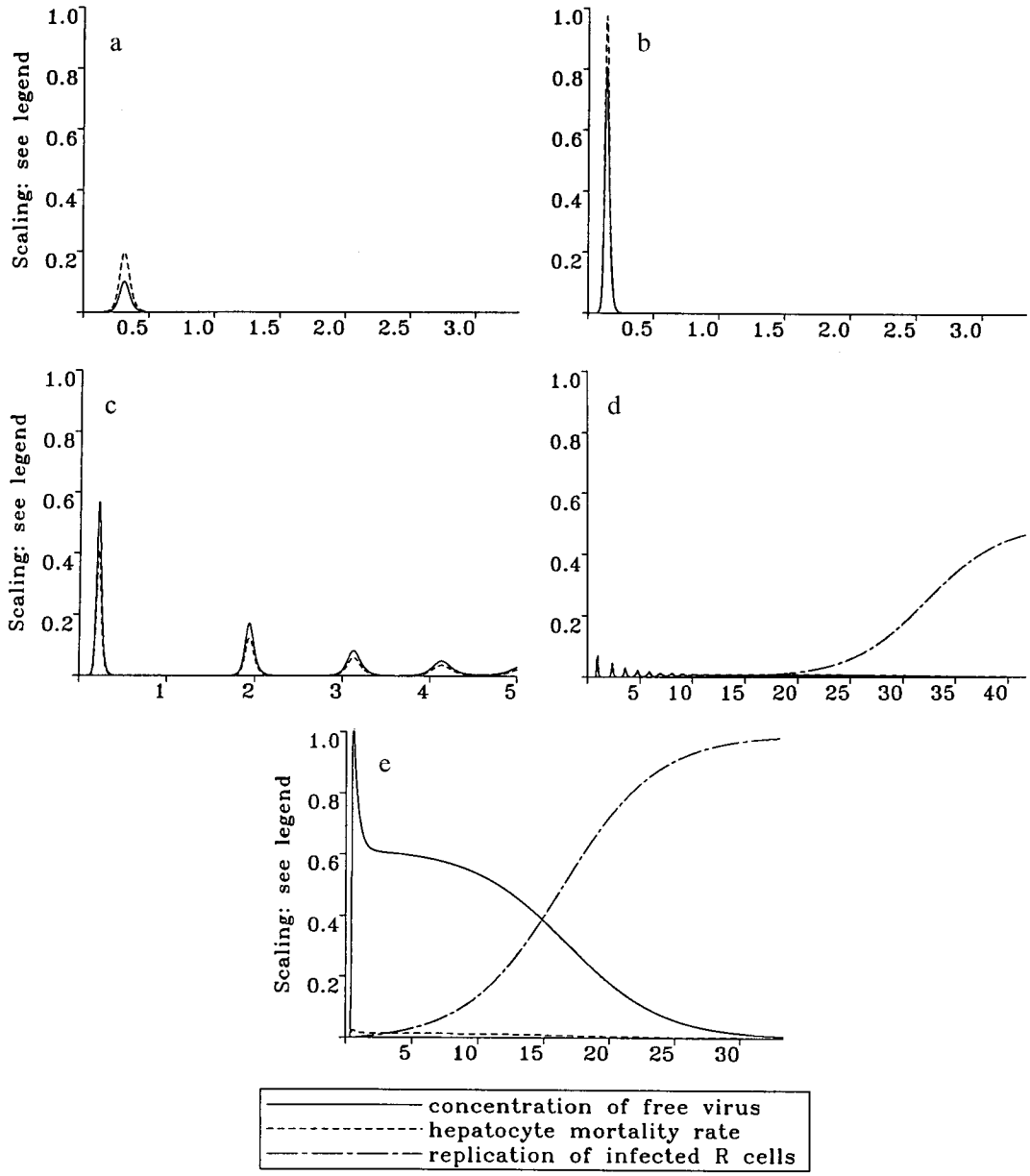

FIG. 1. Computer simulation of the model, based on mathematical formulation and parameter values in Payne *et al.* (12) and Payne (unpublished data). The variables followed are (*i*) concentration of free virus (mean viral particles per hepatocyte ÷ 0.25); (*ii*) the combined excess hepatocyte mortality resulting from infection (mean total cell deaths per hepatocyte per month ÷ 1.5); (*iii*) replication rate of infected R cells (mean infected R cell divisions per hepatocyte per month ÷ 50.0). If the probability of a phenotypic transformation leading to the initiation of PHC is small for each cell division then the overall instantaneous probability of such a carcinogenic event is approximately proportional to the rate of replication of the infected R cells. *a–e* are given in order of decreasing immune effectiveness, reflecting decreasing age at first infection. (*a*) Transient subclinical infection. (*b*) Transient acute hepatitis. (*c*) Recurring acute hepatitis. (*d*) Asymptomatic persistent infection likely to lead to PHC. (*e*) Symptomatic persistent infection likely to lead to PHC.

have to be lost before X becomes smaller than the critical value X_A, and the rate at which infected cells are destroyed. If the immune response against the infected S cells is strong (but still small enough for the virus to take hold), and X_A therefore large, then few cells have to die before X drops beneath X_A, and thus the disease will be subclinical, as in Fig. 1*a*. At the other end of the scale, if the response is very weak then the rate of cell death is slow and this also will be subclinical, as in Fig. 1 *d* and *e*. Between these two extremes, however, there is the possibility of having the immune response against the S cells both small enough that many cells have to become infected before X falls below X_A, and large enough that the rate of cell death will be high; in this case significant damage will be inflicted on the liver and acute hepatitis will occur, as in Fig. 1 *b* and *c*.

Transient or Persistent? Once X becomes smaller than X_A, the viral concentration falls and the liver begins to return to a more normal state, except with a higher proportion of R cells than prior to infection. The differentiation of these R cells into S cells then causes the proportion of S cells to start to increase, until eventually X is again larger than the critical value X_A. At this point one of two things can happen: if there is no virus remaining then recovery will be complete, as in Fig. 1 *a* and *b*; if, however, there is still some free virus present then viral replication starts again and chronic infection sets in. Which of these two courses is followed depends on the ratio of uninfected to infected R cells, which in turn depends on the strength of the immune response against the infected R cells. Thus those patients with less effective immune systems, most probably the very young, are more likely to develop persistent infection. The manner in which the resurgence of viral infection manifests itself depends on the degree of "overshoot" as X rises past X_A. If there is significant overshoot then separate waves of active replication will occur, and recur (compare Fig. 1 *c*, *d*, and *e*). Less extreme oscillations may account for those cases of unresolved acute hepatitis that subsequently have fluctuating levels of serum transaminases with intermittent jaundice. Note, therefore, that the appearance of these recurrent bouts of disease does not necessarily require reinfection, viral mutation, or changes in the patient's immunological status.

Chronic Disease: Asymptomatic or Symptomatic? We consider two features of interest in persistent disease: whether the chronic carrier state is asymptomatic or symptomatic, and whether or not PHC is seen to develop. The latter is discussed in the next section. Asymptomatic chronic disease is characterized by absence of viral replication, low infectivity, and the presence of antibodies against the hepatitis B e core antigen (HBeAg); symptomatic chronic disease is characterized by active viral replication, high infectivity, and failure to seroconvert to anti-HBeAg (21). Although transition to persistent infection is determined by the properties of the R cells, the level of active replication apparent once persistence is established is determined by the properties of the S cells. If the immune response against the infected S cells is large then the chronic carrier state will be asymptomatic, as in Fig. 1*d*, whereas if it is small then the chronic state will be symptomatic, as in Fig. 1*e*. This implies that the mean age of HBeAg-positive patients should be lower than that of those who are HBeAg-negative, a prediction in agreement with the clinical observations of Realdi *et al.* (5).

A Dynamic Origin for the Latency of PHC

The London–Blumberg model was designed to help account for various features of HBV-induced PHC, principally: (*i*) PHC is an outcome of persistent but not transient infection (7), (*ii*) tumor cells do not usually contain HBsAg (15), (*iii*) the concentration of HBsAg in patients with PHC is low (22), (*iv*) HBV DNA can be observed integrated into both tumor and nontumor cells (16), (*v*) HBV DNA can insert into many different sites in the host genome (23), (*vi*) the presence of a carcinogenic cofactor does not appear to be necessary for the occurrence of PHC (24), and (*vii*) there is usually a long (30–50 years) latency period between infection and the development of PHC (22). Examination of the figures shows that most of these observations can, in dynamical terms, be accounted for if the probability of initiating PHC is somehow dependent on the concentration of infected R cells. Such a correlation could occur in two ways: either the random integration of the HBV DNA into the host cell genome may have a small probability of causing a relevant phenotypic transformation, or the transformation could occur by a mutation of the infected cell DNA during cell division. Both the absence of an essential cofactor and the observation that viral DNA integration, random in nontumor cells, is nonrandom in malignant cells (25) would suggest the former scenario to be more likely. The infected S cells could also be potentially tumorigenic, but their limited capacity for cell division means that their role in the promotion of PHC would not be significant. This means that we do not need to look for any differences between the function (or dysfunction) of R and S cells upon integration of the HBV DNA—the differing responses of the two cell types to active replication is sufficient for the working of the model.

Although it should be clear how items *i–vi* are satisfied by the assumptions of our model, it is less obvious why there should be a delay before the rise in the concentration of infected R cells. In the numerical simulations this delay was achieved by choosing the infectivity of the R cells to be much smaller than that of the S cells. To see why this is necessary, bear in mind that the concentration of infected R cells may increase by two different mechanisms: infection and regeneration. If infection were the dominant process then high concentrations of infected R cells would correlate with high levels of free virus. Since, however, active viral replication is usually highest at the start of the disease, this is in contradiction with our required dynamics; in addition, it is in contradiction with item *iii* above. Thus the gradual build-up in numbers of infected R cells must instead be primarily a result of cell division, with the large increase some years after infection coming about when positive feedback takes control of the system, as the rate of cell death becomes overwhelmed by the rate of regeneration. This notion is in accord with the observed focal nature of pre-cancerous lesions in HBV infected transgenic mice (13), and in woodchuck hepatitis virus infected woodchucks (26). Such behavior can only occur if the infectivity of the R cells is small enough for the overall rate of infected R cell division, although low, to be larger than the rate of cell–cell infection. This reasoning indicates how the increase in the concentration of infected R cells can be delayed in one individual, but its extension to cover the overall epidemiological picture is more subtle.

Given that the duration of the delay is controlled by the balance between the addition of infected R cells by cell division and the removal of these cells by immune attack, the question is why the domination of the liver by infected R cells does not occur much more rapidly in those patients with very weak immune responses. Although the level of active viral replication has already been discarded as a determining factor for the initiation of PHC, the S cells do, nonetheless, play a crucial role. The important point to note is that the R cells, whether infected or not, can only increase by multiplying to fill the space left vacant by dying S cells. In the (hypothetical) situation of there being no immune response of any sort against the infected S cells, no extra S cells would die as a result of the disease, the infected R cells would not have an opportunity to prosper, and the likelihood of PHC would be negligible. By considering this scenario we can see that the most rapid accumulation of infected R cells will occur when there is a positive immune response against the infected S cells, for which the long-term mortality of infected S cells is maximized

(in contrast to the immune response against the infected R cells, which would need to be zero). This means that there is a limit to the possible rate of increase of infected R cells, and, when considered over a population of individuals, there is thus a minimum duration to the latency period that precedes the dramatic rise in the incidence of PHC. Given a small enough infectivity of the R cells this minimum delay can be of the order of decades, as demonstrated in Fig. 2. (It should not be forgotten, however, that we are dealing with probabilities, so the possibility of rare cases with an early onset of PHC is not excluded.) This provides us with a purely dynamical explanation for the long latency period characteristic of HBV-associated PHC.

Summary and Implications of the Model

In this report we have described how the London–Blumberg model may be used to provide an understanding of the dynamics of HBV infection—the presence of two subpopulations of cells in differing stages of maturation being crucial in accounting for the variety of manifestations. The severity of the initial phase of disease is determined by the immune response against the infected S cells, which also controls the asymptomatic/symptomatic character of persistent hepatitis, whereas possible progression to persistent disease is determined by the immune response against the infected R cells. This separation of mechanisms is in accord with the observation that protective antibodies against the HBsAg cannot usually be detected until some months after the passing of acute disease (27), the process for the initial subjugation of disease apparently being distinct from that for the elimination of the virus. Although the risk of oncogenesis is proportional to the number of infected R cells, we find that both cell types play a role in determining the expected duration of latency before the onset of PHC. Crudely put, this is because the total number of R cells present depends on the death of the infected S cells, whereas of those R cells present, the proportion that are infected depends on the balance between the infected and the uninfected R cells. The interaction of the two cell types can also lead to oscillatory behavior, which may account for the intermittent symptoms of hepatitis seen in some patients. Oval liver cells, which we have speculated to correspond to the R cells of the London–Blumberg model, have been shown to coexpress transforming growth factor α (TGF-α) and HBsAg (28). Since the gene for TGF-α can lead to hepatic oncogenesis in transgenic mice this supports our inference that R cells act as the principal site for the development of HBV-induced PHC.

Our analysis explains the observed age dependence of the various manifestations of infection (recall that Fig. 1 *a–e* are displayed in order of decreasing strength of immune effectiveness, reflecting decreasing age at first infection). Transient infection is most likely in adults, subclinical chronic infection is most likely in infants, asymptomatic carriers have a lower mean age than symptomatic carriers, and the probability of progression to PHC is higher the lower the age at infection. Cirrhosis of the liver is also a known risk factor for the development of PHC (29), regardless of whether the cirrhosis is caused by alcohol, HBV infection, or other active hepatitis. In our model this can be attributed to the cirrhotic cell necrosis providing further room for the proliferation of infected R cells.

The differing roles of the two cell types has implications for the treatment of HBV infection. For example, to alleviate acute hepatitis the S cells should be targetted, whereas for the prevention of progression to a carrier state it is the R cells that are pertinent. Reducing the likelihood of developing PHC is a more complex matter, with both cell types being of importance, the most appropriate target for treatment depending to a large extent on context. Note, however, that although it would in all cases be beneficial to boost the host's immune response against the infected R cells, tampering with the immune response against the infected S cells could prove to be a double-edged sword: increasing the death rate of infected S cells with the intention of reducing the amount of viral replication during chronic hepatitis is, in the absence of other factors, liable to increase the probability of initiating PHC.

Our model is able to mimic the wide variety of disease behavior without having to include the detailed complexities of immune responses. For example, following transient infection the patient will be immune regardless of any change in immunological status (the subsequent differentiation of R cells into S cells means that such immunity may only be temporary), and the reappearance of disease following apparently transient infection, such as that in Fig. 1*c*, may occur for dynamical reasons rather than from reinfection. Similarly, it is possible to have a resurgence of disease even in the absence of viral mutation or changes in immunological status. That is not to say that these other possibilities will not happen, just that they are not necessary in accounting for the disease dynamics. More significantly, our explanation for the delay in the onset of PHC is also based on purely dynamical considerations and does not depend on either the existence of a carcinogenic cofactor or the occurrence of an early event with a delayed manifestation [but does not preclude the effect of additional carcinogens, such as aflatoxin (30)]. Provided that the infectivity of the R cells is very small compared with that of the S cells, no

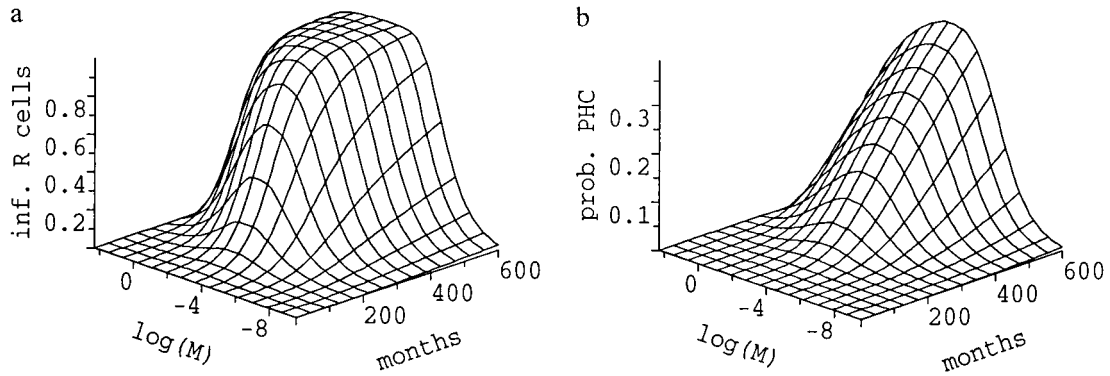

FIG. 2. Duration of PHC latency period in a population. Change with time of (*a*) proportion of liver made up of infected R cells and (*b*) cumulative probability of developing PHC (approximately proportional to the time integral of the number of infected R cells), taken over a range of M (a measure of the immune response against the infected S cells). Considered over a population—that is, over the range of all possible M—there is a minimum duration of the delay before the proportion of infected R cells in the liver begins to rise significantly. If, as here, this minimum delay is of the order of decades, then the typical latency period before PHC in a population of carriers will, as required, also be of the order of decades. To achieve this the infectivity of the R cells must be very small.

additional mechanisms, over and above the basic assumptions of the London–Blumberg model, are necessary to account for the typically long delay before the development of HBV-induced PHC. It would be interesting to know whether the type of dynamic behavior described here could also be the reason for the delayed development of other virus-associated tumors.

We thank W. Thomas London for his critical reading of the manuscript. This work was partially supported by the Science and Engineering Research Council and the Natural Environment Research Council of the United Kingdom (R.J.H.P.), by a Wellcome Trust Senior Research Fellowship and the E. P. Abraham Junior Research Fellowhsip at Keble College (M.A.N.), and by Public Health Service Grant CA-06927 from the National Institutes of Health and by an appropriation from the Commonwealth of Pennsylvania (B.S.B.).

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