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ESTIMATION OF HIV AND HBV INFECTIOUS TITERS IN HUMAN FLUIDS AND TISSUES

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ABSTRACT

This is the report of the first in a four-task project to assess the risks of HIV and Hepatitis B virus infection for health care workers. Here we describe the basic biology of the two viruses and assess available information on the distributions of infectious titers in the patients that might be sources of occupational exposure.

For HIV there are two potential vectors of occupational infection--free viral particles, and infected cells. Based on data on the distribution of HIV antigen titers in blood, it appears that later, symptomatic stages of HIV infection are associated with both increased median titers of free virus, and reduced variability in viral titers. (These trends may reflect significant features of the pathogenic process.) We were fortunate to have available one set of serial dilution measurements that we were able to interpret in terms of distributions of cell-mediated infectious titers for one specific population group--asymptomatic seropositive people. The indication is that for this group, the median cellular infectious titer may exceed the titer due to free viral particles by something like 500 fold. This relationship may not hold for later stages of HIV infection.

In the case of Hepatitis B virus, we had available some limited counts of free viral particles in a relatively high-titer set of sera, and some older experiments on deliberate experimental inoculation of people with known jaundice-producing sera. Comparison of these data with the HIV titer data go a considerable way in explaining the much greater frequency of occupational transmission for HBV than for HIV.

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1. INTRODUCTION

Acquired immunodeficiency syndrome, or AIDS, is already posing heavy demands on the health care system. These demands are likely to increase as individuals infected with the human immunodeficiency virus (HIV, the etiological agent for AIDS), but not yet clinically ill, progress to frank AIDS.

As the demands on the health care system grow and change, the importance of various routes of occupational transmission of AIDS to health care providers may change. To reduce the incidence of occupational transmission of HIV, it is important to understand which bodily fluids and tissues from HIV infected individuals may be infectious, how infectious they are, and whether the degree of infectiousness changes with time. It is also important to know the rate at which the virus loses its infectiousness under various environmental conditions likely to exist in a health care situation.

In this report, efforts are made to estimate the infectiousness of HIV, in terms of tissue culture infectious doses, for blood, semen, saliva and other body tissues known to harbor the virus. These estimates are made by several independent routes, either based on HIV data alone, or by comparing HIV with Hepatitis B Virus (HBV). HBV was selected as a point of comparison because it appears to have similar transmission routes and high risk populations, but higher transmission rates in occupational settings and a longer history of being studied. The literature on the stability of HIV and HBV under various environmental conditions is also reviewed.

This report is part of a larger project to develop predictions of the extent and routes of HIV transmission in health care workers. The data reviewed and analyzed here include the molecular and cell biology of HIV, but not epidemiological studies--these will be assessed in later work.

In the next section of this report, pertinent aspects of HIV are summarized, including its molecular biology and assays used to detect its presence in various tissues. In the third section, the amount of both free HIV and HIV-infected cells in various tissues at various disease stages is estimated. The fourth section discusses factors affecting occupational transmission of HIV, including its stability under environmental conditions, infectious dose, and the variability of susceptible populations. The fifth section provides a general description of the Hepatitis B virus, including methods used to detect its presence and its stability under environmental conditions. The sixth section provides estimates of the amount of infectious HBV in human tissues and fluids, including materials, such as dialysis wastes, that have been in close contact with human tissues. The final section draws comparisons between HIV and HBV, and summarizes the findings of this report.

2. DESCRIPTION OF HIV

2.1 Viral classification

HIV is a member of the lentivirus subfamily of retroviruses. Lentiviruses are known to cause slow, degenerative neural disease in several animals. The visna virus, which causes a chronic degenerative disease in sheep, is the prototype lentivirus (Fauci, 1988; Weber and Weiss, 1988a). HIV also has biological, morphological and molecular similarities with the equine infectious anemia virus and the feline immunodeficiency virus (Fauci, 1988). Visna virus infects monocytes/macrophages and may possibly be spread by aerosols (Weber and Weiss, 1988a).

2.2 Molecular Biology

The genomic organization of HIV has been carefully characterized during the last several years. HIV has the three structural genes common to all retroviruses and regulatory genes that seem to be specific to the lentivirus subfamily.

The structural genes are:

1) gag, which codes for the core and shell proteins (p24, p18, and p15);

2) pol, which codes for the protease (p66), reverse transcriptase, and endonuclease (p34); and

3) env, which codes for the outer spike glycoprotein (gp120) and the transmembrane glycoprotein (gp41) (Weber and Weiss, 1988a; Fauci, 1988).

Nonstructural regulatory genes include tat (transactivation of transcription), art (also called trs), 3' orf (also called nef), and sor. The tat gene is required for viral replication and acts as a transactivating factor, at both the transcription and post-translational levels, on all viral proteins. The art gene, which encodes an 18 kd protein that is found only in the cell nucleus, enhances the synthesis of viral structural proteins by acting at the translational level. 3' orf seems to act as a negative regulator, reducing HIV expression. The sor gene influences virus transmission in vitro and is critical to the efficient formation of infectious particles (Weber and Weiss, 1988a; Fauci, 1988).

Long terminal repeat sequences, LTR's, at each end of the provirus contain promoter, enhancer, integrator and possibly other sequences that regulate viral gene expression and replication. These DNA sequences respond to tat and art, and possibly cellular proteins, to enhance the transcription of viral genes (Weber and Weiss, 1988a; Fauci, 1988).

2.3 Detection Assays

Several assays have been developed to detect HIV in culture and in body fluids. Some of these detect viable virus, others detect the presence of viral proteins or nucleic acid.

Many investigators use an assay that detects reverse transcriptase activity in the culture supernatant. In this assay, a permissive cell line or primary cell culture (such as peripheral blood mononuclear cells, PBMC's) is inoculated with an HIV containing fluid. The cell culture supernatant is then monitored for reverse transcriptase (RT) activity by adding a primer/template and radioactive DNA precursors for RT, and testing for the formation of radioactive DNA products. The time to appearance of RT activity depends on the original titer of the virus and can vary from several days to more than one month. This feature of the assay is demonstrated in Figure 1, taken from Resnick et al. (1986), in which the RT activity is plotted vs days in culture for a stock virus solution and four sequential hundred-fold dilutions.

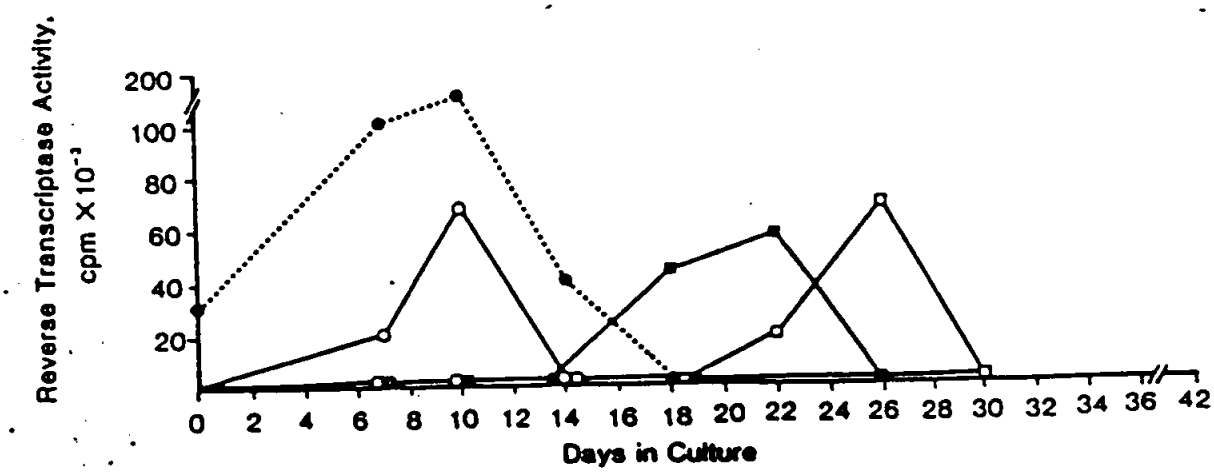
In the experiment shown in Figure 1, the virus stock was obtained from the culture supernatant of normal peripheral blood mononuclear cells (PBMC) infected with virus from a patient with AIDS. It had 10^8 viral particles/ml as judged by electron microscopy (EM). One ml of each dilution of the stock solution was used to infect 5-6x10^6 fresh PBMC's. After infection, the PBMC's were resuspended in fresh growth medium supplemented with 10% interleukin-2 and carried in triplicate cultures maintained at 0.5-1x10^6 cells/ml. Reverse transcriptase activity was detected after 6-8 days in infectivity assays using both the undiluted virus stock and the 10^{-2} dilution. RT activity was detected 18-22 days after infection with the 10^{-4} dilution and 22-26 days after infection with the 10^{-6} dilution. No RT was detected with a 10^{-8} dilution up to 42 days after infection. Based on this, the dilution that would have about a 50% chance of causing an infection in tissue culture, the $TCID_{50}$, was judged to be approximately 10^7/ml--about one tenth of the apparent concentration of viral particles by electron microscopy.

Data from this assay indicate that on average it takes more than 1, but less than 100 viral particles to generate an infection in this tissue culture system and to produce detectable RT activity in PBMC's. It would have been possible to narrow this range if more dilutions had been tested (however, this was not the purpose of these experiments).

The infectivity assay using the 10^{-6} dilution indicated that infection resulted after incubating 100 viral particles (based on EM count) with 5x10^6 cells. We can use this information to determine the number of viral particles that are likely to be required to infect a cell. To do this, we assume that the attachment/infection of cells by viral particles can be described by the Poisson

Figure 1

Effect of Sequential Dilution of HIV on the Pattern of Development of Reverse Transcriptase Activity in Cultures of Normal Human Peripheral Blood Mononuclear Cells



Solid circles--undiluted viral preparation; open circles--10^{-2} dilution; solid squares--10^{-4} dilution; open squares--10^{-6} dilution. The 10^{-8} dilution yielded no detectable response after 42 days in culture. The original stock had 10^{8} particles/ml as judged by electron microscopy and a TCID of about 10^{7}/ml, as judged from this serial dilution evidence.

Source: Resnick et al. (1986).

approximation for the binomial distribution. This assumes that the attachment of a virus to a cell is random and the virus particles act independently of each other. The Poisson equation can be written as:

$$P(Z=k) = e^{-a}a^k/k!$$

where P(Z=k) is the probability that an individual cell will be infected by k virus particles and a is the number of viruses per cell in the PBMC mixture that is susceptible to viral infection.

The susceptible cells include T4 lymphocytes (averaging $8x10^{-5}$ /ml of blood according to Redfield and Burke, 1988) and about 10-40% of circulating monocytes* (based on a range of .24-$9.6x10^{-5}$ monocytes per ml, we assume a geometric mean of $1.5x10^{-5}$ total monocytes; of which $.3x10^{-5}$ might be expected to bear the CD4 antigen). Total mononuclear cells in the PBMC mix would be likely to include the total $1.5x10^{-5}$ monocytes plus the total of all lymphocytes, which reportedly number $6.6\text{-}46x10^{-5}$ in typical blood samples (geometric mean = $17.4x10^{-5}$) for a total PMBC count of about $18.9x10^{-5}$, implying that of PBMC's, the proportion susceptible to HIV infection is about 8.3/18.9 = .44. Using this factor, the number of susceptible PBMC's among $5x10^6$ used in the experiment depicted in Figure 1 is $2.2x10^6$.

We can now use this figure in the Poisson distribution calculations suggested earlier. The ratio of viruses to susceptible cells (a) is

$$100/(2.2x10^6) = 4.54x10^{-5}.$$

The proportion of cells receiving 1 viral particle (k = 1) is therefore $4.54x10^{-5}$, and the proportion receiving 2 viral particles (k = 2) is about $1.0x10^{-9}$. If there are $2.2x10^6$ susceptible cells in an experimental dish, then the probability that even one cell in a dish is infected with two viral particles at the 100 particles/dish inoculation level is about 0.002. Clearly only one viral particle must be required to produce an infection.

An antigen capture assay, also known as an enzyme immunoassay or EIA, has been used more recently to detect HIV in fluids and tissues (c.f., Goudsmit and Paul, 1987). In this assay, human (polyclonal) antibodies to virus proteins, primarily p24 (core antigen), are used to capture the virus proteins from cell culture supernatants or directly from body fluids. The human antibody, which is attached to beads, is incubated with the sample. The beads are then washed, incubated with rabbit IgG antibody to HIV, and washed again. The presence of the rabbit IgG on

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* All data on the normal blood concentrations of different types of white cells come from Hamilton (1986).

the beads is then monitored by adding horseradish peroxidase-conjugated goat antirabbit antibody, reacting the complex with ortho-phenylene-diamine, and measuring the optical density at 492 nm.

The limit of detection of the antigen capture assay using 0.2 ml of sample is a concentration of 10 cells/ml of HIV infected HT-9 cells or 9-17 pg/ml of purified HIV lysate (of which p24 constitutes approximately 10%; Goudsmit and Paul, 1988). If the antigen capture experiments are conducted in parallel with infectivity assays, the amount of p24 can be correlated with the amount of infectious virus. Namikawa et al. (1988) have found that 1 pg of p24 corresponds to 1-10 $TCID_{50}$ infectious units of virus.* The RT assay and the antigen capture assay have comparable sensitivities in the detection of virus in culture supernatants (McDougal et al., 1985).

Some studies have also used nucleic acid hybridization assays to detect HIV. These have been conducted with either isolated nucleic acids using Southern blot techniques, or using in situ procedures to detect infected cells (Harper et al., 1986).

All of these detection assays can be used to directly test for the presence of HIV in the original material sampled. As has been illustrated for the RT assay, they can also be used, in combination with tissue culture techniques, to assay serial dilutions of the original material to determine a tissue culture infectious dose. The original material can be co-cultivated with activated (mitogen stimulated) peripheral blood mononuclear cells which will support virus replication. The co-cultivation serves to amplify the amount of virus present, thus giving a more sensitive assay of the presence of the virus.

3. AMOUNT OF INFECTIOUS HIV AND INFECTED CELLS IN HUMAN TISSUES AND FLUIDS

3.1 Cell types supporting HIV replication

The cellular protein, CD4, has been identified as the major cellular receptor, or binding site, for HIV. Initial observations indicated that HIV infects primarily lymphocytes and other cell types that have CD4 receptors on the cell surface (Fauci, 1988; Weber and Weiss, 1988a). The importance of CD4 was confirmed in experiments in which non-CD4 containing cells that are

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* Taken together, these results suggest that infected HT9 cells release between 5 and 100 $TCID_{50}$ infectious units of virus. This estimate is fourfold less than estimated based on the work of Harper et al. (1986) for in vivo isolates of infected cells (see Section 3.2). However, since these two sets of data come from different laboratories, the exact procedures differ and so may the sensitivity of the assay system defining the $TCID_{50}$ units. It should also be noted that the calibration is complicated by the presence of incomplete, inactive HIV particles that contribute p24 but not infectivity.

normally resistant to HIV infection, such as HeLa cells, were transfected with DNA encoding CD4. The subsequent expression of CD4 rendered these cells sensitive to HIV infection. However, CD4 may not be sufficient for infection. Other experiments with CD4 transfected into mouse cells demonstrated that although the virus could bind to the CD4 containing cells, it could not enter into the cells. Thus, an additional factor appears to be necessary for infection (Fauci, 1988).

CD4 bearing cells are principally the helper or inducer subset of T lymphocytes. Other immune cells which express CD4 include 10-40% of circulating monocytes (and the subsequent tissue macrophages) and 5-10% of B lymphocytes (Weber and Weiss, 1988a,b). HIV infection has been reported in specialized tissue macrophages such as pulmonary macrophages (type II pneumocytes), Langerhans cells in the dermis, and microglia (astrocytes) (bone marrow derived macrophages within the CNS) isolated from HIV infected individuals (a). These macrophages have CD4 receptors, although the level of expression of CD4 appears to be lower than for T4 cells (Weber and Weiss, 1988a; Fauci, 1988).

Infection has also been demonstrated in cell lines that appear to be CD4 negative, as determined by immunofluorescence. These include a colon-carcinoma cell line, glial cells in the CNS (Cheng-Mayer et al., 1987), myeloid progenitor cells (Folks et al.; 1988; a) and chromaffin cells in the gut (Weber and Weiss, 1988b). However, CD4 may be present in these cells in quantities lower than are detectable by immunofluorescence (>200 molecules/cell) and only a small number of CD4 molecules are required for infection (Weber and Weiss, 1988a). On the other hand, phagocytosis, another nonspecific route of entry, or a different receptor may be playing a role (Folks et al.; 1988; Weber, 1988; Cheng-Meyer et al., 1987). For example, glial cells can be infected with HIV in the presence of monoclonal antibodies known to block the CD4 binding site (Weber, 1988).

3.2 Tissues and Fluids Containing HIV

Table 1 summarizes selected data on human tissues, fluids, and cell lines from which HIV has been isolated. These include blood, plasma, bone marrow, lymph nodes, semen, saliva, cerebrospinal fluid, brain, and the colorectum. In most cases, HIV was detected by the reverse transcriptase (RT) assay or the enzyme immunoassay (EIA). In some cases, in situ nucleic acid hybridization techniques were used, as described in the next subsection. The populations from which the samples were taken are also indicated in Table 1. Most were drawn from patients with AIDS or ARC (AIDS related complex), although some were taken from patients during initial seroconversion or from healthy seropositives. Most of the groups tested were adult male homosexuals.

Table 1
Demonstration of HIV Infection in Human Tissues and Fluids

Tissue/fluid	how detected*	frequency of detection	population source of specimen	reference
blood	RT	7/17	healthy seropositive gay men	Ho et al., 1985
blood	RT	14/21	ARC-gay men	Ho et al., 1985
blood	RT	7/12	AIDS-gay men	Ho et al., 1985
plasma	RT	3/6	AIDS or at risk	Markham et al., 1985
plasma	RT	6/7	initial HIV infection (7-28 days)	Albert et al., 1987
serum	EIA	19/58	seroconverting gay men	Goudsmit and Paul, 1987
serum	EIA	2/16	seroconverting hemophiliacs	Goudsmit and Paul, 1987
serum	EIA	24/145	gay men, Europe, CDCII&III	Goudsmit and Paul, 1987
serum	EIA	0/16	hemophiliacs, Europe, CDCII&III	Goudsmit and Paul, 1987
serum	EIA	2/60	iv drug users, Europe, CDCII&III	Goudsmit and Paul, 1987
serum	EIA	3/4	children, Europe, CDCII&III	Goudsmit and Paul, 1987
PBMC	RT	12/12	AIDS patients	Albert et al., 1987
PBMC	RT	13/16	ARC patients	Albert et al., 1987
PBMC	RT	15/22	LAD patients	Albert et al., 1987
PBMC	RT	10/39	asymptomatic, Ab+	Albert et al., 1987
PBMC	RT	5/7	initial HIV infection	Albert et al., 1987
PBMC	RT	90/176	AIDS or at risk	Markham et al., 1985
PBMC	in situ hybrid	2/2	ARC	Harper et al., 1986
PBMC	in situ hybrid	5/12	AIDS	Harper et al., 1986
rectal mucosa	RT, viral antigen	2/4	AIDS patients w/chronic diarrhea	Nelson et al., 1988
bowel epithelium	in situ hybrid	5/10	AIDS patients	Nelson et al., 1988
saliva	RT	0/7	initial HIV infection	Albert et al., 1987
saliva	RT	8/20	AIDS or at risk	Markham et al., 1985
saliva	RT	0/20	healthy seropositive-gay men	Ho et al., 1985
saliva	RT	0/38	ARC-gay men	Ho et al., 1985
saliva	RT	1/25	AIDS-gay men	Ho et al., 1985
semen	RT	3/3	AIDS or at risk	Markham et al., 1985
bone marrow	RT	1/6	AIDS or at risk	Markham et al., 1985
brain	RT	1/1	AIDS/declining mental status	Levy et al., 1985a
brain	RT	2/3	AIDS or at risk	Markham et al., 1985
cerebrospinal fluid	RT	13/14	AIDS/ARCS, 12 with neurol symps	Levy et al., 1985a
cerebrospinal fluid	EIA	7/16	AIDS-gay men	Goudsmit et al., 1986
cerebrospinal fluid	EIA	5/6	AIDS-children 2-6 years	Goudsmit et al., 1986
lymph nodes	RT	4/4	AIDS or at risk	Markham et al., 1985
lymph nodes	in situ hybrid	3/4	ARC	Harper et al., 1986
lymph nodes	in situ hybrid	3/3	AIDS	Harper et al., 1986

* RT is reverse transcriptase assay; EIA is enzyme immunoassay

Table 1 also provides the frequency of isolation, i.e., the number of successful isolations versus total attempts, for each fluid or tissue/cell type. The gross frequency of isolation may be used as a surrogate measure of the concentration of infectious units of HIV under the culture conditions used, although better methods of estimation are available, as described in the next subsection. The frequency of isolation of HIV from tissues, especially blood and its products, has increased over the years because newer protocols such as co-cultivation with healthy peripheral blood mononuclear cells have increased the sensitivity of HIV detection (Markham et al., 1985; Levy and Shimabukuro, 1985).

3.2 Estimation of HIV Titer(s) in Peripheral Blood

There may well be two kinds of entities present in blood that can transfer HIV infection--(1) free virus, and (2) infected cells. In theory, each of these can be used to define an "infectious titer"--and they may have different degrees of persistence in the journey from source patient to recipient, and pose different risks of infection for different recipients. We are unfortunately not in an excellent position to separately evaluate all of these likely differences, but we have some data that can provide some indications of the population distributions of infectious titers in each case.

3.2.1 Infectious Titers Due to Free Viral Particles

Most of the data available for assessment of the serum titer of free viral particles depend on measurements of viral antigens. Goudsmit and Paul (1987) used the antigen capture assay described previously to examine the sera of adult and pediatric patients with AIDS and healthy seropositive adults and children. Figure 2 reproduces the original data, which provides the distribution of the optical density values measured in the HIV antigen capture assay for individual patients. Approximate estimates of the $TCID_{50}$ infectious units of HIV/ml serum can be obtained from these data, the linear calibration curve of optical density vs pg/ml purified HIV lysate provided by Goudsmit and Paul (1987), and other assumptions.* More recently, similar data have

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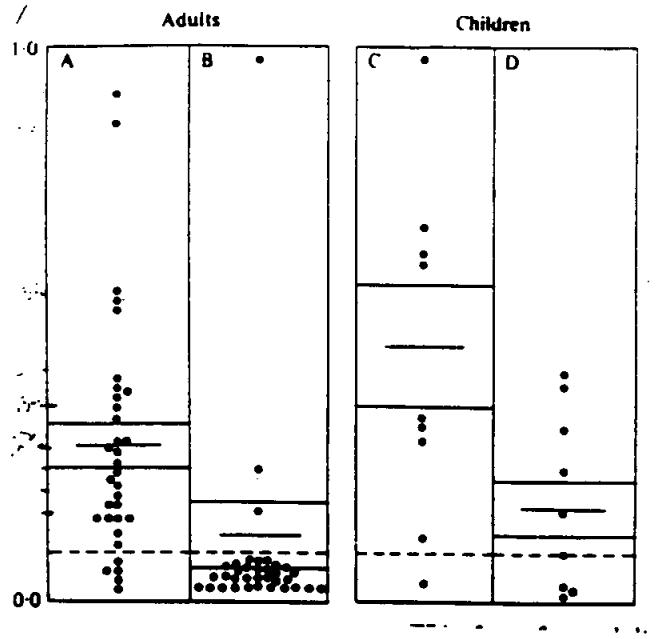
* Optical Density (o.d.) is linearly related to pg/ml purified HIV lysate by the formula, $O.D. = 1.54 \times 10^{-3}$ (pg/ml HIV lysate) + 0.138, as shown in Figure 1 of Goudsmit and Paul (1987). As discussed by the authors, it is assumed that approximately 10% of the purified viral lysate is composed of core protein (p24), the major component with which the antibodies are known to react. The conversion to infectious units is based on the estimate of Namikawa et al. (1988) that one picogram of p24 is equivalent to 1-10 $TCID_{50}$ infectious units of HIV. Our use of this relationship to interpret observations of serum and other body fluids makes an implicit assumption that the ratio of p24 antigen and viral particles will be similar in these media as it has been observed to be in tissue culture. For this reason, our estimates of tissue culture infectious units in serum must be regarded as highly tentative.

Figure 2

Population Distributions of HIV-ag in Serum



Distribution of optical density values in the HIV-ag EIA of sera from adult (panel A) and pediatric (panel C) patients with AIDS, from asymptomatic anti-HIV seropositive adults (panel B) and children with ARC (panel D). The mean optical density (± S. E. M.) is indicated for each group by horizontal bars. The dashed line indicates the cut-off value for detection (0.07).

Source: Goudsmit and Paul (1987)

been published by Goudsmit et al. (1988) for individuals with early to intermediate stages of HIV infection (asymptomatic seropositive to persistent lymphadenopathy--CDC Stages II and III).

A key initial question for analysis of these data is the statistical form(s) of the population distributions of antigen (and presumably viral) titers. Figures 3-7 show the logarithms$_{10}$ of the titers plotted against the "Z-score." Essentially, the Z-score is the number of standard deviations above or below the population median (middle) value, inferred simply from the order of the different values when arranged from high to low levels.* The fact that most of the distributions are reasonably (although not exactly in all cases) approximated by a straight line in this type of plot indicates that the distributions are reasonably well described as "lognormal." That is, the logarithms of the titers in different people (within the groups shown in the figures) have close to a normal gaussian distribution.

Based on the regression lines shown in Figures 3-7,** we can make highly tentative estimates of the median and 95th percentiles of the distributions of estimated HIV infectious titers per µl of serum*** (Table 2). The results in Figure 3, 6, and Table 2 indicate that overall, most adults whose HIV infection has proceeded to the point where it is producing symptoms have considerably higher serum infectious titers than most asymptomatic seropositive adults. However,

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* To find the Z-score for each ordered value we first calculated the "percentage score"--the estimated percentage of an infinite sample of values from the same distribution that would be expected to fall below the value under considerations. For the ith value in a distribution of N observations, this is 100*(i - 0.5)/N (Wilk and Gnanadesikian, 1968). Then, using tables in Finney (1971), we found the number of standard deviations above or below the median represented by each percentage score.

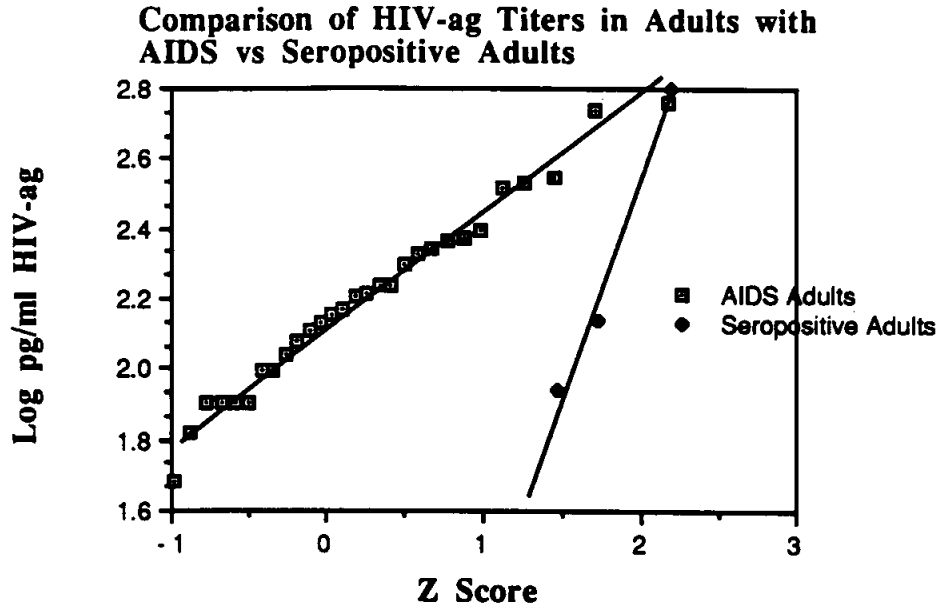
For purposes of these analyses, we plot only those values that are above the cutoff for detection. Thus although the N used for calculating the Z scores for the seropositive adults in Figure 3 is 35, there are only three points, representing the three individuals with detectable levels of HIV-ag. This distributional analysis technique allows us to estimate the geometric mean and standard deviation of the data without making arbitrary assumptions about the precise titers in the "undetectable" samples.

** Where log(pg/ml) = b + mx, the raw regression coefficients are:

	b	m	R^2
AIDS Adults	2.1152	.32954	.982
Ser+ Adults	.14106	1.1999	.987
AIDS Children	2.3176	.36866	.850
ARC Children	1.9431	.38419	.800
CDC II/III Adult (Goudsmit et al., 1988)	1.8870	.70211	.947

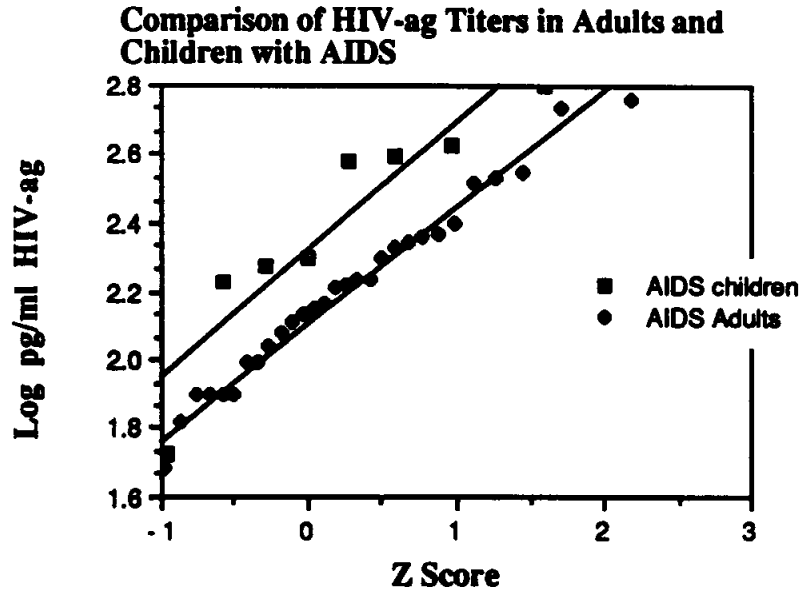
*** Here and elsewhere we will be translating infectious titers into concentration per µl because the average amount of blood transferred in a needlestick is a little bit more than 1 µl (Napoli and McGowan, 1987).

Figure 3



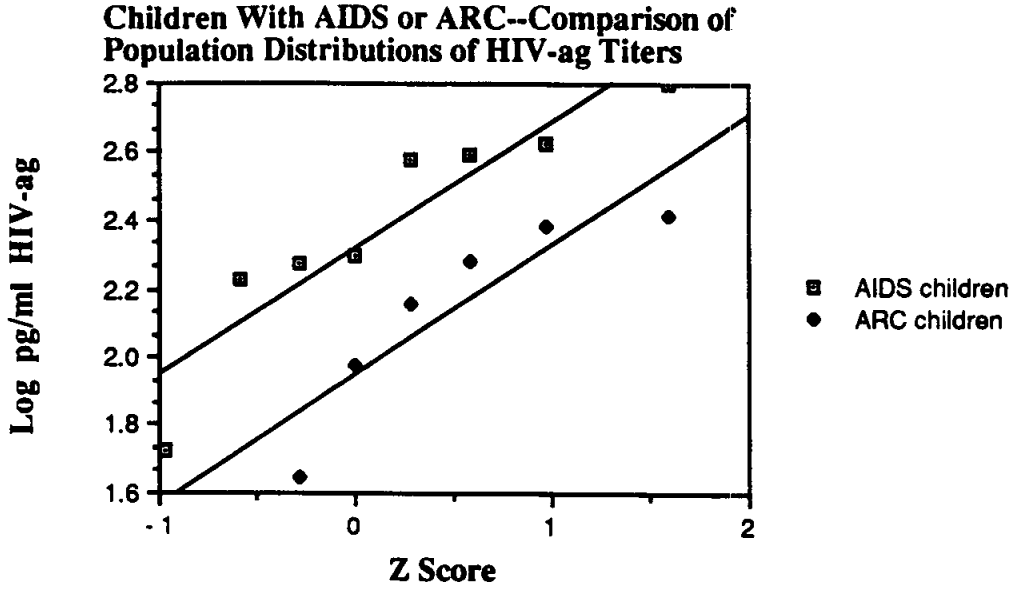
Data Source: Goudsmit and Paul (1987)

Figure 4



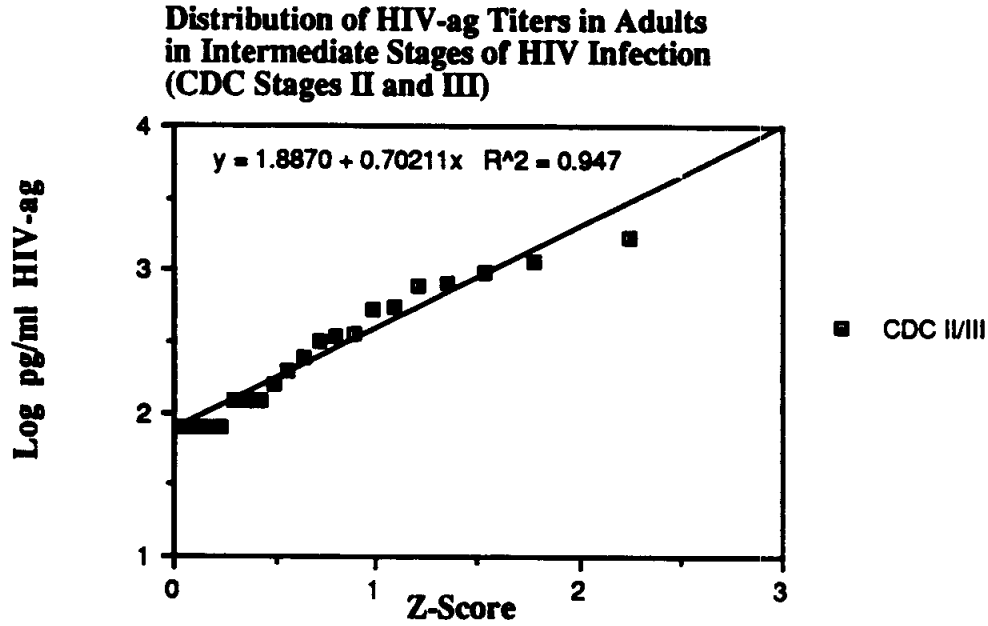
Data Source: Goudsmit and Paul (1987)

Figure 5



Data Source: Goudsmit and Paul (1987)

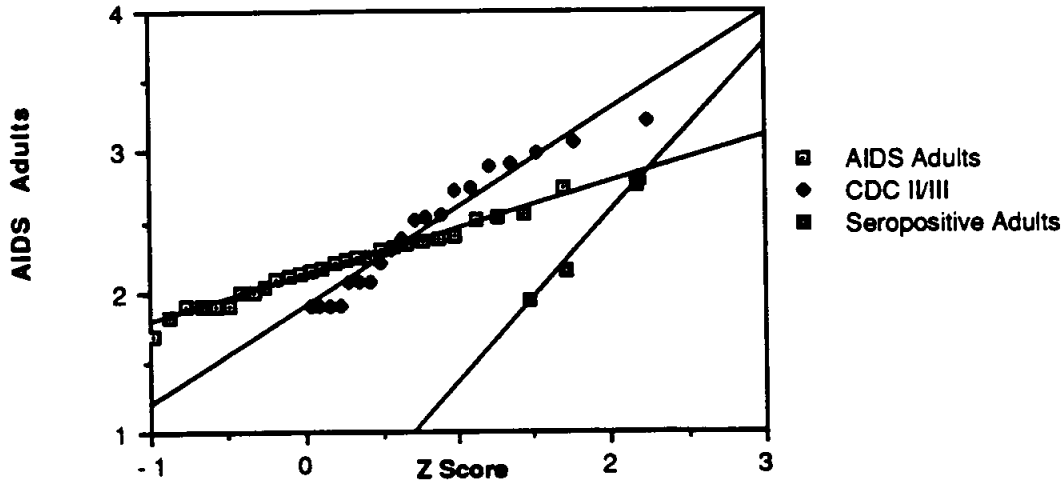
Figure 6



Data Source: Goudsmit et al. (1988)

Figure 7

Threefold Comparison of HIV-ag Titers in AIDS Adults and Seropositive Adults with Newer Data for CDC Stage II/III Adults



Data Sources: Goudsmit et al. (1988) for Stage II/III patients, other data from Goudsmit and Paul (1987).

Table 2
Projected HIV Infectious Titers in Serum

Group	----pg/ml HIV-ag--- (approx. 10% p24) Median	95th %tile	Highly Tentative Best-Estimate ---of TCID's Per µl of Serum-- Median	95th %tile
AIDS Adults*	130	454	0.041	0.144
CDC II/III Adults**	77	1100	0.024	0.35
Ser+ Adults*	1.4	130	0.00044	0.041
AIDS Children*	208	839	0.066	0.265
ARC Children*	88	376	0.028	0.119

*Based on data of Goudsmit and Paul (1987).

**Based on data of Goudsmit et al. (1988)

the titers of the seropositive adults and, to a lesser extent, the CDC Stage II/III patients, are more variable than those of the adult AIDS cases. The estimated geometric standard deviations* of the titers of the adult seropositives is 15.8; for the stage II/III patients this is 5.04; in contrast to 2.14 for adults with frank AIDS (CDC Stage IV.) Overall, there is thus an orderly progression to higher median titers and lesser variability with more advanced clinical stages of infection.

By contrast, the comparison of AIDS children with ARC children (Figure 5) does not indicate greater variability for the ARC children (geometric standard deviations of 2.34 and 2.42, respectively). Overall, the median and 95th percentiles of the serum titers of the children with clinical AIDS are about double the corresponding values of children with ARC. Similarly, children with AIDS tend to have a population distribution of HIV-ag titers that is almost parallel to the distribution of adult titers, but at levels that are 50-90% greater than for adults.

We can obtain some additional insight into the distribution of HIV-ag titers for patients at different stages of their disease with the aid of data recently published by Abrams et al. (1989). Table 3 and Figure 8 show data from baseline** measurements of p24 antigen for patients classified as in CDC's stage III category (persistent generalized lymphadenopathy--formerly such patients might be designated as having "ARC") vs those in various stage IV subcategories. As with the Goudsmit et al. data, the regression lines*** indicate generally higher antigen titers in the Stage IV patients (the indicated medians are 189 and 17 pg p24/ml, respectively--a greater difference, in this case, than was suggested earlier). There is also at least moderately more variability in the observed titers for the Stage III than for Stage IV patients (geometric standard deviations of 7.93 and 4.19, respectively).

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* These geometric standard deviations are simply 10^m where m is the slope of the regression lines of the titers vs. the "Z-score".

** Before therapy with dextran sulfate.

*** Where log(pg/ml) = b + mx, the raw regression coefficients are:

	b	m	R^2
Stage IV (AIDS)	2.2757	.62211	.878
Stage III (persistent lymphadenopathy)	1.2323	.8995	.960

Note, however that what was measured here is labelled p24 antigen, rather than the more generic HIV-ag--the latter consisting only of approximately 10% p24. Taking this difference in labelling literally, however, would lead us to predict considerably higher viral titers from the Abrams et al. (1989) data than from the Goudsmit et al. data. Because Abrams et al. (1989) do not describe their antigen measurement methodology, however, we are uncertain as to whether the difference between the results of the two sets of authors may be more apparent than real.

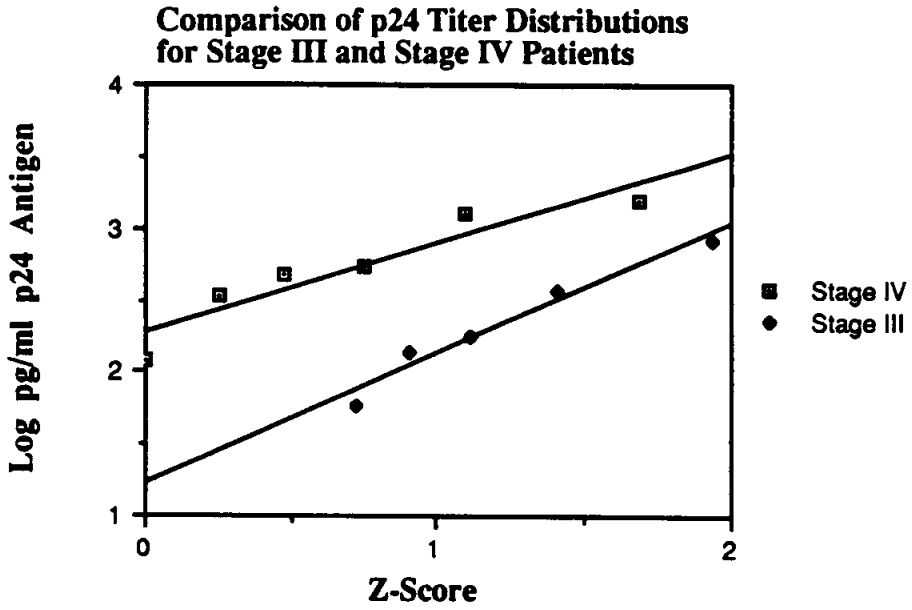
Table 3

Individual Data of Abrams et al. (1989) on Baseline P24 Antigen Titers in Patients Entering a Drug Trial

Stage III Patients pg/ml p24 Antigen*	Stage IV Patients pg/ml p24 Antigen*	Stage IV Subcategory
0	0	C2
0	0	C2
0	0	C2
0	0	C1
0	0	C2
0	119	C2
0	333.9	D
0	469	A
0	540	C1
0.2	1289	C2
1.1	1549	C1
4.9		
6		
7.1		
57.7		
139		
174		
369		
835		

*Only antigen measurements over 50 pg/ml were considered "positive" detections, and only these points are used for constructing the distributions shown in Figure 8

Figure 8



Data Source: Abrams et al. (1989)

Because of possible differences in assay procedures and calibration, we are reluctant to combine the data from these two laboratories in projecting absolute values of median infectious titers of HIV. However, it will be helpful for calculations later in our study to have a set of overall estimates of the interindividual variability of antigen (and presumably viral) titers at different stages of HIV infection. To do this, we use the observation that all sets of data appear to be lognormal, and compute a combined variance (and therefore geometric standard deviation) of the two samples, weighted according to the number of observations:

$$\text{Combined GSD} = 10^{[(n_1 m_1 + n_2 m_2 + \ldots)/(n_1 + n_2 + \ldots)]^{1/2}}$$

The results of these calculations are shown in Table 4. It is intriguing to speculate that the reduction in interindividual variability in antigen titers at later stages of HIV infection may reflect the ongoing pathogenic process. In early stages, when the immune system is not greatly impaired, episodic releases of viral particles and fragments containing viral antigens induce responses that cause relatively rapid clearance, and generally low but fluctuating viral antigen titers. In later stages of HIV infection it may be the case either that the episodes of viral release are more frequent, or the responses to those releases are weaker, or both, giving rise to less extreme fluctuations in viral antigen titers. This speculation might be tested by analysis of the variability of serial measurements of HIV-ag titers within individuals as their disease progresses.

3.2.2 "Infected Cell" Titers

Harper et al. (1986) have used in situ hybridization with a probe specific for HIV RNA.to examine the frequency of infected lymphocytes in peripheral blood and lymph nodes AIDS/ARCS patients. It is clear from their work that infected lymphocytes are relatively rare, but the precise counts are unfortunately not provided--instead Harper et al. describe their findings of the frequency of labeled cells in peripheral blood lymphocytes as "< .01% and usually < .001%" (that is, $< 10^{-4}$ or $< 10^{-5}$) in the 7 out of 14 patients where labeled cells were detected at all. Fauci (1988) has noted that latently infected cells may not be picked up in this assay (the amount of HIV RNA may be below the limit of detection), and thus this assay might tend to underestimate of the number of infected cells. However, studies using Southern blot technology to detect HIV DNA in lymph nodes suggested that the number of infected cells is probably less than 1 in 10^3 (Shaw et al., 1984, cited in Harper et al., 1986). Harper et al. (1986) estimated that between 20 and 300 copies of viral RNA were expressed per virus infected cell in the peripheral blood lymphocytes and in lymph nodes.

Table 4

Calculation of Combined Geometric Standard Deviations for Patients at Various Stages of HIV Infection

Calculation Based Only on Data From Adults:

Group	Goudsmit et al. data		Abrams et al. (1989) data		Combined GSD
	n_1	m_1	n_2	m_2	
AIDS (CDC IV)	34*	0.32954*	11	0.62211	3.47
	16**	0.57977**			
CDC III			19	0.8995	
CDC II/III	40***	0.70211***			5.90
Seropositive	35*	1.1999*			15.85

Calculation Including Data From Both Adults and Children:

Group	Goudsmit et al. data		Abrams et al. (1989) data		Combined GSD
	n_1	m_1	n_2	m_2	
AIDS (CDC IV)	34*	0.32954*	11	0.62211	3.24
	9*	0.36866*			
	16**	0.57977**			
	6**	0.45631**			
CDC III	9*	0.38419*	19	0.8995	
CDC II/III	40***	0.70211***			5.39
Seropositive	35*	1.1999*			15.85

*From Goudsmit and Paul, 1987

**From Goudsmit et al., 1986

***From Goudsmit et al., 1988

These values, in conjunction with the number of infected lymphocytes, can be used to make a very preliminary estimate of the range of concentrations of potentially infectious particles per ml of blood due to infected lymphocytes. Normal adults have approximately 2.7×10^6 lymphocytes/ml in blood, and similar amounts in lymph* (Smith et al., 1986). If, on the average, $<10^{-5}$ to $<10^{-4}$ of these were to be infected with HIV, the number of infected cells per ml is clearly on the order of <30-300, or <.03 - .3 per µl (in patients with more advanced stages of HIV infection these ranges might be too high because they would be expected to have a lower proportion of CD4 lymphocytes in their blood). Placed in a suitable environment for viral expression, of course, each infected cell can in turn produce about 20-300 infectious particles. However what is primarily relevant for assessment of potential infectiousness to exposed health care workers is the concentration of infectious cells themselves before transfer (assuming that potentially one infectious cell could give rise to an HIV infection).

These lymphocyte based estimates do not include the titer of HIV that may be associated with monocytes in whole blood. Normal adults have approximately 5×10^5 monocytes/ml of blood (Smith, 1986), about 20% of the number of lymphocytes. HIV is not cytopathic for monocytes, instead virus are released from infected cells slowly through the budding process (Fauci, 1988). If a similar fraction of monocytes were infected with HIV as the fraction of lymphocytes, then of course the "infectious cell" titer might be increased by a relatively small (20%) proportion. However, monocytes might well provide an important contribution to cell infectivity during the end stages of the disease when T4 lymphocytes are severely depleted.

Fortunately, we are able to make some better estimates of the frequency of infected cells in peripheral blood of healthy seropositive patients by using serial dilution isolation results of Ulrich et al. (1988). These workers cultured various numbers of PBMC's from 23 individual seropositive people with normal uninfected lymphocytes after the method of Gallo et al. (1987). Viral growth was detected by the production of reverse transcriptase. The number of positive cultures found when various numbers of potentially infected PBMC's were used is shown in the first three columns of Table 5.

In the next three columns of Table 5, the observed numbers of positive or negative cultures are compared with the numbers that would be expected, either (A) optimizing** the fraction of

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* This value includes T cells and B cells, but not granulocytes and monocytes. Among the T cells are approximately 800,000 T4 cells/ml which have large amounts of CD4 on the surface (Redfield and Burke, 1988)

** These "optimizations" were simply iterative trials where we honed in on the value(s) of mean titer and geometric standard deviation that produced a minimum value of the chi-squared statistic. The calculations were done in an Excel spreadsheet in which the lognormal distribution of individual infected-cell titers was approximated with a 100-interval step function. Within each of the 100 intervals, for each number of PBMC's cultured, the probability of a positive culture was

Table 5

Analysis of Ulrich et al. (1988) PBMC Culture Data*

A. Analysis Assuming No Interindividual Variability in the Frequency of Virus-Producing Cells in the Donors (Best-Fitting Proportion of Virus-Generating PBMC's is $5.87X10^{-6}$)

specimen PBMC's per culture	No. Trials (1 per person)	Obs. Positive Cultures	Expected Pos Cultures	Expected Neg Cultures	Chi-Squared
1E+02	23	1	0.01	22.99	72.15
1E+03	23	4	0.13	22.87	111.65
1E+04	23	14	1.31	21.69	130.21
1E+05	23	19	10.21	12.79	13.60
1E+06	23	21	22.94	0.06	57.84
				Total	385.44

B. Analysis Allowing Interindividual Variability Among the Donors (Best-Fitting Geometric Standard Deviation is 14.3; Best-Fitting Proportion of Virus-Generating PBMC's is $5.36X10^{-5}$)

specimen PBMC's per culture	No. Trials (1 per person)	Obs. Positive Cultures	Expected Pos Cultures	Expected Neg Cultures	Chi-Squared
1E+02	23	1	1.52	21.48	0.19
1E+03	23	4	5.19	17.81	0.35
1E+04	23	14	11.71	11.29	0.91
1E+05	23	19	18.22	4.78	0.16
1E+06	23	21	21.78	1.22	0.53
				Total	2.15

*The data shown in the first three columns are the results of culturing various numbers of peripheral blood mononuclear cells from the same 23 individuals in a standard HIV growth/detection system. The latter three columns show the results of comparing the observed number of positive or negative cultures (whichever was less) with the numbers which would be expected, assuming a lognormal distribution of infected-cell frequencies in the donor population, and a one-hit model of culture viral production (a culture would be positive if it received one virus-producing cell).

calculated as a one-hit function of the dose of truly infected PBMC's for individuals with titers in the specific range covered.

infected cells among the donor PBMC's but assuming that there is no interindividual variability in donor titers (upper panel) or (B) assuming that donors had a lognormal distribution of cell titers (fraction of PBMC's that would produce virus in culture), and optimizing both the median and geometric standard deviation of the assumed distribution of donor titers. It can be seen that no single uniform value of the fraction of infected PBMC's can be found which would produce a good fit to the data (the value of the chi-squared statistic which would provide a barely acceptable fit at p = .05 with five degrees of freedom is 11.07).

The best-fitting parameters for cell-based infectious titers (those which gave rise to the close description of the observations in Table 5B) can now be compared with our assessments of viral titers among asymptomatic seropositive individuals:

- The best fitting estimates of the interindividual variability in tissue culture infectious titers for the two types of measurements are remarkably similar--geometric standard deviations of 15.8 for the antigen-based estimates of free viral titers, vs 14.3 for the cell-based titers.

- If we assume 2.7×10^6 lymphocytes/ml in blood, and that 5.36×10^{-5} of the lymphocytes are productively infected (as assessed in tissue culture), then the median titer of infected cells comes to about 140 per ml, or 0.14 per µl. This is similar to the range of <.03 - .3 per µl calculated from the observations of Harper et al. (1986). Referring back to Table 2, it can be seen that our best estimate of the median viral titer in healthy seropositive people is only 4.4×10^{-4} per µl of serum, or about 2.4×10^{-4} per µl of blood.* If these calculations are even roughly correct, therefore, the indication is that for this particular population group the median cellular infectious titer may exceed the titer due to free viral particles by a very considerable amount--something like 500 fold. Given this, it may be productive to explore whether blood collection systems can be modified to rapidly destroy the viability of infectious cells without compromising the intended use of the sample for chemical measurements, etc.

It is unfortunate that we do not have available similar serial dilution measurements for later stages of HIV infection which would allow us to make similar comparisons for symptomatic patients (CDC stages III and IV). In the absence of such evidence, it seems likely that the increasing titers of free virus at later stages of HIV infection (Table 2) might become more important (and perhaps predominant) vectors of potential infection in fresh blood from patients with advanced disease.

3.3 Estimation of HIV Titers in Cerebrospinal Fluid

In an earlier paper, Goudsmit et al. (1986) measured the concentration of HIV antigen in cerebrospinal fluid (CSF) and serum of adult and pediatric AIDS patients using the enzyme

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* Assuming an average hematocrit of approximately 45%.

immunoassay (EIA) described previously. For unknown reasons, however, these titers apparently are not directly comparable to the HIV-ag titers given in the later Goudsmit and Paul (1987) paper.

The raw results are reproduced as Table 6. HIV antigen was detected in the CSF of 7 out of 16 adult AIDS patients and of 5 out of 6 pediatric AIDS patients. The concentration of HIV antigen in CSF is generally 2-20 fold less than the corresponding serum. However, for four individuals (including two children) the measured concentration of HIV antigen is higher in the CSF than in serum.

Figures 9-10, and Table 7 show comparative analyses of the distributions of serum and CSF titers using the same methods as were used for Figures 3-8 and Table 2, except that in this case we emphasize only the relative concentrations of HIV-ag in the different fluids. The regression equations in this case* indicate more variability in population distribution of serum titers than was found in the later Goudsmit et al. (1987) assays, and generally somewhat greater interindividual variability in CSF than in serum titers. Overall it can be seen in Table 7 that median titers in CSF are 4 - 11 fold less than in serum. Because of the greater variability suggested for CSF titers, the difference is less (1-4 fold) at the upper 95th percentiles of the distributions.

3.4 Time Course of Viremia

The time course of viremia of HIV is qualitatively summarized in Figure 11, taken from a review by Redfield and Burke (1988). This widely accepted model (c.f., Haseltine and Wong-Staal, 1988; Price et al., 1988) summarizes a growing body of data of HIV concentrations (including infectivity tests and enzyme immunoassays) during the different stages of AIDS.

Figure 11 indicates that there is substantial viremia at the time of initial infection with the virus which lasts between several weeks and several months. When the natural immune response begins to take effect, the concentration of infectious virus in peripheral blood decreases. This lower level (but not nonexistent) viremia persists for several years before the immune response decreases. When the measurable immune response decreases, the viremia increases, and clinical symptoms of AIDS begin to appear.

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* Where log(pg/ml) = b + mx, the raw regression coefficients are:

	b	m	R^2
Adult Serum	3.30224	.57977	.859
Adult CSF	2.24495	.86848	.916
Child Serum	3.63469	.45631	.915
Child CSF	3.03705	.83045	.800

Table 6

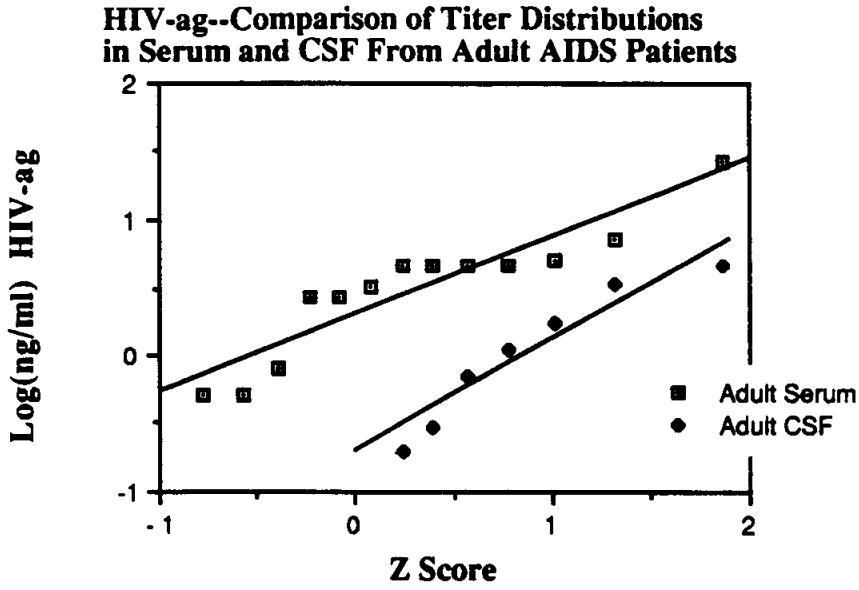
HIV Antigen and Antibody in Serum and Cerebrospinal Fluid of Adult and Pediatric AIDS Patients

Patient No.	Age (yrs) and Gender	HIV Antigen (ng/ml) in Serum	CSF
1	46 M	27.5	--*
2	42 M	4.7	0.2
3	33 M	0.5	--
4	46 M	4.6	1.1
5	38 M	0.5	--
6	54 M	2.7	--
7	36 M	--	1.7
8	35 M	--	--
9	41 M	5.1	0.3
10	39 M	--	--
11	25 M	4.7	3.4
12	40 M	7.4	--
13	38 M	0.8	--
14	32 M	3.3	0.7
15	25 M	4.6	--
16	38 M	2.7	4.7
17	3 F	9.0	--
18	1.9 M	17.3	3.9
19	2.5 M	3.6	7.6
20	2.6 F	3.9	0.1
21	6 M	4.2	5.2
22	2 M	0.7	1.4

*Not detected.

Source: Goudsmit et al. (1986)

Figure 9



Data Source: Goudsmit et al. (1986)

Figure 10

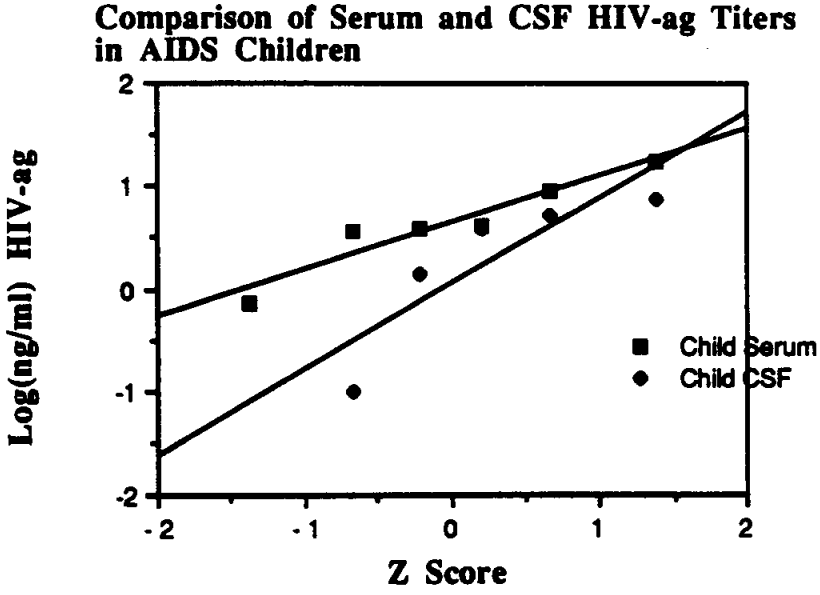
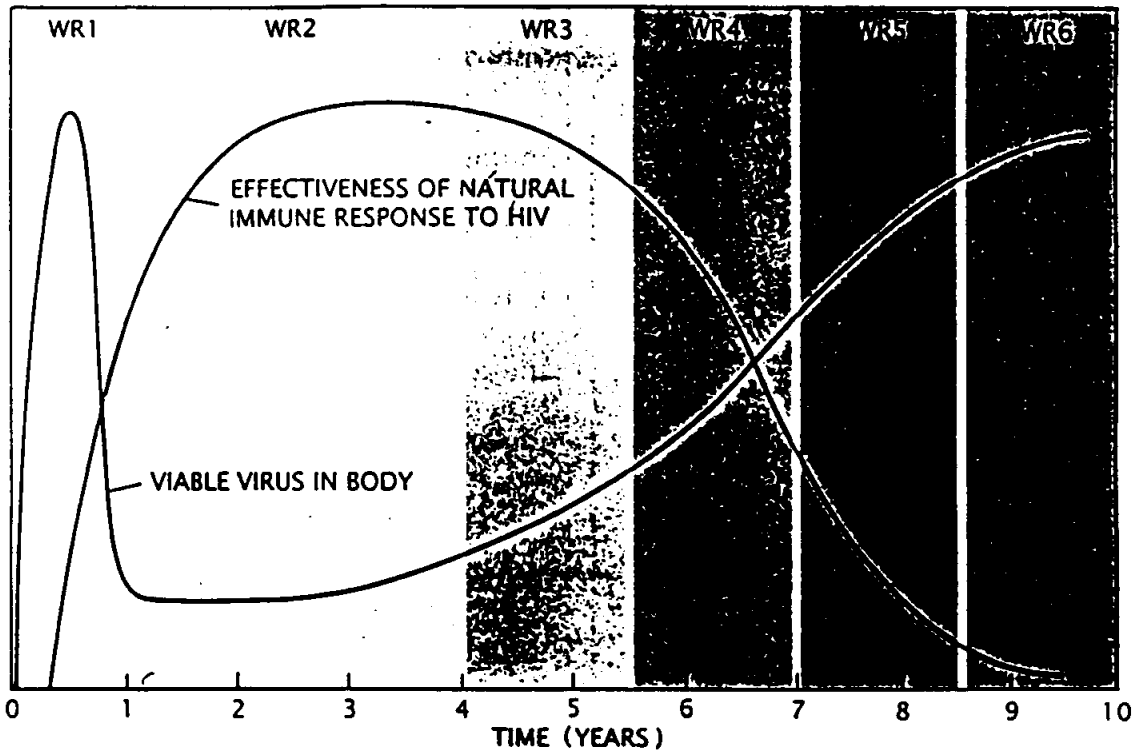


Table 7
Comparison of HIV-Ag Titers in Serum and CSF From AIDS Patients, Based on Older Goudsmit et al. (1986) Data

Group	----ng/ml HIV-ag--- Median	95th %tile	-----Serum/CSF Ratio---- Median	95th %tile
AIDS Adult Sera	2.0	18.0		
AIDS Adult CSF	0.18	4.7		
Adult Comparisons			11.4	3.8
AIDS Child Sera	4.3	24.3		
AIDS Child CSF	1.1	25.3		
Child Comparisons			4.0	1.0

Figure 11

Schematic Conception of the Change in HIV Titer During the Course of Infection



Balance of power between HIV ("viable virus" curve) and the immune system shifts during the course of infection, according to a model proposed by Redfied and Burke (1988). The amount of HIV in the body soars in the first days of infection, but once the immune system "kicks in," it initially operates normally and reduces the amount of virus. The immune system remains in good control of the virus for several years, but HIV gains ground slowly. At some point the T4 cells that orchestrate the immune response become so depleted that the balance of power switches. HIV then replicates wildly, killing the remaining T4 cells and hence any vestiges of immune defense.

Source: Redfield and Burke (1988)

4. FACTORS AFFECTING OCCUPATIONAL TRANSMISSION

4.1 In Vitro Stability of HIV

Several groups of investigators have examined the stability of HIV, as judged by its continued infectivity, under a variety of conditions likely to be encountered in a laboratory or clinical situation. These studies were primarily conducted as the control experiments in an effort to determine effective decontamination procedures (Martin et al., 1985; McDougal et al., 1985; Resnick et al., 1986).

McDougal et al. (1985) examined the thermal inactivation of HIV (stock titer $>10^5$ $TCID_{50}$/ml) in cell culture media. The thermal inactivation appeared to follow first order decay kinetics. The virus titer was reduced ten-fold after incubation at 37°C. for 116 hours. This corresponds to a half-life of 35 hours. Higher incubation temperatures resulted in shorter half-lives.

Resnick et al. (1986) diluted high titer HIV stock (10^7 $TCID_{50}$/ml) into 50% human plasma and then subjected the virus to room temperature (23-27°C.) or higher temperatures for time periods ranging from two to fifteen days. The treated samples were then tested for infectivity by plating onto PBMC's or H9 cells and monitoring for reverse transcriptase activity. Virus activity could be detected even after 15 days at room temperature and up to 11 days after storage at 37°C. Assuming a first order rate of decrease in titer throughout this time period, the half-life of the virus at 37°C in this matrix is approximately 13 hours and the half-life of virus at room temperature is more than 15 hours.

Resnick et al. (1986) also dried 1 ml aliquots of HIV (in 50% human serum) on culture plates, incubated the dried material at room temperature for 3 to 168 hours, and then tested for infectious virus. In this experiment, the inactivation rate was 6 to 9 hours per log reduction in titer, corresponding to a half-life of 2 to 3 hours.

Unfortunately we have no data available that would allow us to make analogous estimates of the inactivation half life of infected cells. Presumably, however, these entities would be much more rapidly destroyed under a variety of in vitro conditions than free viral particles.

4.2 Infectious Dose in Mouse/Human Chimeras

Namikawa et al. (1988) used a hematochimeric mouse as an animal model to study the initial stages HIV infection. The mouse, termed SCID-hu, is an initially severely immunodeficient strain into which human lymphoid organs were implanted. The human fetal thymus and lymph node implants were essentially indistinguishable from those of their normal human counterparts.

Namikawa and colleagues (1988) inoculated various dilutions of an HIV strain isolated from cerebrospinal fluid directly into the thymus or lymph nodes of SCID-hu mice. Infection was determined by in situ hybridization of infected tissue sections with an RNA probe specific for the genomic transcript. An inoculum corresponding to 400-4000 infectious units (in 20 μl), as measured in tissue culture, produced a detectable infection after two weeks (but not after one week). The authors report that an inoculum corresponding to 4-40 tissue culture infectious units did not produce signs of infection, but did not specify the details of the experiment (i.e., the number of mice used, the number of weeks post-inoculation before examination of thymus tissue, etc).

4.3 Interindividual Variability of the Susceptible Population

Folks et al. (1986) tested normal human peripheral blood lymphocytes (PBLs) for their susceptibility to infection with HIV. The PBLs of all ten healthy individuals tested (laboratory personnel at the NIH) became infected and produced HIV. However, the amount of virus produced, as determined by the amount of reverse transcriptase activity, differed approximately twofold among the ten individuals. The differences seemed to correlate with the fraction of cells with the Leu-3 marker, which is indicative of T4 helper cells. It seems unlikely that this relatively modest observed difference among individuals is a reasonable estimate of the true interindividual variability in susceptibility to HIV infection. In Task 3 of this project, we will draw inferences on this point from other data.

5. DESCRIPTION OF HEPATITIS B VIRUS

Hepatitis B virus (HBV) is a member of the hepadna family of viruses. The virus is a spherical particle with a diameter of 42 nm. It has a 27 nm inner core composed of the hepatitis B core antigen (HBcAg), partially single stranded, circular DNA, and DNA polymerase. The outer shell is a lipid membrane (envelop) which bears the hepatitis B surface antigen (HBsAg). HBV is only synthesized in hepatocytes (Sarver, 1986; Gerety, 1987). The disease now known as hepatitis B was formerly known as serum hepatitis and the viral particle known as a Dane particle.

Although HBV is a DNA virus, it usually replicates by reverse transcription as the RNA containing retroviruses do. Chronic infection is often accompanied by integration of the HBV into the genome of the infected liver cells (Gerety, 1987). Again, integration into host cellular DNA is a characteristic associated with retroviruses.

HBV is a major cause of chronic hepatitis. HBV infection can also lead to cirrhosis, hepatocellular carcinoma, or immune complex diseases such as necrotizing vasculitis and

glomerulonephritis (Sarver, 1986). Clinical disease caused by hepatitis B is usually manifest approximately 12 weeks (the typical incubation period range is 40-180 days) after infection (Francis and Maynard, 1979; Sarver, 1986). The length of the incubation period is inversely related to the dose of the virus (Barker and Murray, 1972), as discussed in section 6.1.

HBV can cause both acute and chronic infections, although, unlike HIV, acute infection does not necessarily lead to chronic infection. Hepatitis B infections become chronic in 5-10% of the infected adults and 70-90% of the infants infected at birth (Gerety, 1987).

Blood (or blood derivatives), semen, and saliva have been shown to transmit the disease. The demonstrated modes of disease transmission are (Bond et al., 1982):

- o percutaneous inoculation by a needle containing contaminated serum or plasma;
- o percutaneous transfer of infective material though a scratch or abrasion;
- o contamination of mucosal surfaces by infective serum or plasma, or bodily fluid containing serous material, including splashes to the eye, skin or mouth; and
- o transfer of infective material via inanimate environmental surfaces.

As pointed out by Peterson (1980), the ability of HBV to cause infection by penetration of mucosal surfaces allows at least the theoretical possibility that airborne transmission can occur. However, the epidemiological evidence is inconclusive and attempts to infect susceptible gibbons with an aerosol spray of infectious saliva (as determined by subcutaneous injection) were unsuccessful (Scott et al., 1980). This result cannot be considered especially definitive, however, because saliva has a virus particle titer one thousand to ten thousand-fold less than the corresponding serum (see below) and only a few attempts were made. Thus, if higher doses are required to generate observable responses by the inhalation than subcutaneous route, then infection may result from blood or serum aerosols.

5.1 Detection Assays

It is not yet possible to culture HBV in vitro (Gerety, 1987). Instead, HBV is usually detected by radioimmunoassays specific for different viral antigens. Most frequently, HBV is characterized by the surface antigen, HBsAg, formerly called the Australia antigen. Active disease is characterized by the presence of HBeAg, and this appears to be the best serological marker of infectivity (Francis and Maynard, 1979; Bond et al., 1982; Sarver, 1986). HBV can also be monitored by the presence of HBcAg, the core antigen.

In a few studies, the presence of infectious virus has been demonstrated by HBV transmission causing disease in humans or animals (chimps or gibbons). More recently, the presence of HBV in body fluids such as blood, semen, and saliva has been determined by

Southern blot analysis (Jenison et al., 1987). This nucleic acid hybridization technique can detect viral DNA sequences.

5.2 Stability of HBV

A detailed examination of the stability of HBV under environmental conditions has been hampered by the lack of a cell culture system to test virus infectivity. In a literature review, Bond et al. (1977) summarized the results of several investigators, including Barker and Murray (1972), showing that a serum can remain infectious, based on human and primate studies, after storage for 15 years at -20°C., 6 months at 27°C., and more than 4 hours at 60°C.

Favero and colleagues (reviewed in Bond et al., 1977) examined the heat stability of HBV using a specific radioimmunologic detection of HBsAg as a surrogate measure for infectious virus. By determining the linear region of the radioactive counts vs sample dilution curve, quantitative results were obtained. The investigators estimated that the D_{60} value (the time required for a ten fold decay in activity at 60°C.) was 227 hours. They concluded that HBsAg was remarkably heat stable, but this probably did not reflect virus infectivity. Transmission of HBV from environmental surfaces, such as computer cards, demonstrates that HBV is reasonably stable in dried blood (Francis and Maynard, 1979).

6. AMOUNT OF INFECTIOUS HBV IN HUMAN TISSUES AND FLUIDS

HBV has been detected in blood and blood products, semen, and saliva, as judged either by clinical symptoms of infection (in humans or primates), the presence of viral antigens, or by Southern blot hybridization (Barker and Murray, 1972; Scott et al., 1980; Jenison et al., 1987). In addition, investigators have detected the presence of HBsAg in other materials, such as waste dialysis fluids. The quantitative results of these studies, in terms of infectious particles per ml of fluid, are presented below.

6.1 Blood

Murray and colleagues conducted studies with pooled human plasma known to be icterogenic (cause jaundice) on inmates of federal penitentiaries. Inmates were inoculated with 1 ml of either the initial plasma, serial dilutions of the initial plasma, or the plasma treated by various disinfection procedures. They were then monitored for symptoms of hepatitis. Fifteen years later,

frozen serum samples from these studies were assayed for the presence of HBsAg (Barker and Murray, 1972). The results of this serial dilution portion of this study are reproduced in Figure 12.

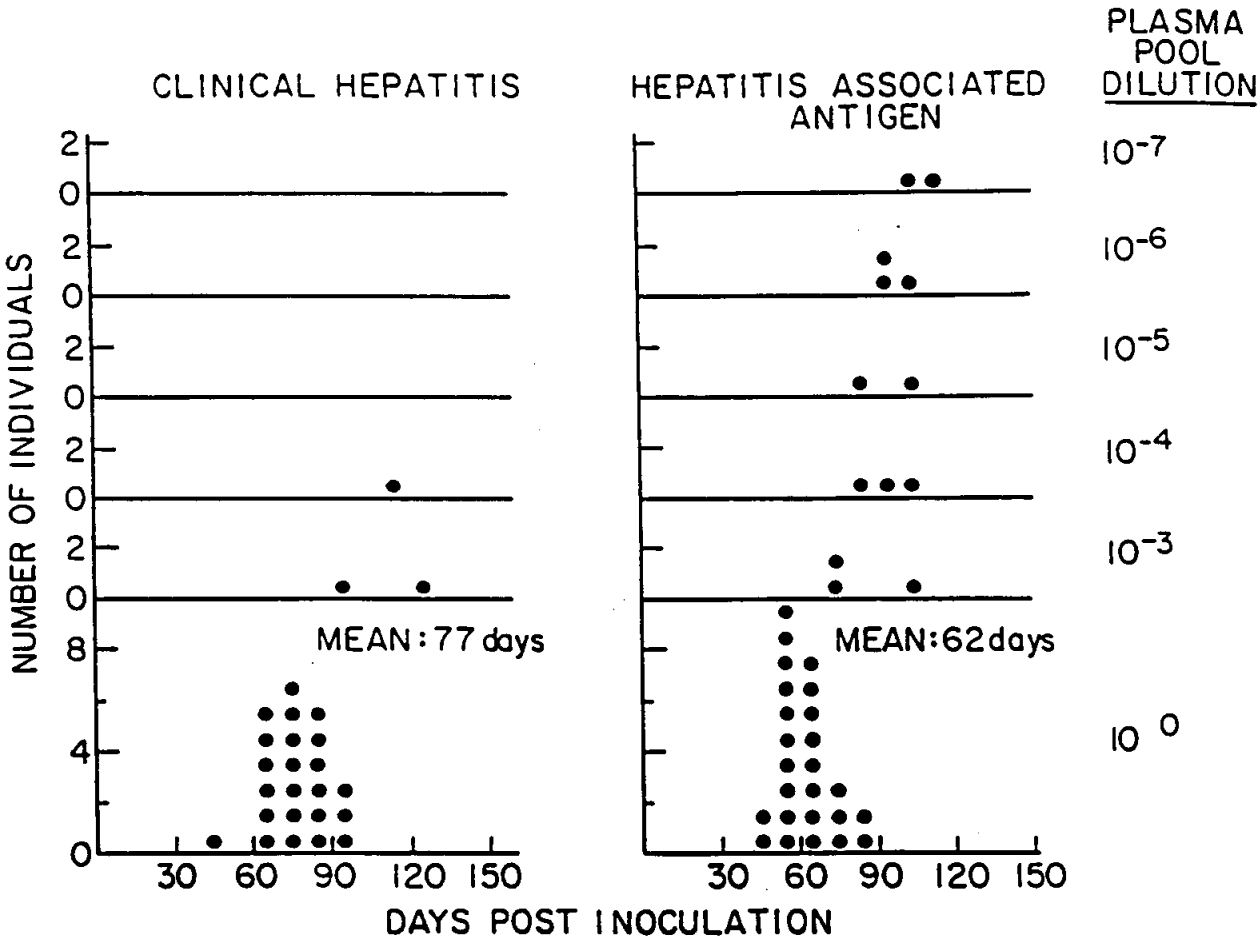
Of the 37 individuals who received the undiluted plasma pool, 22 developed clinical hepatitis and 25 tested positive for HBsAg. The time to onset of clinical disease ranged from 45 to 92 days with a mean of 77 days. The time to detection of HBsAg ranged between 42 and 82 days with a mean of 62 days. It appears that five individuals were inoculated with each of the plasma pool dilutions. At the 10^{-3} dilution, two individuals developed clinical disease and three had detectable levels of HBsAg (presumably the two with clinical disease and one other) in their serum. At the 10^{-4} dilution, one individual developed clinical disease while a total of three had detectable levels of HBsAg. Two individuals had detectable HBsAg after inoculation with the 10^{-7} dilution of the original serum pool, which indicates an original titer of at least 10^7 infectious units per ml. Later in this project, as part of the work under Task 3, we will analyze the human interindividual variability in susceptibility of HBV seroconversion and clinical disease implied by these data.

Jenison et al. (1987) used the Southern blot technique of nucleic acid hybridization to assay quantitatively for the presence of HBV DNA in the semen, saliva, and serum from 15 homosexual men known to be chronic HBsAg carriers. From these data, they were able to estimate the number of virus particles/ml of fluid. Their results are presented in Table 8. It should be noted that the men in this study had been recruited as plasma donors [from the New York (City) Blood Center from 1978-1982] for the preparation of Hepatitis B vaccine because of the high titer of their serum HBsAg antigen. Thus, the resulting serum, saliva, and semen virus particle estimates may show a skewed distribution toward the high end of the infected population.

Jenison et al. (1987) found that the patients could be divided into two groups on the basis of serology: those who had high serum titers of HBeAg ($\geq$1:625), and those who had low titers of HBeAg ($\leq$1:5). These serological groups correlated with the measurement of minus strand HBV DNA in the serum and subsequent estimates of virus particles in the fluids. Seven of the nine individuals with high serum titers of HBeAg had greater than 2×10^9 virus particles/ml serum. One individual appeared to have greater than 10^{10} virus particles/ml serum. The other two individuals with high serum titers of HBeAg appeared to have 5×10^8 virus particles/ml serum. HBV DNA was detectable in only two of the six individuals with low serum titers of HBeAg, and the estimated concentrations of virus in the serum was substantially lower, roughly 8×10^6 virus particles/ml serum.

Figure 13 shows a distributional analysis of the serum data in Table 8. It can be seen that in this case, the lognormal model does not produce as accurate a fit to the data as we observed in general for the HIV antigen titers. Moreover, the indicated degree of interindividual variability (geometric standard deviation = $10^{1.4378} = 27.4$) appears to be somewhat greater even than the

Figure 12
Hepatitis B Virus Dose-Response Relationships for Induction of Clinical Hepatitis and Chronic Infection



Incubation time of clinical hepatitis (left) and appearance time of HAA (right) in individuals inoculated with 1 ml subcutaneously of an icterogenic pool of human plasma, undiluted and diluted from 10^{-3} to 10^{-7}.

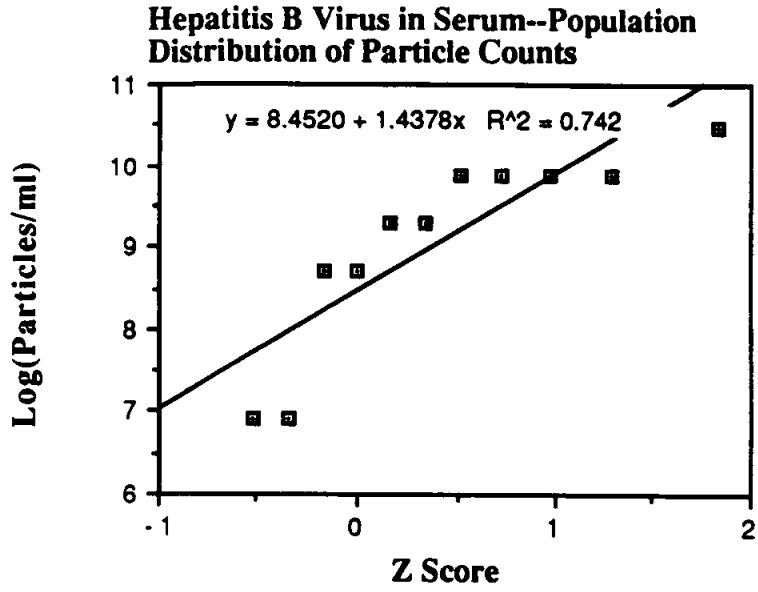
Source: Barker and Murray (1972).

Table 8
Quantitative Comparison of Serum HBeAg and Estimated Virus Particles/ml in Serum, Saliva, and Semen

		---------Estimated virus particles/ml------		
Patient	Serum HBeAg	serum	salivary fluid	semen
A1-049	≥1:625	3×10^{10}	10^6	10^6
A1-012	≥1:625	8×10^9	10^5-10^6	-
A1-039	≥1:625	8×10^9	10^5-10^6	-
A1-037	≥1:625	8×10^9	10^5-10^6	10^5-10^6
A1-136	≥1:625	8×10^9	10^6	-
A1-057	≥1:625	2×10^9	10^5-10^6	10^6-10^7
A1-045	≥1:625	2×10^9	10^5-10^6	-
A1-140	≥1:625	5×10^8	-	-
A1-093	≥1:625	5×10^8	10^5	-
A1-043	1:5	8×10^6	-	-
A1-047	1:5	8×10^6	-	-
A1-114	1:5	-	-	-
A1-128	1:5	-	-	-
A1-060	1:5	-	-	-
A1-164	-	-	-	-

Adapted from Jenison et al. (1987).

Figure 13


Hepatitis B Virus in Serum--Population Distribution of Particle Counts
y = 8.4520 + 1.4378x R^2 = 0.742
Log(Particles/ml)
11
10
9
8
7
6
-1
0
1
2
Z Score

relatively large variability of HIV-ag titers observed for asymptomatic anti-HIV seropositive people. The indicated median viral particle titer ($10^{8.452} = 2.8 \times 10^8$) is consistent with the results of Barker and Murray (1972) of $>10^7$ infectious units/ml serum, and suggests that it may not be very wrong to assume that an infectious dose of HBV sufficient for seroconversion (although not clinical illness) may correspond to a single viral particle. As indicated earlier, the results of Jenison et al. (1987) are likely to be skewed toward the high end of infectious titer of serum while Barker and Murray used a pooled serum sample. In addition, Barker and Murray (1972) required infection to occur in order to observe a response while Jenison et al. only required the presence of HBV DNA to estimate infectious units. Thus, the concentration of infectious units determined by Barker and Murray would be less than the concentration of virus particles if not all of the HBV DNA resulted in infectious particles, or if more than one infectious HBV particle is necessary to produce an infection.

6.2 Semen and Saliva

As can be seen in Table 8, Jenison et al. (1987) used the Southern hybridization experiments described in Section 6.1 to test for the presence of HBV DNA in semen and saliva from the same individuals who were tested for HBV DNA in their serum. HBV DNA was detected in eight of the nine samples of saliva from individuals with high serum titers of HBeAg, but not in any individuals with a low titer of HBeAg. Quantitative estimates of virus particles were made by comparing the intensity of the signal on the Southern blot with signals from 10-fold dilution series of the matched serum samples. The estimated levels of virus particles were 10^3-10^4 fold less than those in the corresponding serum.

HBV DNA was detected in three of the nine samples of semen from individuals with high serum titers of HBeAg, but not in any individuals with a low titer of HBeAg. The estimated levels of virus particles were roughly comparable to those observed with saliva, and thus 10^3-10^4 fold less than those in the corresponding serum.

Figure 14 and Table 9 show a comparative distributional analysis of viral particle titers in serum, semen and saliva. The regression lines for semen and saliva* indicate 3-4 orders of

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* Where log(viral particles/ml) = b + mx, the raw regression coefficients are:

	b	m	R^2
Saliva	5.2306	.454	.755
Semen	4.4774	1.1179	.975

Figure 14

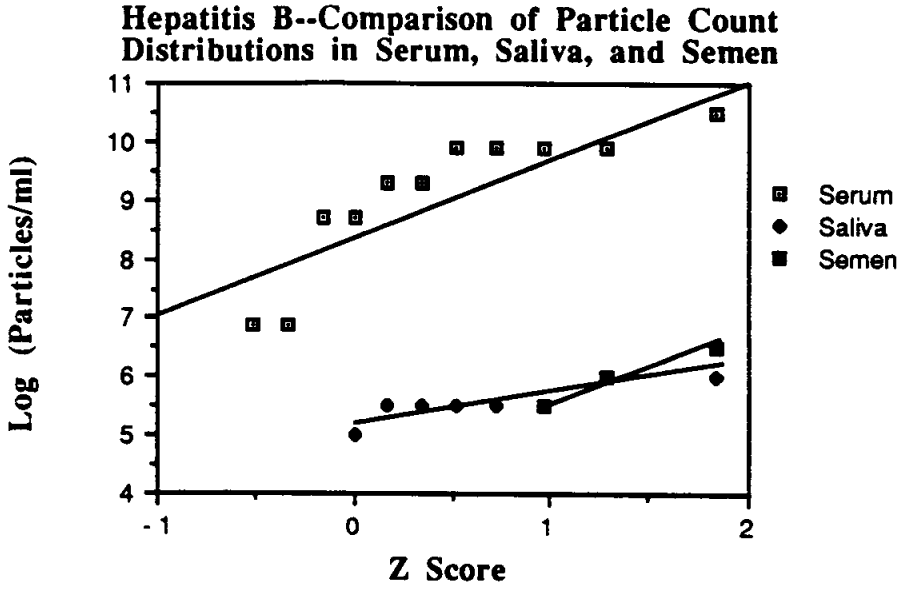
Hepatitis B--Comparison of Particle Count Distributions in Serum, Saliva, and Semen
Log (Particles/ml)
11
10
9
8
7
6
5
4
-1
0
1
2
Z Score
Serum
Saliva
Semen

Table 9
Serum/Saliva/Semen Comparisons Based on Jenison et al. (1987) Hepatitis-B Southern Blot Hybridization Data

	Estimated Virus Particles/µl		-----Comparisons------	
	Median	95th %tile	Median	95th %tile
Serum	2.8E+05	6.6E+07		
Saliva	1.7E+02	9.5E+02		
Serum/Saliva Ratios			1.7E+03	6.9E+04
Semen	3.0E+01	2.1E+03		
Semen/Saliva Ratios			9.4E+03	3.2E+04

magnitude lower median titers and possibly less interindividual variability for these media than was seen for serum.

These results are consistent with earlier studies of Scott et al. (1980) and Bancroft et al. (1977) in which human semen and saliva were observed to transmit hepatitis B infection to gibbons. Scott et al. (1980) detected the presence of HBsAg 10-16 weeks after exposure of two gibbons to 5 mls (2.5 ml doses on consecutive days) of saliva by subcutaneous inoculation. A similar dose delivered to three gibbons by aerosolization directly into the nose and mouth or two additional gibbons by aerosolization into the mouth followed by toothbrushing, did not produce signs of infection. The donors of the saliva had serum HBeAg titers greater than 1:1000, a level at least as high as the donors used in the study by Jenison et al. (1987). If the titers of virus particles in the saliva in the Scott et al study were similar to those determined by Jenison et al., then the gibbons were inoculated with $5x10^5$-$5x10^6$ virus particles.

One gibbon was exposed to HBV by sc inoculation of 1.8 mls of semen. The gibbon developed HBsAg in 8 weeks, although death occurred before infection could be ascertained. The serum of the semen donor was positive for HBsAg and HBeAg, but the titer was not reported (Scott et al. 1980).

6.3 Other fluids

Bond et al. (1982) have examined the potential infectivity of peritoneal dialysis fluid by determining the level of characteristic HBV antigens, HBsAg and HBeAg. Wastes from both intermittent peritoneal dialysis (IPD) and continuous ambulatory peritoneal dialysis (CAPD) were tested. For patients who were seropositive for HBsAg, waste dialysates from both IPD and CAPD were also positive for HBsAg. In addition, during early times in the dialysis, hepatitis B virus particles could be observed by electron microscopy (EM) in the dialysis fluids. Detection by EM usually requires a minimum of 10^6 HBV particles/ml (Bond et al., 1982). Bond et al. (1982) recommend that waste peritoneal dialysis fluids from HBsAg positive patients be handled in the same manner as HBsAg blood.

Bond et al. (1977) described experiments in two remote Alaskan villages to determine whether transmission of Hepatitis B could be associated with contact with bodily secretions or contaminated surfaces. As part of the study, gingival swab samples were taken. The investigators found that a higher percentage of the swab samples were positive for HBsAg in the absence of detectable blood than were corresponding saliva samples. According to Bond et al. (1977), this suggests that HBsAg is concentrated at the gum line crevicular fluid, which is a serum-derived material originating at the tooth root-capillary interface.

6.4 Time Course of Viremia

Figure 15 shows the two serological profiles typical of chronic carriers of Hepatitis B. The dotted line shows the relative concentration of HBeAg, the antigen closely associated with infectivity, over time. HBeAg appears during acute infection, a stage that lasts approximately six months, and can persist at high levels for years after the initial infection. Some chronic carriers show a decrease to undetectable levels of the antigen after years of chronic infection while others maintain the high level of serum antigen levels (Sarver, 1986; Abbott, 1984).

7. SUMMARY COMPARISON OF DISTRIBUTIONS OF INFECTIOUS TITERS IN CHRONIC HIV- AND HBV-INFECTED PEOPLE

Table 10 summarizes our quantitative conclusions. The data are generally consistent with a lognormal form for the distributions of titers, and are therefore conveniently summarized as geometric means and geometric standard deviations. An important caveat to the HIV results is that we cannot be very certain at this point of the relationship between the "Tissue Culture Infectious Doses" represented in Table 10 and the human in vivo infectious doses to workers who might receive parenteral inoculations of blood from HIV-infected people. We will make some inferences on this issue in Tasks 3 and 4 of this project after examining the existing epidemiology on worker seroconversions and the volume of blood that might be transferred in needlesticks. Tentatively, however, we can note that the indication of fractional tissue culture infectious units per microliter of blood, and the data of Napoli and McGowan (1987) indicating that the median needlestick may involve transfer of about 1.4 μl of blood, may well suggest that a modest number of tissue culture infectious units (on the order of 10) could be expected to produce a 50% probability of infection for exposed workers.*

One interesting finding that emerged from the analysis of the HIV "free virus" titers is the trend toward both higher median "free virus" titers and reduced variability of titers at later stages of HIV infection. It is possible that this may reflect some aspects of the ongoing pathogenic process. In early stages, when the immune system is not greatly impaired, episodic releases of viral particles and fragments containing viral antigens might induce responses that cause relatively rapid clearance, and generally low but fluctuating viral antigen titers. In later stages of HIV infection it

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* Namikawa and colleagues (1988), working in the SCID-hu mouse system report that an inoculum corresponding to 400-4000 tissue culture infectious units produced a detectable infection after two weeks, but that an inoculum corresponding to 4-40 tissue culture infectious units did not produce signs of infection.

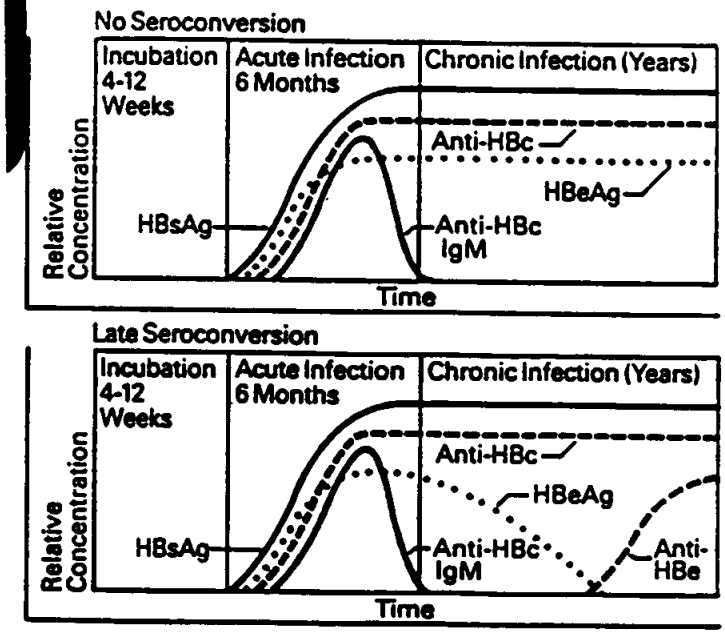
Figure 15

ABBOTT

Hepatitis B Diagnostic Profile

Serological profile in 75-85% of patients with acute type B Hepatitis

Hepatitis B Chronic Carrier Serological Profiles:



Source: Abbott (1984).

Table 10
Preliminary Estimates of HIV and HBV Titer Distributions

Blood HIV Titers Attributable to Free Virus Particles:

Group	pg/ml HIV-ag (10% p24, Based Solely on Recent Goudsmit et al. Results) Median	Geom. S. D.	Highly Tentative Estimate of Tissue Culture Infectious Doses Per µl of Blood Median***	Geom. S. D.****
AIDS Children*	114	2.33	0.036	
AIDS Adults*	72	2.13	0.023	3.47
ARC Children*	48	2.42	0.015	
CDC II/III Adults**	42	5.03	0.013	5.9
Ser+ Adults*	0.76	15.8	0.00024	15.8

Blood HIV Infectious Titers Attributable to Infected Cells:

AIDS (CDC IV)	No Data	
CDC III	No Data	
Ser+ Adults*****	0.14	14.3

Hepatitis B Virus Titers:

Medium	Estimated Virus Particles/µl Median	Geom. S. D.
Serum	156,000	27.4
Saliva	94	2.84
Semen	16.5	13.1

*Based on data of Goudsmit and Paul (1987).

**Based on data of Goudsmit et al. (1988)

***These estimates of median infectious titers are based on the more recent Goudsmit et al. HIV-antigen data summarized in the previous two columns, an assumption that the "HIV-ag" measured is about 10% p24, and an assumption from tissue culture experiments that 1 pg of p24 corresponds to 1-10 tissue culture infectious doses of HIV. (The center of this range was used for calculations.) If we had used the recent data for serum p24 antigen levels of Abrams et al. (1989), or older Goudsmit et al. (1986) results, the estimated median titers would be about 10-fold higher. A hematocrit of 45% was assumed in converting serum data to blood equivalents.

****Geometric standard deviations in this column are based on all available data, including earlier Goudsmit et al. (1986) results and the recent results of Abrams et al. (1989)

*****Based on our best-fit analysis of the serial dilution experiments of Ulrich et al. (1988).

may be the case either that the episodes of viral release are more frequent, or the responses to those releases are weaker, or both, giving rise to less extreme fluctuations in viral antigen titers. This speculation might be tested by analysis of the variability of serial measurements of HIV-ag titers within individuals as their disease progresses.

An important part of our overall evaluation of "infectious titers" for HIV-infected individuals is an assesssment of the likely relative roles of infected cells and free viral particles in transferring infections. Unfortunately we can only offer a tentative comparison of cell-based titers with "free virus" titers in the case of one population group. For asymptomatic seropositive individuals, however, it appears clear that cells represent a much greater potential vector of infection than free virus. The indication is that for this particular population group the median cellular infectious titer may exceed the titer due to free viral particles by something like 500 fold. Given this, it may be productive to explore whether blood collection systems can be modified to rapidly destroy the viability of infectious cells without compromising the intended use of the sample for chemical measurements, etc.

In the case of Hepatitis B virus, we have more direct reason to believe that infectious titers are at least of the same order of magntidue or so as the particle counts on which we based the titer estimates in Table 10, although there is still appreciable uncertainty. Even though the population that gave rise to the Hepatitis B titer estimates in Table 10 is likely to be unrepresentatively high in titer relative to a typical group of HBV carriers, the data go a considerable way in explaining the much greater frequency of occupational transmission of infection for HBV than for HIV.

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