SOLVING AND CREATING PROBLEMS: HEPATITIS B VIRUS AND THE PUBLIC HEALTH*†

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INTRODUCTION

PROFESSOR Sivaraj Ramaseshan, Professor V. Radhakrishnan, Professor Sharat Chandra, colleagues, ladies and gentlemen:

I am honoured to have been invited, in my capacity as Raman Visiting Professor of the Indian Academy of Sciences, to deliver the Gandhi Memorial Lecture for 1986. I have been told that the Raman Trust, which sponsors this lecture, requires that it be held each year, often on the anniversary of the death of Mahatma Gandhi, and within the precincts of the Raman Research Institute.

It is very appropriate that the names of two outstanding Indians, Gandhi and Raman, should be linked. Gandhi was a nationalist in the sense that he recognized the immediate and special problems of his own country and adopted original political and practical solutions to these problems. But he also had the vision to recognize that his methods and his goals had a universal appeal. They inspired many outside India and gave to the world of the oppressed the often-successful political approach of non-violence and the principle of truth-force *Satyagraha*. In particular, he inspired our own Martin Luther King, whose great contributions have been further recognized this year by the establishment of a United States national holiday to honor his achievements.

Professor Raman also was dedicated to his nation and his homeplace. He maintained his Indian identifications in dress and style even when among his fellow scientists of the West. But he also had the vision to recognize his contributions to world science, which have become even more apparent in recent years. He established the Raman Research Institute and served as the Director of the Indian Institute of Science, institutions of the highest intellectual standards; they have set a demanding standard for the young people of India and offer a powerful means of interaction with scientists in other parts of the world. They can provide the means by which natural observations of phenomena peculiar to India can enter the corpus of scientific problems of interest to the world scientific community.

Before proceeding to the main text of my presentation, I would like to show a photograph (figure 1) which provides an interesting link between Professor Raman and my own institution, the Institute for Cancer Research, Fox Chase Cancer Center, in Philadelphia. It is a picture of Professor Raman and other distinguished x-ray crystallographers, including the late Dr A. Lindo Patterson, much of whose career was spent at the Institute for Cancer Research in Philadelphia. It was taken in 1948 at the First Congress of the International Union of Crystallography at Harvard University, Cambridge, Massachusetts. It pictures Professor C. V. Raman along with J. D. Bernal (England), C. Patacke (U.S.), P. P. Ewald (Germany and U.S.), and the afore-

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Figure 1. Professor C. V. Raman with Dr A. L. Patterson (Institute for Cancer Research, Fox Chase Cancer Center) and other scientists at the First Congress of the International Union of Crystallography, Cambridge, Massachusetts, 1948. From left to right: J. D. Bernal, C. V. Raman, C. Palache, P. P. Ewald, A. L. Patterson.

mentioned A. L. Patterson (Canada, England, U.S.).

In my talk today I plan to cover several topics.*

1) A description of the research on hepatitis B virus [HBV] and how it can contribute to the public health in India and elsewhere. I will also try to show how broad the research has become over the 20 years it has been in progress and will cite findings of biological interest which have developed along the pathways of research.

2) A review of aspects of scientific process in an attempt to show the problem solving and problem creating character of the scientific method.

3) A discussion of how the the solution of an important medical problem concerning HBV generated another problem which then had to be approached by additional attempts at problem solving.

The discovery of hepatitis B virus: Medical applications.

The research which led to the discovery of the hepatitis B virus began as an investigation of inherited variation in humans, specifically by the examination of inherited proteins in the blood. One of the most impressive features of medicine is the great variation in response of this host to disease-causing agents. We reasoned that if we initially studied such serum variation in normal individuals, we could eventually determine how people differ in respect to disease susceptibility and this in turn could help in prevention and therapy. Several of the blood proteins were known to be polymorphic, hence patients requiring multiple transfusions are likely to receive in their blood serum proteins that they themselves had not inherited or acquired. We hypothesized that if some of these proteins were antigenic, a transfused

patient might develop serum antibodies against them and his or her blood could be used as a "reagent" to study an inherited serum protein system.

While testing this hypothesis, we identified, in 1961, inherited antigenic variation of the serum low density lipoprotein. Subsequently, we and others found that the sera from selected transfused patients defined an elaborate multi-loci system with many alleles controlling serum lipoprotein specificities. This has been used in genetic and forensic studies and may be of value in an understanding of inherited differences in susceptibility to cardiovascular diseases. Since our hypothesis had been successful in revealing a hitherto unknown polymorphic system of variants, we continued to test the serum of transfused patients against a panel of normal sera with the expectation that we would find additional antigen-antibody systems of interest.

The frequencies of genes determining polymorphisms are known to vary greatly from population to population. Hence, to increase the probability of finding unknown phenotypes, we tested the transfused serum not only against sera from the United States (where our work was being done), but also from Europe, Africa, Asia and Oceania.

In one of these studies we detected a precipitin band different from any we had seen before. It developed between the serum of a transfused hemophilia patient from New York and the serum of an Australian Aborigine (figure 2). Our problem now consisted of determining the nature of this protein present in the Aborigine[3].

In order to provide data from which to generate hypotheses to begin unraveling the nature of this unknown material (called "Australia antigen," after the population in whom it was first found, and abbreviated as Au), we tested a large number of sera and found that Australia antigen was rare in normal Americans and north Europeans but common in populations from several areas of the tropics. It was also common in people with leukemia and

* A few references to cited work will be given in this review. A more complete bibliography can be found in references 1 and 2.
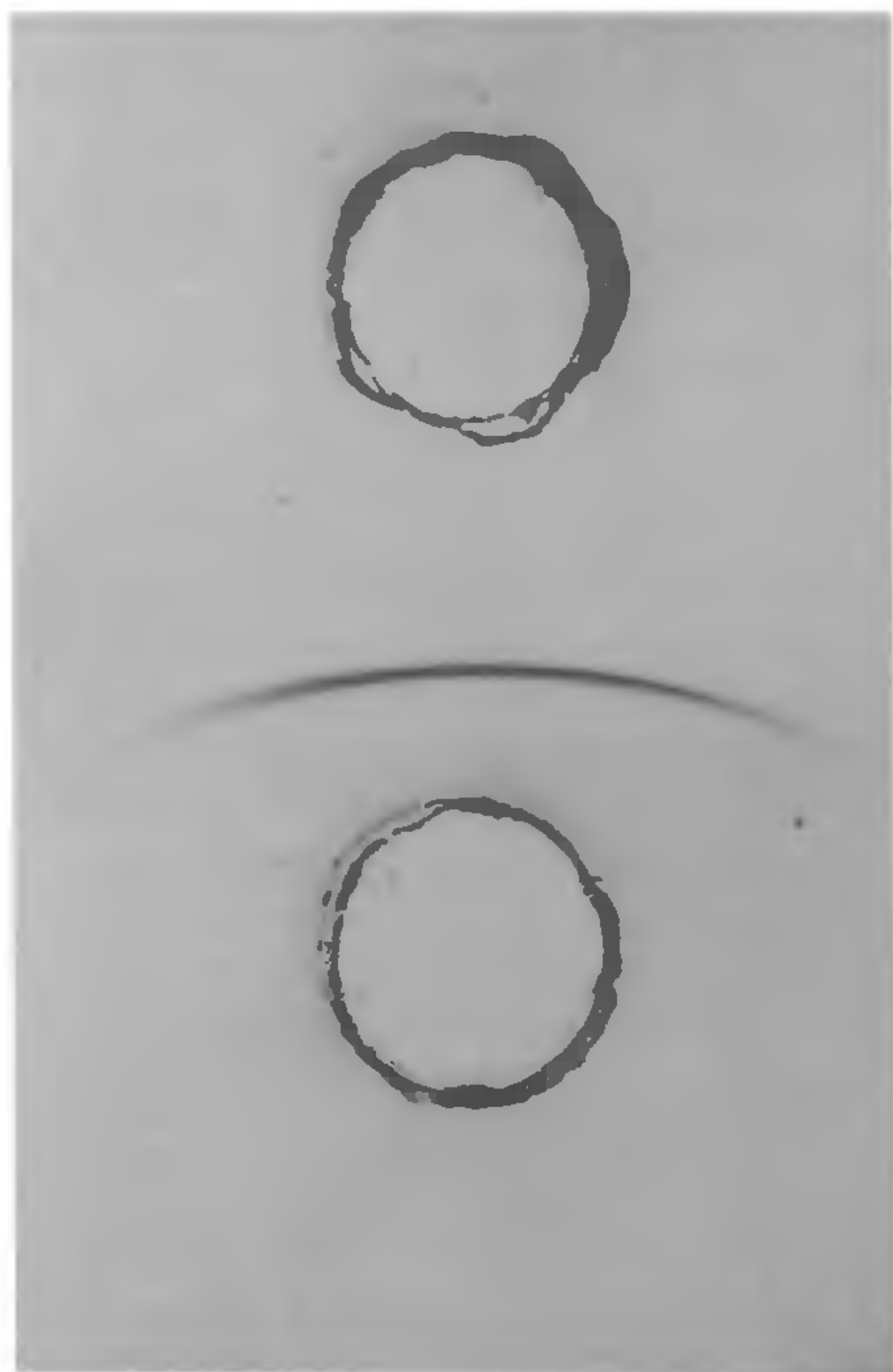
Figure 2. A precipitin band between a serum from a leukemia patient containing Australia antigen (top well) and an antibody against it in the serum of a haemophilia patient (bottom well).

this generated an additional hypothesis. Since patients with Down's syndrome (DS, trisomy of chromosome 21 associated with mental retardation) have a greater risk for leukemia than control groups, we predicted that they too would have a high frequency of Au. We tested sera from Down's patients and found that about one third of institutionalized patients had Australia antigen.

Studies were made of DS patients with and without Au in order to determine the factors which led to this "condition". On one occasion we observed that a young man who did not have Au initially, developed detectable amounts of the antigen over a period of weeks. We soon discovered that the appearance of the Australia antigen was associated with labora-tory evidence of the development of chronic, asymptomatic hepatitis. This gave us an important clue as to the nature of Au and generated the hypothesis that Au was associated with viral hepatitis.

We tested this hypothesis in turn (in 1967) by simply testing the serum of hepatitis patients, both acute and chronic, for the presence of Au. We soon established that there was a significant association between Au and hepatitis. Having supported this hypothesis, we then created another, namely that Au was on, or was part of, the hepatitis virus. By 1968 we had reported the visualization in the electron microscope of particles associated with the putative virus (figure 3). A series of other observations supported the hypothesis that Australia antigen was part of what soon became designated as hepatitis B virus (HBV)[4]
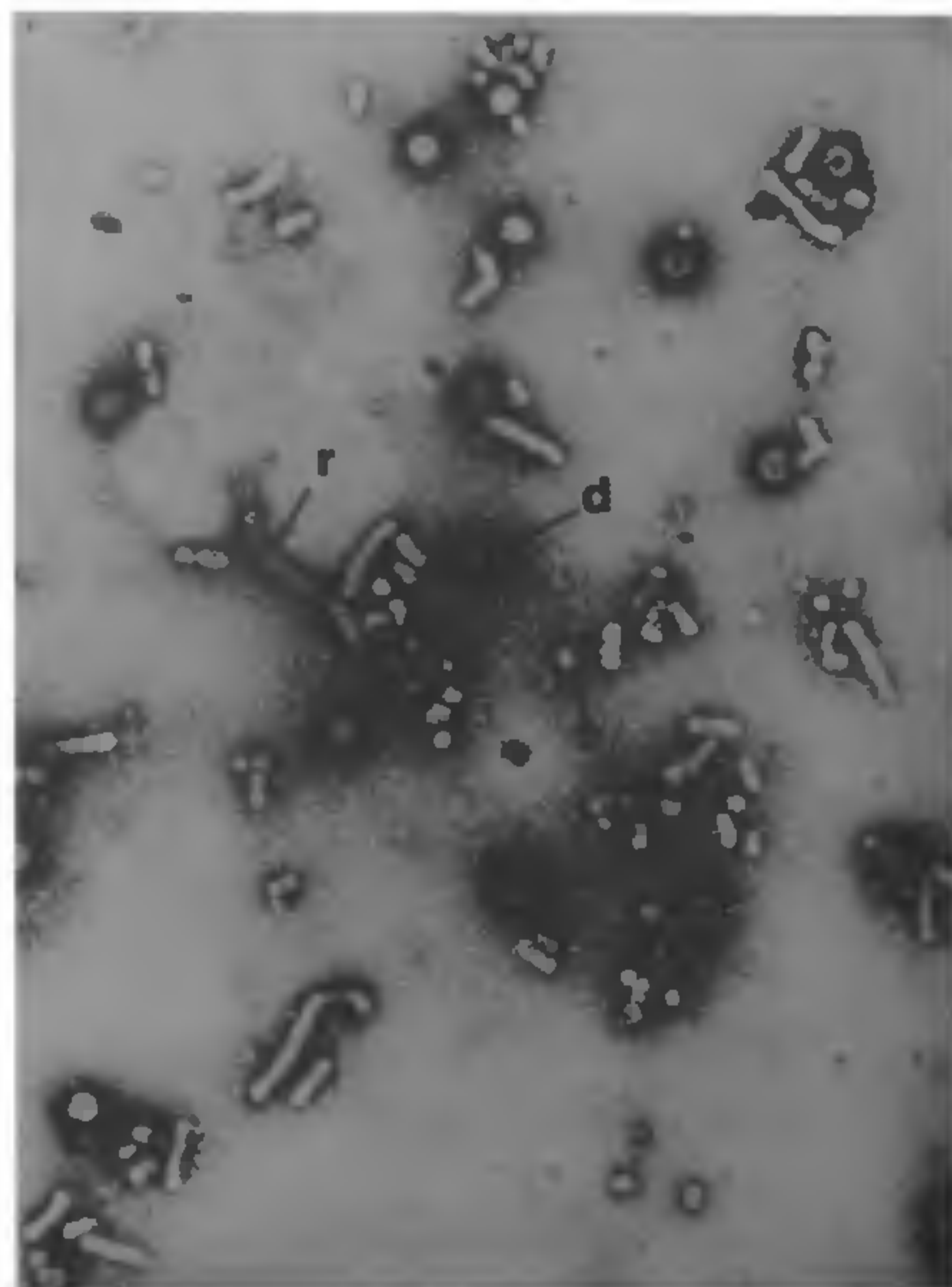
Figure 3. Electron micrograph of the hepatitis B virus particles. d = the whole virion (~ 42 nm); s = the surface antigen particle (about 21 nm); r = elongated particles containing surface antigen (about 21 nm diameter variable lengths).

Even before we had concluded the testing of the virus hypothesis, it became clear that the test we had developed for Australia antigen had a very important application. For many years, a serious and sometimes deadly complication of surgery was the development of hepatitis in patients who had been transfused with blood from a donor who was an occult carrier of hepatitis virus. The donors themselves could be asymptomatic; but if their blood were transfused to a patient, the recipient might develop clinical hepatitis.

In medical research when a finding derived from a basic scientific observation can be used for therapy or disease prevention, an obligation arises to utilize the discovery. By 1969, we advocated[5] donor blood testing and were doing this for the Philadelphia General Hospital. (This was the first hospital in the United States in which donor testing was performed. It illustrates a feature of medical research: the institution in which research is done is often the first place where the beneficial findings are applied.) The "Au test" was also valuable for the diagnosis of acute and chronic hepatitis and became widely used for these purposes.

It appeared then that a medical problem, that is, the prevention of post-transfusion hepatitis due to hepatitis B, had been, to a large extent, solved (particularly when a sensitive radioimmunoassay for HBV was later introduced by ourselves[6] and others). However, the solution had raised other problems, namely the personal, psychological and sociological complications connected with the identification of the carriers. This point will be discussed in greater detail later, after the discussion of other aspects of the hepatitis B research.

Introduction of the vaccine

In 1969, we had received advice from the U.S. Government, the sponsor of much of our research, that we should seek additional funding by finding applications for our basic research results. By that time we realized that antibodies against the surface antigen of the HBV (termed HBsAg) were probably protec-

tive. We had rarely seen individuals who had both HBsAg (indicating infection) and the antibody, anti-HBs, in their blood. Further, Okochi in Tokyo[7] had shown that patients who had anti-HBs before they were transfused with donor blood containing HBV were much less likely to develop hepatitis than those who did not have anti-HBs.

Further, we realized that it might be possible to produce a vaccine from the peripheral blood of carriers of the virus. They carried enormous numbers of particles which appeared to be composed only of HBsAg. We devised a method of separating these particles from the whole virus (which was not identified until later by Dane, Almeida and others[8] in England) by centrifugation, column separation, proteolytic enzyme treatment and other methods. The resultant material is treated with formalin and other agents to inactivate any remaining hepatitis virus or any other virus; appropriate adjuvants are added and this constitutes the vaccine.

In 1969, the Institute for Cancer Research filed a patent for the vaccine and its method of manufacture in the United States and foreign countries and it was issued by the United States patent office in 1971. At the time, this was a unique method for producing a vaccine. However, the medical and scientific world was not quite ready for a vaccine since the virus had been discovered only recently, and the validity of the concept was not universally accepted.

By 1975, sufficient amount of work had been done in laboratories in the United States and elsewhere to encourage us to recommend production of the vaccine by an experienced manufacturing company. A licensing agreement was concluded with Merck and Company, a large U.S. drug firm (which had agreed to work on the project as early as 1971) and later others. The vaccine was produced in quantity and in due course, subjected to intensive field testing in the United States. Volunteers from among male homosexuals in New York City were the subjects for the initial trial[9]. It was learned that the vaccine, which

requires three injections over a six-month period, was highly effective (greater than 90% protection) and, within the limits of the trials that had been evaluated, without any side effects. Several million doses of this vaccine have now been used and, to date, there have been no reports of detrimental effects; it is a very safe and effective vaccine. In this case, a vaccine whose major use has been and will be in developing countries, was initially tested on United States populations.

Solution of the vaccine problem raises other problems

The introduction of the vaccine provided an example of how the solution of one problem raised others; in this case, spurious.

In 1980, about the time the use of hepatitis vaccine was beginning to become widespread, the first cases of Acquired Immune Deficiency Syndrome (AIDS) were reported in the United States. Within a few years it became apparent that a terrible epidemic had developed in the susceptible groups, primarily male homosexuals and drug abusers. A small number of cases were also reported in haemophilia patients who had received human blood products, and more rarely in people who had received transfusions or had sexual contacts with people in the high susceptibility groups.

An inordinate fear of human blood products developed; this was extended to the hepatitis vaccine despite the fact that the methods of manufacture destroy all known viruses and that there was no evidence that AIDS had been transmitted by the vaccine. The wide use of the vaccine in the United States (and to a lesser extent elsewhere) was delayed. Subsequently, the agent which is thought to be responsible for AIDS was identified. It was then shown in a variety of ways that AIDS, even if present in the blood from which vaccine is prepared, could not survive the process of manufacture[10,11]. A further point was the finding that male homosexuals who had received vaccine did not have a different incidence of AIDS than those who had not received the

vaccine. Based on these findings, the short-lived fear of the vaccine diminished and it has not impaired its use in areas of the world with high frequencies of hepatitis carriers.

The etiologic role of HBV in the causation of primary hepatocellular carcinoma

There is now a substantial body of evidence that persistent infection with HBV is required for the development of primary hepatocellular carcinoma (PHC). The evidence is sufficiently impressive to have generated large regional and national vaccination programs based on the inference that HBV "causes" PHC. This, therefore, represents the first example of what appears to be a direct, widely used preventive program for a virus-caused cancer and is, in effect, a "cancer vaccine". It is important to stress that the vaccine is not a treatment for cancer that has already commenced.

In the 1950's investigators in Africa and elsewhere had, on the basis of clinical and pathological observations, suggested that hepatitis might precede PHC and be related to its etiology. It was not until the late 1960's when methods for detecting HBV were introduced that it became possible to test this hypothesis directly.

In 1971, at a meeting on cancer in Africa held in Uganda, several studies showing a striking association between HBV and PHC were presented. This event appeared to be a turning point, at least in our laboratory, in the development of interest in this relationship, and the intensity of the work in the field increased. In 1975, we wrote[12]:

"During recent years, there have been parallel developments in understanding, on the one hand, the pathogenesis of primary hepatic carcinoma . . . and, on the other, the biology of Australia antigen . . . and the infectious agent, hepatitis B virus (HBV), to which it is intimately related. Recently, the paths of these developments have begun to converge and from this it is possible to design a preliminary strategy which could, if the interpretations of these

data are correct, result in the prevention of many, and perhaps most, cases of one of the most widespread and deadly cancers of humans".

The evidence that was then available and that supported the statements was presented. We discussed the vaccine we had introduced and the possible prevention strategies. In the relatively short time since that article appeared, a large body of data supporting the hypothesis that persistent infection with HBV is required for the development of most cases of PHC has been amassed. This data will now be briefly reviewed [for references see (2)].

Carriers of HBV are common in those parts of the world where PHC is also common, including such surprising locations as Alaska where HBV and PHC are found in high frequency in the native American population. A large series of case-control studies has been completed in which the frequency of the virus in patients with PHC has been compared with the frequency in controls. In essentially all of these, the frequency of persistent HBV infection is higher in the PHC patients than in controls. In studies, which utilized the most sensitive methods for the detection of the virus, the frequency of infection among the patients approaches 100%.

The virus can be identified by immunohistologic methods in the liver tissue of people with PHC. An interesting feature of this observation, shown by Popper and others who have pioneered in the pathological work, is that the virus is most abundant in the cells that do not appear to have undergone malignant transformation. The neoplastic cells themselves often have sparse or no evidence of viral protein, while the apparently non-cancerous cells surrounding them may have large amounts of virus.

Currently, one of the most exciting areas of research is the molecular biology of HBV. The entire base sequence of the virus has been determined and confirmed by several groups. HBV DNA is integrated into the DNA of the host liver cells in a very large percentage of

PHC cases—in some studies, all the cases. However, there is also integration of HBV DNA in the liver cells of patients without PHC, including patients with chronic liver disease and asymptomatic carriers of HBV who have been infected for several years. Hence, integration itself cannot explain the pathogenesis of the cancer. The site of integration of HBV DNA appears to be the same in every cell in an individual tumour (i.e. integration is clonal), but the points of insertion of viral DNA are not the same in different tumours. Based on present knowledge, there does not appear to be a simple pattern of integration.

In one of the most convincing studies on cancer. etiology, Beasley and coworkers[13] in Taiwan studied prospectively 22,707 male government workers between the ages of 40 and 59 years. Recruitment into the study population began in November 1975 and was concluded in June 1978. Of the total, 3435 men were asymptomatic carriers of HBV and the remainder were not. As of December 31, 1983, 116 cases of PHC had developed in the study population and 113 of these were in HBV carriers. The annual incidence for the whole population was 82.4/100,000, but for the carrier group it was 527.7/100,000. Beasley and Hwang estimated the relative risk for the carrier men to be 217 times that of the noncarriers. The relative risk showed little variation from year to year and there was no secular trend. This is probably the highest odds ratio for any known environmental cause for a common cancer. On the basis of life table projections, they estimated the lifetime risk of death from PHC for a Chinese male carrier in Taiwan to be about 40%, a remarkably high figure.

Beasley's study established that the association between HBV and PHC was closer than that for any other virus and a site-specific cancer, probably for any environmental factor and a specific cancer. It has been shown also that chronic infection with the virus, rather than cirrhosis, carries the risk for cancer.

Patients with cirrhosis due to causes other than HBV have a low risk for PHC, while those with cirrhosis due to HBV have a much higher risk[14].

Prospective studies similar to Beasley's are now in progress with Eskimos in Alaska, Chinese in Hong Kong and Singapore, and Japanese in Tokyo. Early reports confirm Beasley's findings i.e. a greatly increased risk of PHC in the carriers.

In 1971, we had proposed, on the basis of unusual population distribution and clinical and physical characteristics of HBV, that it represented the first identified member of a new group of infectious agents. We termed these "Icrons," an acronym on the name of the Institute for Cancer Research—where the research on HBV was initiated. Several viruses in this "class" have now been identified in woodchucks, ducks and other species.

The finding of the woodchuck hepatitis virus (WHV) provided another convincing piece of evidence for the hypothesis that HBV and similar viruses cause cancer of the liver. Woodchucks (*Marmota monax*) (figure 4) persistently infected with WHV have a very high probability (80% or greater) of developing PHC. Uninfected animals or transiently infected animals never or rarely develop tumours. The duck hepatitis B virus (DHBV) found Chinese ducks and in the Pekin breed of domestic ducks (figure 5) is similar to HBV and WHV and it is also associated with pri-

Figure 4. Woodchuck or groundhog (*Marmota monax*).

Figure 5. Pekin ducks (*Anas domesticus*).

mary cancer of the liver, at least in China. Recently, a fourth member of this class of virus which appears to cause primary hepatocellular carcinoma has been identified. Ground squirrel hepatitis virus (GSHV) chronically infects the California ground squirrel (*Spermophilus beecheyi*) and, based on long-term observations, appears to play an etiologic role in this species. These viruses will be discussed in greater detail later. This appears to be one of the most remarkable examples of a series of viruses causing similar cancers in four different species and provides substantial support to the hypothesis that HBV is a necessary cause of most (or many) cases of PHC.

It has been shown both directly and indirectly that many individuals who develop PHC are infected at a very young age. In some parts of the world, particularly in Asia, the transmission may be maternal. In other locations, transmission is more likely to be horizontal. It is also known that infection early in life

increases the probability of persistence. These observations favour early childhood vaccination and this will be discussed later.

The hepatitis problem in India

Before proceeding to a discussion of mass prevention of hepatitis B infection, it would be appropriate to discuss the general problem of hepatitis in India.

There are several viral agents which cause the common forms of viral hepatitis. Hepatitis A is caused by an enteric RNA virus quite different in shape and structure from HBV. It can cause acute but rarely chronic hepatitis and is often spread in epidemics by water or food contaminated by the faeces of individuals infected with hepatitis A. There is no vaccine for hepatitis A, but the administration of human gamma globulin can provide protection for about 3 months following the injection.

Hepatitis B can cause both acute and chronic hepatitis, as already noted, and many people may become asymptomatic carriers for long periods of their lives. HBV is spread by many means, by transfer of blood from an infected individual to another, sexually, from mother to child, intimate contact as in families, and probably by insects. It may also be spread by the fecal-oral route i.e. contaminated food and water, but this is not generally thought to be an important mechanism of epidemic diffusion; this question is still unresolved.

There are also other viruses which affect the liver which have not been characterized. These are called "non-A non-B" hepatitis virus since they are neither A nor B; but since they have not yet been identified, a letter designation has not been assigned to them. One of these viruses has characteristics of hepatitis A and can be spread by water and food. Large epidemics of this disease occur in India and in some of these there is a high mortality rate. This is probably the single most common form of viral hepatitis in India.

There is another form of the non-A non-B virus which is similar to B in that it is blood transmitted and chronic. In India this appears to be less common than the non-A non-B virus which is similar to hepatitis A.

Probably the most important single public health measure which could be taken to decrease hepatitis in India would be the control of human waste and the protection of drinking water and food from fecal contamination. Since fecal contamination is the cause of a great deal of disease in addition to hepatitis in India, its control would probably be the most effective method available to help achieve the goal of "Health for all" in India by the year 2000. This is, of course, well known to medical and public health authorities, but the immense logistic effort required has prevented its complete implementation.

I have learned from colleagues in India that, in urban areas, the problem may have increased in recent years because of the rapid growth of city populations. The infrastructure for water supply and piped sewage disposal were in many cases installed years ago and are now inadequate for the increased population. Multiple breaks may exist in the water and sewage lines and, if they are proximate to each other, contamination can occur. This is particularly true when the water supply is inadequate and the pipes are empty for a portion or all of a day. A partial vacuum can result and sewage can enter the water lines if they too are ruptured.

In rural areas, deep bores may provide uncontaminated water, but its distribution after it reaches the surface may allow fecal contamination if proper facilities for control of waste are not used. A major regional and national program directed to providing adequate waste management and clean water and food would result in enormous rewards in health improvement. One approach to this could include the upgrading of training in public health engineering and the improvement in the status of the profession. This might be achieved by attaching schools for training in these disciplines to the high status scientific and engineering institutions in India, such as

the Indian Institute of Science and the Indian Institutes of Technology.

Prevention of hepatitis infection

It has been estimated that HBV is probably the second most common cause of viral hepatitis (after A-like non-A non-B), and it is probably the most common cause of chronic liver disease, including the deadly primary hepatocellular carcinoma. There are estimated to be about 25 million carriers of HBV in India making it, after China (with about 100 million carriers), the second largest concentration of this chronic infection in the world.

Hepatitis B infection from medical procedures

There are several specific strategies that can be used for the prevention, and possibly the eventual elimination of HBV. Prior to the discovery of the methods for detection of HBV in blood, post-transfusion hepatitis (PTH) was extremely common in the United States. In some patients receiving large numbers of transfusions (such as heart surgery), the incidence of PTH events reached 50%. Beginning in the 1970's, testing of donor blood for HBV was required by regulation and/or law in many United States jurisdictions and in other countries. PTH due to HBV has now essentially disappeared, although PTH due to other viruses (non-A non-B) still occur.

A law enacted by the Government of India in 1976 requires the testing of all donor blood, but for a variety of reasons this has not been accomplished; the Indian Council for Medical Research estimates that only 30% of donor bloods are tested. There are relatively few systematic studies, but the frequency of carriers varies from about 4% in some volunteer blood donor groups to over 20% in professional donors. It has been difficult for me to obtain a figure for the number of blood donor units used in India; 10 million per year has been one estimate. If we assume that an average patient receives two units of blood, then there will be about 5 million recipients of these 10 million units. If we assume a 5% frequency of carriers

among the donors (probably a low figure since many donors are professionals), then about 10% of the 5 million recipients will receive at least one unit of blood containing HBV Based on a prospective study, Dr Jacob John of Vellore has estimated that about one third of patients receiving positive blood from donors will develop clinical hepatitis. Hence, it can be estimated that about 165,000 patients per year will develop hepatitis B from the blood transfusion system.

Another mode of hepatitis infection is the re-use of venipuncture or injection needles or other equipment exposed to human blood. It is difficult to remove encrusted blood from a needle and ordinary sterilization will not kill HBV. The amount of virus which can cause hepatitis in susceptible hosts may be extremely small; an amount contained in the dregs of the needle or other instruments (i.e. blood syringes) could be sufficient.

It is difficult to estimate how many cases can be caused in this manner, but it is probably not less than that caused by transfusions. The preventive measures for these cases are obvious. All donor bloods require testing for HBV using the sensitive enzyme assay (ELISA), radioimmunoassay (less convenient), or a method of equivalent sensitivity. At present there is inadequate indigenous production of the reagents for these assays and the imported reagents are too expensive for widespread use. The encouragement of local manufacture could remedy the problem.

Facilities for the manufacture of sharp disposable needles at low cost would decrease the dependence on re-used needles and instruments. Local manufacture of other disposable blood collecting equipment (i.e. syringes, plastic containers for the units of blood) will also be of value in preventing this mode of infection. It is obvious that the collection of blood from donor groups with a low frequency of HBV carriers (i.e. volunteer donors) would alleviate the situation and probably decrease infection with non-A non-B and possibly other viruses as well.

Prevention of infection with the virus which causes AIDS

There are remarkable similarities between the modes of transmission of HBV and the virus which is thought to cause Acquired Immune Deficiency Syndrome (AIDS). This virus is now called Human Immuno-deficiency Virus (HIV) but was formerly called LAV/HTLV-III. Methods similar to those used for preventing blood borne transmission of HBV can be used for preventing blood borne transmission of HIV. A serological test of donors for HIV (and antibodies against it) can be performed on the same serum specimen required for testing for HBV. Much of the same equipment can be used for the two tests. The techniques described above to prevent transmission of HBV by needles and other equipment will also be effective for preventing transmission of HIV.

There is great demand in India for the prevention of blood borne AIDS. The implementation of both the HIV and HBV programs can be done together, thus effecting large savings in public health expenditures.

Vaccination against hepatitis B

The major preventive progrmme involves the use of the hepatitis B vaccine. Several strategies have been used in different countries and regions. In areas of low frequency (i.e. United States, northern Europe), routine use of the vaccine has been recommended for high-risk groups such as health care workers, families of carriers, newborn children of carrier mothers, male homosexuals and others. In areas of moderate frequency (say 1–2%, i.e. Japan), the major program is directed to the offspring of carrier mothers. They are given gamma globulin at the time of birth and a second dose of gamma globulin, and the first of three doses of hepatitis B vaccine are given at two to three months of age. A second dose of vaccine is given one month and the third dose three months after the first. In some cases a fourth dose of vaccine may be used. Th

program has been in progress in Tokyo for several years; it is to be extended to all of Japan within a few months.

In areas of high frequency (such as China), the plan is to vaccinate all newborn children in the high-risk areas. In People's Republic of China this would amount to more than 20,000,000 courses of vaccinations per year! To accomplish this large goal, indigenous production of vaccine from human blood is being undertaken in newly built factories.

A vaccination program has been in progress on the island of Taiwan since 1984. Taiwan has one of the highest frequencies of carriers in the world; the mean frequency of carriers (among pregnant women) is 18% with a range of 14–22%. Testing was made available to all pregnant women and some 78% were tested (352,721 tested of 450,585 pregnant women). Women who were HBeAg(+) or who had high titers of HBsAg were identified and they were given high titer anti-HBs gamma globulin (HBIG) within 24 hr of delivery; vaccination injections were given at 1, 5 and 9 weeks and at 12 months. A very high percentage (84%) completed three doses and a smaller percentage (71%) the entire course. In July 1986 all newborns will be included in the vaccination program. (Pregnant women will still be tested to ascertain highly infectious mothers and their children will be offered HBIG as before.) In due course, vaccination will be extended to household members of carriers, to medical personnel and eventually to all other susceptibles. National and regional programs are also in progress in the Gambia, Italy, South Korea, United States and elsewhere.

Vaccination program for India

What is the appropriate vaccination strategy for India? A vaccination program could proceed as follows.

1) A systematic survey of Indian populations in different regions of the country to determine the prevalence of HBsAg and anti-

HBs carriers. This would provide knowledge of high infection areas and also an estimate of the population still at risk for HBV infection (i.e. those who have no serological signs of infection).

2) A systematic survey, in different regions of India, to determine the frequency of HBV infection in patients with various kinds of liver disease. This survey could be combined with studies of hepatitis A and non-A non-B hepatitis and would provide some estimate of the disease load of HBV in hospitalized patients.

3) Based on these surveys, strategies for vaccination (possibly different for different parts of the country) could be designed. A decision could then be made concerning target populations for different regions i.e. (i) all newborn children; (ii) only the children of carrier mothers; (iii) susceptibles in the families of carriers and (iv) all susceptibles. Strategies could then be designed, based on the cost of vaccine, disease load imposed by the virus, cost of identifying susceptibles and other variables.

4) The survey and vaccination activities for HBV could be combined with disease surveys and prevention programs caused by other infectious agents. Since there is currently an interest in the prevalence of AIDS virus in India, both surveys could be combined. Eventually, hepatitis B vaccination could be made part of the routine pediatric vaccinations.

5) The cost of vaccine sold in the United States and Europe is very high (about 100 U.S. dollars for the course). However, the cost of vaccine produced in Korea is about one third of this and the Chinese are said to be producing vaccine at about one to three dollars per course. Several studies have now shown that intradermal injections are effective and require only one tenth the amount of vaccine required for the intramuscular route (the method currently widely in use). This brings the cost down to about 10 to 30 cents (equivalent to one rupee 30 paise to three rupees 90 paise), a reasonable amount even for large vaccination programs.

Vaccine prepared by recombinant DNA methods has recently been approved by the Food and Drug Administration of the United States. The cost, however, is not less than the currently available product (that is about 100 U.S. dollars) and it may be some years before methods of production bring it down to the costs of the blood-produced product.

Public health authorities will have to balance the pros and cons of the possible strategies of vaccine production and application and arrive at a judicious decision as to which methods to use.

The biology of hepatitis B virus: Sex differences in response to HBV

Physicians and microbiologists usually encounter viruses and microbacteria primarily in their disease-causing phase. But micro-organisms have a rich interaction with humans and other organisms beyond their ability to cause distress and woe. Examples of how HBV interacts with human sex will now be presented. (For more detailed information and references see refs. 15, 16.)

In many of its responses, HBV interacts differently with human males and females. One of the earliest observations on "Australia antigen" (that is, hepatitis B surface antigen) was the higher frequency of HBV carriers in males compared to females. It was shown that, when infected, males are more likely to become carriers and females are more likely to develop antibody to the surface antigen (anti-HBs). This has an important bearing on the fate of these individuals, since carriers are, in general, more likely to develop chronic liver disease and primary cancer of the liver and those who develop anti-HBs are often immune to further disease. Hence, females are favoured over males in this regard.

Another curious observation emerged from a study of patients receiving treatment on a renal dialysis unit. Prior to the use of sensitive methods for the detection of HBsAg, HBV infection was extremely common in renal

dialysis patients and in some artificial kidney units nearly all patients became infected. (This situation is now controlled and hepatitis infection in dialysis units in the United States and elsewhere is rare. This was achieved by epidemiological control and restricting carrier patients to their own machines in special dialysis units or sections of units.) Many patients on dialysis units were transplanted with kidneys from cadavers and living donors. London and his colleagues found that patients who developed anti-HBs rejected the transplanted kidneys more rapidly than transplanted patients who were carriers of HBV. Furthermore, they found that this effect, although independent of the sex of the recipient, was seen only when the kidney donor was a male. There appeared to be a specific relation between anti-HBs and an antigen present in male but not female kidneys.

The most perplexing observation and possibly the most important, concerns the effect of the response of parents to HBV infection on the sex of their offspring. The original observations were made in a community in northern Greece selected because of the high frequency of HBV carriers. If a mother or father were carriers, the sex ratio of their offspring (number of male offspring divided by the number of female offspring multiplied by 100) was greater than in families in which one of the parents (in particular, the mother) was anti-HBs. Similar observations were made in four other communities; in France, in Kar Kar, Papua New Guinea, in northern Luzon, in the Philippines and in two villages in eastern Greenland. In several of these the increased sex ratio in the carrier families was a consequence of a decreased number of females. There was also an overall decreased fertility in the carrier families.

We have developed several models in an attempt to explain these sex effects. It was suggested that HBV viruses contained an antigen that was present in males but not in females. Hence, when males were infected they would be more likely to regard the antigen

as "self," would not develop anti-HBs and hence would be more likely to develop the carrier state. Male kidneys would contain a HBV-like antigen and would be more likely to be rejected by a kidney recipient with anti-HBs. Another model was that the virus was more likely to replicate in a female than in a male fetus and therefore, the female conceptus, infected by the carrier mother or father, was less likely to come to term than the male conceptus. This would explain the observed decrease in the number of girls (and children overall) in the carrier families.

HBV and insects

There are other biological interactions of HBV with other species in the environment. The virus is borne by mosquitoes and the field infection rate can be[17,18] as high as 1:200. It is also found in over 50% of bedbugs (Cimex sp.) (figure 6) captured in beds whose main occupants are carriers of HBV[19]. The virus has also been reported in house flies and other insects.

There is no evidence that the virus replicates in insects; nevertheless, they may be an important source of transmission since, in susceptible individuals only a small amount of virus may cause infection. Insect-borne diseases can be controlled and this may have a bearing on the planning of extended public health programs. This may be particularly important if there is a campaign to totally eliminate the virus; this may be feasible even now in circumscribed island or isolated populations.

Figure 6. Bedbug (Cimex sp.) SEM.

Insect infection may also be of biological and evolutionary interest. Insects can provide a mechanism by which genetic information can be carried from one human to another via the viral genome. Since HBV integrates into the host DNA of long-term carriers, the infectious virus may carry with it to an infected person small portions of the genome of the previous host, and this could, theoretically, be expressed in the new human host. An interesting kind of epidemiology may emerge from this in which one is concerned with the infectious agent, the host and the immediately prior host.

Hepatitis B-like viruses in other species

One of the most convincing pieces of evidence that HBV is a necessary cause of primary hepatocellular carcinoma in humans is the existence of a series of viruses, very similar to HBV, which appear to cause PHC in other species. In our earlier investigations, we had postulated the existence of viruses similar to HBV, which we termed Icrons.

Four of these viruses have been identified at the Fox Chase Cancer Center by several investigators. These include the human virus (HBV), the virus found in the north American woodchuck or groundhog (*Marmota monax*) called woodchuck hepatitis virus (WHV), the duck hepatitis B virus (DHBV) first identified in China and the tree squirrel hepatitis B virus (THBV). In addition, a virus has been reported in the California ground squirrel (*Spermophilus beecheyi*) (GSHV) and recently in the Palm Tree squirrel of India. (The last species is hallowed in Indian tradition. It is the squirrel which assisted Rama when he crossed the sea to Sri Lanka to rescue his consort Sita from the demon Ravana.) HBV-like viruses have also been reported in kangaroos and possibly other species. Primary cancer of the liver, caused by the virus, has been reported in four species (humans, woodchucks, ducks, ground squirrels). These animals are a rich resource for observational and experimental research. In addition, they provide a fascinating body of information to study the evolution of the virus and the diseases they cause in both closely related and distant species.

Solving and creating problems in clinical research

I would like to conclude this presentation with a brief discussion of how, in clinical research, the solution of problems often creates other problems. A brief discussion of certain aspects of scientific process which relate to this concept will be followed by an example from our own experience in research on HBV[20].

Comments on scientific process in clinical research

A hypothesis is a declarative statement concerning the scientist's view of a state of nature confined to a specified subject matter. In the inductive phase of scientific process, the data are collected first and the hypothesis induced from them. In the deductive phase, the hypothesis is stated first and then data are collected to test it. After the hypothesis is stated, a study is designed to test the hypothesis (usually an attempt is made to reject the hypothesis) and during the course of testing, a body of data accumulates. Irrespective of the support or rejection of the hypothesis, the data can be used to generate other hypotheses. These, in turn, can be tested by additional data. Again, a decision can be made as to whether the hypotheses of the second cycle are supported or rejected and the data used to formulate a third series of hypotheses, which are also available for testing. This process may be continued through many cycles. As the process proceeds, more and more hypotheses are tested and support or rejection is determined; that is, more and more questions are answered. At the same time, an even greater number of new questions are asked. The more that is known, the greater are the number of unknowns. If perfect knowledge means that the investigator knows everything about a

subject, then, if this model of scientific process is valid, solutions will always be imperfect. The solution of one problem will often raise others.

To illustrate this concept, we will use the experience which resulted from the introduction of testing of donor blood to prevent post-transfusion hepatitis.

Problem solving and problem creation: The prevention of post-transfusion hepatitis

The test for what was then known as the Australia antigen, was reported in 1964. By 1966, as described above, there was substantial evidence that Australia antigen was on the surface of hepatitis B virus. In 1967, screening of blood donors for occult carriers of hepatitis virus was routine in some hospitals in Philadelphia and by 1970 it was widely applied in the United States and other countries.

In 1968, disturbing reports were heard from persons, usually health care personnel, who had been identified as carriers of HBV as a result of a blood donor or hospital staff testing program. For example, a hospital nurse identified as a carrier was told that she would be dismissed because of the possibility that she might infect others by personal contact. An applicant for a hospital position was deemed ineligible for employment because he was a carrier. A homosexual man was informed that he was a carrier but given no specific instruction on how he should conduct himself socially.

Along with many incidents of this nature, policy questions about hepatitis B virus carriers were raised by institutions. Military medical authorities in a foreign country where hepatitis B virus carriers were common asked if they should screen applicants for admission to medical school and disqualify those found to be carriers. The same question was raised with respect to admission to officers' training school and graduation from nursing school: should these applicants and graduates be screened for the carrier state to determine if they should be allowed to enter or undertake the practice of their chosen professions? (We recommended that screening should not be done.) Still

another difficult policy problem arose in relation to the adoption of Southeast Asian refugee children. In the mid 1970's, many children from Indochina were placed for adoption. Because hepatitis B virus carriers were known to be prevalent in Vietnam, the advisability of screening these children for the virus became an issue. Should the results of this screening test determine who would be accepted for adoption and who would not? (United States health officials decided that carrier testing should not be done as a qualification for immigration to the USA from Southeast Asia.) These carrier-related questions surfaced in increasing numbers as the use of the hepatitis B virus test became more widespread.

What appeared to be emerging was the possibility that a new class of stigmatized persons and groups—hepatitis B virus carriers—was being created by the introduction of a single laboratory test. These were persons who did not have any recognizable external characteristics. Their carrier state was "occult," only discernible by use of a blood test. Many carriers already had been and even more would be, identified as a result of the donor and other blood-testing programs that had been launched. (Tens of millions of donor blood samples are collected each year, and, in due course, most of these would be tested.)

It was known that some carriers could transmit hepatitis readily by blood transfusion. From this knowledge, but without quantitative data on actual transmission, it was inferred that carriers could also convey hepatitis through social interaction. This assumption began to take hold despite the fact that it was apparent that most carriers were not very infectious. It had been estimated that there were about 700,000 carriers throughout the United States. If they were infectious—for example, to the degree that people infected with smallpox are—there would have been far more hepatitis in this country and elsewhere than there is known to be. Furthermore, several preliminary studies of health care workers known to be carriers showed that they had not transmitted

hepatitis B virus to their patients. The scientific evidence strongly suggested that, although some carriers might be infectious, many were much less so if at all, and that the danger to public health was probably not immediate or enormous. Nevertheless, persons who had been identified as hepatitis B virus carriers were being medically and socially marked in potentially disadvantageous ways. They were having personal, family and career difficulties as a result of their disclosed carrier status.

Consideration of these problems made it clear that an evaluation of a general screening program should be done before any such program was executed. (This program refers to the screening of persons other than blood donors. Blood donor screening had been evaluated, found to be justified and accepted.) What was called for was a judicious, well-informed set of decisions about screening that would potentiate its public health benefits and protect individual hepatitis carriers from undue economic, psychic and social harm. It became apparent that sufficient data were not available to make sound decisions that would appropriately balance collective needs and individual rights. It was not at all clear what the rules for a screening program ought to be. Should screening be compulsory? Should the activities of carriers be distinguished from those who were not infectious? What instructions should be given to identified carriers? What kind of protection could and should be offered to those with whom carriers came in contact? The history of previous medical applications strongly suggested that these issues should be addressed before the screening procedure acquired the routine familiarity and authority of an established practice; once a procedure has been instituted it becomes increasingly hard to question or freshly evaluate it.

There were also broader issues that had been raised and required consideration. Most of the infectious diseases that people contact are transmitted either directly or indirectly from other people, in many cases from carriers. However, screening for these agents is difficult and not done routinely on large segments of the general population. For example, *Staphylococcus aureus* carried on skin surfaces may be spread from person to person and has caused large and calamitous epidemics in hospital nurseries. Nevertheless, routine screening is not done for this bacteria except after infection has been found. *Salmonella* species may be spread by food handlers and cause serious epidemics of diarrheal disease, but routine screening is not done because of its expense and difficulty. Should hepatitis carriers be targetted for screening simply because the test is easy to do and widely available? Beyond this, how much should biological knowledge be allowed to influence and control our social relationships? To what extent should medical and public health practices be allowed to affect our social behaviour—particularly in the face of the kind of inadequate information that existed at this point about hepatitis B virus carriers and the infectious risk they constitute?

For various reasons, hepatitis screening for blood donors was accepted quickly, whereas screening for many other infectious agents has not been accepted. There was an unambiguous, long-standing need to screen blood donors for hepatitis. Post-transfusion hepatitis was a real and significant problem that had been recognized for years and any solution was bound to be accepted quickly. The tests for hepatitis B virus (particularly the radioimmunoassay that was introduced relatively early in the program) were sensitive and specific. There was a large commercial interest in these tests. Test reagents for hepatitis B virus to the value of tens of millions of dollars are sold yearly, aided by extensive advertising and skilled promotion. Further, several law suits had been brought against hospitals, blood banks, and physicians by defendants who developed post-transfusion hepatitis and claimed that the institution and health care workers were liable because they had not used the screening test for hepatitis B virus. In addition, blood has a powerful symbolic meaning in our culture. It is associated with life and

vigor, lineage and kinship in ways that are likely to confer special positive significance on technical procedures that guarantee its "purity."

The question of a general screening program (the testing of persons who were not blood donors) had to be viewed very differently. We, and others in the field, took the position that there had not yet been enough research on hepatitis B virus carriers to justify general screening programs, except for those that were part of a research protocol. It was obvious that additional research was necessary to resolve these medical and bioethical problems.

Advances in research and their impact on ethical questions

Since our original publications on these issues, there have been scientific and technical advances that have changed the ethical issues in hepatitis B virus carrier screening and altered our views in the process. In 1972, Magnius and Espmark reported their finding of the hepatitis B e antigen (HBeAg). This antigen appears to be a part of the core of hepatitis B virus and its presence in a carrier indicates that significant amounts of whole infectious virus are present in the blood. Mothers who are hepatitis B surface antigen (HBsAg) and HBeAg positive can transmit hepatitis to their offspring, particularly in Asian populations. Carriers with HBeAg are in general more likely to transmit the virus than those without. Furthermore, HBsAg carriers who also have antibody to hepatitis B e antigen (anti-HBe) in their blood are much less likely to transmit the agent than those with HBeAg or those without any sign of HBe antigen or anybody. By separating the carrier group into those who are potentially infectious and those unlikely to transmit hepatitis, the problems of screening were narrowed and focused.

The introduction of the vaccine against hepatitis B virus by Blumberg and Millman (described above) has also markedly changed the medical and ethical picture. If the vaccine continues to be as safe and effective as it now

appears to be, it will be possible to protect persons with whom carriers come in contact. In time, in some regions of the world where hepatitis prevalence is high, all or nearly all of the population will either have natural protection against hepatitis B virus (they will have developed the antibody to hepatitis B surface antigen [anti-HBs] after natural infection with the virus), or they will have been vaccinated. When this happens, the public health impact of the carriers will be greatly minimized in these areas. As a consequence, the chief ethical problem of the carrier will have been eliminated.

In areas with low intensity of hepatitis B virus infection, the vaccine will probably be used only in high-risk populations, including health care personnel, travellers, military personnel, blood handlers, homosexual men, drug abusers, family members of carriers, immigrants from HBV high frequency areas and certain other groups. In some of these high-exposure populations, the frequency of naturally occurring anti-HBs may be common (15 to 50%). Because the cost of vaccine is high, it would be prudent in some areas to screen these populations for the presence of anti-HBs (and possibly HBsAg), because they would not profit from vaccination. Under these changed circumstances, the screening of general populations would be warranted. The initial concerns about general population surveys would be set aside, because measures of known value could be taken as a consequence of the survey.

There are other situations in which screening surveys may be warranted. Current policy encourages the "mainstreaming" of mentally retarded children by placing them in small, home-like settings in the general community and in regular schools with other children. There is a relatively high frequency of carriers among children and young adults with Down's syndrome, who make up a sizable portion of deinstitutionalized, mentally retarded people. Research should continue to determine the risk that these children may impose on their class-

mates and on the feasibility of vaccine protection.

Other advances that would further alter the ethics of screening are likely to occur. Research is now being directed toward an understanding of how to either eliminate hepatitis B virus from carriers, or to decrease virus replication so that the carrier is not infectious. If such measures become possible, they would probably also decrease the likelihood of the carrier developing chronic liver disease or primary cancer of the liver. Under these conditions, identified carriers could be offered a therapeutic procedure. This would provide a powerful new medical and moral rationale for general hepatitis B virus carrier screening that might further offset the negative personal, social, and cultural side effects associated with it.

CONCLUSION

The research on HBV has led to many interesting and curious findings. These have had practical applications and also are of theoretical interest in biology. The search is continuous since the unravelling of one problem generates even more. We hope that it will continue to provide information which will improve health in India and the world.

1. Blumberg, B. S., *Science*, 1977, **197**, 17.
2. Blumberg, B. S. and London, W. T., *Cancer*, 1982, **50**, 2657.
3. Blumberg, B. S., Alter, H. J. and Visnich, S., *JAMA*, 1965, **191**, 541.
4. Blumberg, B. S., Gerstley, B. J. S., Hungerford, D. A., London, W. T. and Sutnick, A. I., *Ann. Int. Med.*, 1967, **66**, 924.
5. Blumberg, B. S., London, W. T. and Sutnick, A. I., *Bull. Pathol.*, 1969, **10**, 164.
6. Coller, J. A., Millman, I., Halbherr, T. C. and Blumberg, B. S., *Proc. Soc. Exp. Biol. Med.*, 1971, **138**, 249.
7. Okochi, K., Murakami, S., Ninomiya, K. and Kaneko, M., *Vox Sang.*, 1970, **18**, 289.
8. Dane, D. S., Cameron, C. H. and Briggs, M., *Lancet*, 1970, **1**, 695.
9. Szmuness, W., Stevens, C. E. and Harley, E. J., *N. Engl. J. Med.*, 1980, **303**, 833.
10. Stevens, C. E., *N. Engl. J. Med.*, 1983, **308**, 1163.
11. Wallbank, A. M., *Lancet*, 1985, **1**, 642.
12. Blumberg, B. S., Larouze, B., London, W. T., Werner, B., Hesser, J. E., Millman, I., Saimot, G. and Payet, M., *Am. J. Pathol.*, 1975, **81**, 669.
13. Beasley, R. P., Linn, C-C., Hwang, L-Y. *et al.*, *Lancet*, 1981, **2**, 1129.
14. Nishioka, K., Hirayama, T., Sekine, T., Okochi, K., Mayumi, M., Juei-Low, S., Chenhui, L. and Tong-Min, L., *GANN Monograph on cancer research*, 1973, **14**, 167.
15. Drew, J. S., London, W. T., Lustbader, E. D., Hesser, J. E. and Blumberg, B. S., *Science*, 1978, **201**, 687.
16. Blumberg, B. S., *Arthritis Rheum.*, 1979, **22**, 1261.
17. Blumberg, B. S., Wills, W., Millman, I. and London, W. T., *Res. Commun. Chem. Pathol. Pharmacol.*, 1973, **6**, 719.
18. Wills, W., Saimot, G., Brochard, C., Blumberg, B. S., London, W. T., Dechene, R. and Millman, I., *Am. J. Trop. Med. Hyg.*, 1976, **25**, 186.
19. Wills, W., Larouze, B., London, W. T., Millman, I., Werner, B. G., Ogston, W., Pourtaghva, M., Diallo, S. and Blumberg, B. S., *Lancet*, 1977, **2**, 217.
20. Blumberg, B. S. and Fox, R. C., *Ann. Int. Med.*, 1985, **102**, 390.