Toward Control of Viral Infections of Man

Such control is viewed from the standpoint of the possible, the probable, and the practical.

Maurice R. Hilleman

Not infrequently one reads or hears spectacular prophesies about control of the maladies of mankind—predictions that its achievement lies at the near horizon or just around the corner. Such predictions, often made by those who contemplate or who have reached retirement, reveal a flattering confidence in those who inherit the legacy and are charged with bringing about its fulfillment. The practice of science, like the practice of politics, is a game which is best played with one's sights set on the possible and probable. Hence, I discuss here the prospects for the control of viral infections within the limits of possibility and probability, giving such attention to the practical as may be appropriate. I present principally my own point of view; limitations of space preclude presentation of all opposing arguments. I do not pretend to give a complete bibliography, citing only reviews and a few specific individual reports.

At present there are only three approaches to the specific control of infections caused by viruses: immunologic control, host resistance, and chemical control (see Table 1). Immunologic control has been remarkably effective in affording protection for the long or relatively long term, but with the disadvantage that the spectrum of viral strains against which it gives protection is very narrow. Host resistance, as exemplified by the interferon mechanism, promises broad-spectrum antiviral activity, but with the disadvantage that the effect is of short duration. Chemical methods for controlling viral infection have thus far given meager reward and have two disadvantages: the spectrum of strains against which they give protection is narrow, and maintenance of a protective effect requires continuing administration of the chemical substance.

Immunologic Control

The most effective and economically efficient procedure attempted or utilized to date for the prevention and control of acute viral diseases has been specific immunization by vaccines (*1–3*). Live virus vaccines have generally proved best for preventing diseases caused by viruses whose antigenic types are few, in which there is systemic invasion of the host and in which lifelong immunity following natural infection is the rule. Diseases caused by viruses whose antigenic types are numerous, in which the infection is superficial and in which immunity is not lasting, may be controlled best by the use of killed virus vaccines, especially in association with immunologic adjuvants which enhance the immune response. Table 2 gives a summary of vaccines that are currently licensed or under investigation. Here I briefly discuss current progress and problems associated with the vaccine approach. Human immune globulins are of very limited usefulness in the control of viral infections and are not considered further here.

Smallpox, Rabies, Arbovirus

Jennerian prophylaxis against smallpox, practiced successfully for 17 decades, provided the foundation for the vaccination concept. Over the years the quality and purity of the vaccine have been gradually improved; today's vaccine, which is produced on calf skin or in embryonated hens' eggs, is bacteriologically sterile and has its potency preserved by drying. The not-infrequent clinical complications of vaccination, including central-nervous-system involvement and generalized vaccinial infection, have led to continuing attempts to reduce the occurrence of such complications through prior administration of killed virus vaccine, concurrent administration of human immune globulin containing antivaccinial antibody, or development of viruses that are more highly attenuated but still afford lasting immunity. It is sometimes argued that smallpox vaccination should be discontinued in "smallpox-free" countries, since the use of vaccine is not without danger and the vaccine is not needed. This view might be dangerous in practice, since a principal means for keeping a nation "smallpox-free" is immunization of a substantial portion of the population.

The vaccine against rabies—the second viral vaccine to be developed—was first tested by Pasteur in 1885. Though of venerable lineage, this vaccine has remained until recently the crudest of the preparations injected into human subjects. The most commonly used vaccines are but crude suspensions of the brains of animals infected with an attenuated (fixed) rabies virus which is partially or completely inactivated by chemical or physical procedures and preserved chemically or by drying. With all such brain vaccines there is a chance that the vaccine will induce allergic encephalomyelitis due to organ-specific immunization against nervous tissue—a condition so serious that the risk of an untoward effect of vaccination might exceed the danger from rabies itself. Substantial improvement in the vaccines was made possible by the introduction of avian embryo propagation of the virus in the 1930's and the use of brains of suckling animals, which contain less organ-specific antigen. The modern approach to rabies vaccine is propagation of the virus in cell cultures. This makes it possible to produce highly purified killed virus vaccines (sometimes used with an immunologic adjuvant) and attenuated live virus vaccines. One hopes that the next decade will see the introduction of completely safe vaccines which will provide long-term prophylactic immunity when administered to individuals highly exposed to the virus.

The arboviruses are members of an extremely diverse group of agents which multiply in, and are transmitted by, arthropod vectors. There are at least 230 different arboviruses, which comprise more than 28 immunological subgroups. The normal transmission cycle in nature is in arthropods and lower

The author is the director of the division of Virus and Cell Biology Research, Merck Institute for Therapeutic Research, West Point, Pennsylvania.

vertebrates, with man an alternative and nonessential host. Preventive measures are limited to arthropod vector control and specific immunization in vertebrates. The first and only virus of this group which has yielded satisfactorily to vaccine control is the yellow fever virus; the vaccine used is virus attenuated and propagated in chick embryo or in mouse brain. Killed virus vaccines have afforded only limited protection, and the use of live virus vaccines has been impeded by the extensive testing needed to establish efficacy and safety. Recent cell-culture procedures provide the potential for making purified killed virus vaccines, perhaps with adjuvants, and for preparing live virus vaccines such as the swine cell-culture Japanese B encephalitis vaccine (*4*) now undergoing clinical evaluation in man. An approach which has not achieved spectacular success to date is that of administering a live or killed virus vaccine against a virus of a particular subgroup and then administering a limited number of killed or live virus vaccines against other members of the same subgroup in the hope of providing a broader spectrum of protection against agents of the entire group than would be afforded by the individual agents alone. The problems of arbovirus vaccines might be overcome but for the overwhelming economic problem of the need to provide a multiplicity of vaccines for use in very limited geographic areas of occurrence. All this could change rapidly with widespread dissemination of these viruses by means of modern transportation. Until this occurs, the only practical course appears to be the development of improved live and killed vaccines for limited areas of application and for use in conjunction with concerted vector control.

Respiratory Complex

The complex of acute respiratory diseases of man, like the complex of the arbovirus infections, is characterized by a multiplicity of distinct immunologic serotypes of virus, which include several families of viruses (*5*). Hence, the vaccine approach is that of selecting only those few agents which, from the clinical and epidemiological standpoints, are worthy of control by vaccine. The first of the respiratory viruses to yield to the vaccine approach were influenza A and B, for which a vaccine was developed in the early 1940's from virus grown in chick embryo and killed with formaldehyde. When properly constituted, influenza vaccine may effect a reduction in illness of 75 to 90 percent or even more, and it might continue to do so were it not for the capricious antigenic constitution of the prevailing strains of virus in nature. A major change in influenza-A virus, with near-total antigenic alteration, tends to occur at intervals of approximately 10 years, rendering previous herd immunity noneffective. This causes pandemic prevalence of the disease and renders the previous vaccine useless. Minor changes occurring within the 10-year cycle may be of such magnitude as to markedly reduce the effectiveness of the vaccine and necessitate periodic revision of the strain composition of the vaccine. The last great pandemics occurred in 1947 and 1957; the contemporary 1968–69 outbreak of "Hong Kong influenza" has already swept through much of the Northern Hemisphere and will probably cause epidemics in the Southern Hemisphere by mid-1969. The early detection, in July 1968, of the epidemic in Hong Kong and the recognition by the U.N. World Health Organization of the major antigenic alteration of the virus (*6*)

Table 1. Approaches to specific control of viral infections.

Kind	Characteristic		
	Level of effectiveness	Antiviral spectrum	Duration of effect
Immunologic	Usually high	Very narrow	Relatively long to lifetime
Host resistance (interferon)	Moderate to high	Very broad*	Relatively short term
Chemical	Low to moderate	Narrow	Very short term

* However, exogenous interferon is generally highly species-specific with respect to host species.

Table 2. Survey of vaccines against important human viral infections.

Virus	Vaccine status	
	Existing*	Developmental-experimental
Smallpox	Live	Live, further attenuated; killed
Rabies	Killed; live	Killed, cell-culture-grown
Arbovirus		
Yellow fever	Live	None
WEE, EEE, VEE†	Killed (animal)	Live (animal); killed (man)
Japanese B encephalitis	Killed	Killed, cell-culture-grown; live, cell-culture-grown
RSSE‡	Killed (Soviet)	Killed, cell-culture-grown
Kyasanur forest	None	Killed (ineffective)
Dengue	None	Live
Rift Valley fever	None	Killed, cell-culture-grown
West Nile	None	Live, cell-culture-grown
Respiratory complex		
Influenza A and B	Killed; live (Soviet)	Killed, purified; killed, viral subunits
Influenza C	None	None
Adenovirus	Killed §	Killed, viral subunits; live
Parainfluenza 1, 2, and 3	None	Killed, purified and concentrated; live
Respiratory syncytial	None	Killed, purified and concentrated; live
Mycoplasma pneumoniae (bacterial)	None	Killed
Rhinovirus	None	Killed; live
Reovirus	None	None
Enterovirus		
Poliovirus	Killed; live	None
ECHO and Coxsackie	None	None
Systemic myxoviruses ||		
Rubeola (measles)	Live; killed	Killed, viral subunits
Mumps	Killed; live	None
Rubella (German measles)	None	Live
Herpes virus group		
Herpes simplex	None	Killed
B virus	None	Killed
Cytomegalovirus	None	None
Varicella zoster	None	None
Infectious mononucleosis	None	None
Hepatitis	None	None

* Now licensed in the U.S. † Western, Eastern, and Venezuelan equine encephalomyelitis viruses. ‡ Russian spring summer encephalitis. § Removed from commercial distribution because of oncogenicity of most serotypes. || Rubella family position not established.

made possible the development and production of a substantial amount of vaccine before the epidemic had subsided in the United States. The vaccine became available in mid-November 1968, and 20 million doses had been produced by mid-January 1969. The vaccine was used primarily in the high-risk group of persons who had other disabilities and was credited with saving a great many lives. Fortunately, the illness in the Hong Kong influenza pandemic has been relatively mild and not of the very severe form of the 1889–90 and 1918–19 pandemics.

Further efforts to improve killed influenza vaccine have been directed toward increasing potency and purity and reducing toxicity. Most recently, highly purified influenza virus vaccines of 35- to perhaps 90-percent purity have been introduced (*7, 8*). High-purity vaccines relatively free from bacterial substance are of very low toxicity (*7*). The toxicity may be even further reduced by disrupting the virus with ether or deoxycholate (*9*), but whether the end justifies the effort remains to be determined.

The more urgent need is for a vaccine that provides protection against a greater number of viral strains. The first achievement in this direction came in 1957 with the development of a highly effective killed vaccine against adenoviruses of types 3, 4, and 7 (*10*), which cause significant illness among military recruits. This vaccine was withdrawn from commercial distribution in 1964 because it had been shown that most adenovirus types, including vaccine types 3 and 7, cause neoplasia when administered to newborn hamsters, or cause neoplastic transformation in cell culture (*10*). Possible use of protein subunits of the virus which are free of viral nucleic acid is now being explored (*11*).

Efforts have been made to develop vaccines for protection against the respiratory syncytial virus and the parainfluenza 1, 2, and 3 viruses because of their importance in respiratory illnesses in children. *Mycoplasma pneumoniae*, a bacterium, has been given attention because of the high incidence of atypical pneumonia caused by this organism. Extensive studies in our laboratories have resulted in the preparation of highly purified killed polyvalent vaccines against parainfluenza, with potency equal to that of influenza A and B vaccines and with evident protective efficacy (*5, 12–14*). Killed *Mycoplasma pneumoniae* vaccine has been quite effective in protecting military populations and children (*13, 15*), and respiratory syncytial virus vaccines of potency equivalent to parainfluenza virus vaccine have recently been made in our laboratory. We may expect these vaccines to be used in the foreseeable future. Parrott *et al.* (*16*) have recently reported that naturally acquired maternal antibody against respiratory syncytial virus may not give protection against infection with this virus in infancy, and that antibody induced by a particular killed respiratory syncytial virus vaccine may have increased the severity of the clinical response to natural infection with this virus in a small group of vaccinated infants.

The rhinoviruses, which are a principal cause of the common cold in children and in adults, are diverse antigenically, and few strains share the same antigens. Thus there is little hope that a polyvalent vaccine of substantial usefulness can be developed at this time. Though killed virus vaccines may be protective, no small group of serotypes is of sufficient importance to be singled out for a vaccine. Other approaches to the control of these viruses are being sought. The reoviruses apparently cause so little disease in man as not to warrant efforts to develop a vaccine at this time.

Though workers in most countries prefer killed influenza virus vaccines, live virus vaccines given by way of the respiratory tract have been investigated extensively in the U.S.S.R. (*17*) and, to some extent, in Great Britain and Japan. The theoretical advantages of simplicity of administration, economy, and induction of local immunity have not as yet been realized. Though occasional live virus strains have been developed which proved highly effective in field trials, it has been extremely difficult to produce, on a routine basis, strains which are sufficiently and uniformly immunogenic yet do not cause excessive clinical reaction. Clearly, simplified laboratory markers for virulence and immunogenicity in man will be needed if live virus vaccines are to be useful in man, in view of the frequency with which the strains need to be changed. Smorodintsev has recently stated (*18*) that infection and immunization against influenza may follow oral administration of orally passaged live influenza virus vaccine. This finding is of considerable interest and warrants further investigation.

The live virus vaccine approach for protection against respiratory syncytial virus has also been explored recently, with variants selected by propagating the virus at low temperature (*19*). To date, attenuation of the virus has been inadequate, and it is too early to say whether a satisfactory vaccine can be prepared. Immunogenic live *Mycoplasma pneumoniae* vaccines of sufficiently low clinical reactivity have not as yet been developed. In the case of the adenoviruses, which normally multiply in the intestinal tract, live virus vaccines which may be administered orally have shown considerable promise, as revealed in extensive field trials with the type 4 virus (*20*). Unfortunately, the method is of very limited usefulness since most adenoviruses, including type 4, induce neoplasia in animals or in cells in culture (*10*). The rhinoviruses, which are closely related to enteric viruses such as poliovirus and ECHO viruses, were considered reasonable candidates for control by live virus vaccines given orally, but immunization has not been achieved to date, even when the vaccine is given in the form of enteric-coated capsules, which deposit the virus in the intestinal tract rather than in the inhospitably acid stomach (*21*).

Immunity to influenza and to certain other respiratory viruses appears to depend mainly upon the presence of neutralizing antibody in the respiratory secretions. Questions have been raised concerning (i) the best means of stimulating production of such antibody and (ii) the relative efficiency of the various kinds of antibody in protecting against infection with the virus. Studies (*22, 23*) have been carried out in which killed as well as live respiratory virus vaccines were administered directly into the respiratory tract. This was done in the hope that such a procedure would provide more antibody at the site where natural infection takes place and that a greater amount of IgA (immunoglobulin A) antibody, which is currently considered by some to be more protective than ordinary IgG (immunoglobulin G) antibody, would be produced locally. The studies by Waldman and his associates (*23*) have failed to reveal a significantly greater amount of either IgA or IgG antibody in the respiratory secretions after respiratory-tract administration of vaccine, even when the response to a single dose of vaccine administered subcutaneously was compared with the response to two or three doses of vaccine given in larger amount and at spaced intervals by way of the respiratory route. An-

other approach to the problem of increasing the degree and duration of immunity (*24, 25*) following vaccination is parenteral administration of killed vaccine incorporated in a suitable immunologic adjuvant. Adjuvants are discussed more fully below.

Enteroviruses

The remarkable achievement in many countries of reducing poliomyelitis to insignificant levels through the use of killed and live poliovirus vaccines scarcely requires comment. The principal task, now, is continued vaccination to bring the benefit of the vaccine progressively to all of the world's population. Though live virus vaccine offers great advantage in terms of simplicity of administration, low cost, and duration of immunity, there are reports of low effectiveness in tropical areas where improved living conditions have delayed first experience with the virus to a greater age, causing increased incidence of serious disease, but where continuing infection of the enteric tract with a multiplicity of indigenous enteroviruses may interfere with vaccination by preventing growth of the poliovirus vaccine strains (*26*). For such regions, use of killed vaccine, in which interference is not a problem, might be considered. The ECHO and Coxsackie viruses cause a considerable amount of respiratory, enteric, and other illness, but the extreme diversity of serotypes, with no predominance of any small number of types, excludes the vaccine approach. Though it should be possible to develop live attenuated and killed virus vaccines, there is a clear need for a broad-spectrum approach such as might be afforded by the induction of host resistance.

Systemic Myxoviruses

Concerted efforts are being made to develop vaccines for the three systemic myxovirus infections of childhood—measles, mumps, and rubella (*27*). All three infections occur in epidemic form, mainly attacking children. Some people escape infection in early life only to experience more severe clinical disease as adults. Enders' live attenuated measles virus vaccine (*28*) was licensed for general use in 1963, and its wide application has reduced measles to trivial importance in the United States. Great benefits have also derived from its use in tropical and underdeveloped countries, where the death toll from measles in infancy may be quite high. Vaccines of less clinical reactivity—for example, the Schwarz, Beckenham, and Moraten lines (*29*)—are now available and will probably be used on a greatly expanded scale throughout the world. Killed measles vaccine has afforded immunity of only short duration and, additionally, has caused allergic reactions in some people subsequently exposed to measles or given live virus vaccine (*30*). Killed mumps virus vaccine, likewise, affords immunity of only short duration. A vaccine prepared from live virus of the Jeryl Lynn strain (*31*), developed by our group and introduced in the United States in January 1968, has already been administered to more than 2 million persons. It provides better than 95-percent protection, and a careful 3-year serological and epidemiological follow-up has provided a substantial basis for the belief that the immunity will be lasting (*32*). Highest priority has been given the development of a vaccine from live attenuated rubella virus. It is expected that this will be introduced before the next widespread occurrence of rubella, expected in 1970, with its resulting high fetal mortality and congenital malformation when maternal infection occurs in the first 3 months of pregnancy. Candidate virus strains grown in different cells have shown great promise (*33*). Our laboratories have prepared vaccine from the HPV-77 strain of attenuated rubella virus grown in cell cultures of duck embryo. We have completed tests of the vaccine in more than 18,000 children, including more than 13,000 who were without previous immunity. All data regarded as necessary for scientific review prior to general use have been gathered. It seems likely that the vaccine will be released for routine vaccination in man by mid-1969. Extensive and proper administration of vaccines against measles, mumps, and rubella should result in near elimination of these illnesses from the United States within a few years.

Herpes Virus Group

Killed virus vaccines claimed to be effective against recurrent herpes simplex or claimed to stimulate the development of antibodies against B virus infection, an occupational hazard in monkey handlers, have been developed, but they are little used (*2*). Development, from live attenuated virus, of a vaccine effective against chickenpox (varicella) and herpes zoster is within sight now that the virus can be propagated readily in cell culture, and such a vaccine may be generally available within the next several years. There is present doubt as to the medical and economic justification for development of a cytomegalovirus vaccine. The recent finding of apparent propagation of the infectious mononucleosis agent in cell culture (*34*) and the suggestion of a remote relationship of this agent to Burkitt lymphoma and other neoplasias in man will probably stimulate intensive study of this agent, and intensive efforts to develop a vaccine, during the next decade.

Viral Hepatitis

The lack of significant progress, to date, toward specific control of viral hepatitis is due to failure to achieve satisfactory propagation in the laboratory of the elusive causal agent or agents. The reported (*35*) development of hepatitis in marmosets inoculated with specimens from human hepatitis patients may be a major breakthrough in hepatitis research, if an etiologic relationship between human hepatitis and the agent propagated in marmosets can be established. However, significant progress toward development of a vaccine must await propagation of the virus in a suitable laboratory medium, such as cell culture. We cannot foresee when this will be achieved.

Combined Vaccines

The availability, in recent years, of many new viral vaccines and the expectation that many others will be available in the near future make it imperative that methods be developed that will simplify administration, reduce costs, and keep to a minimum the number of the patient's contacts with the physician. Certain vaccines have already been given in combination; examples are combined polyvalent influenza virus vaccines, combined poliovirus-DPT (diphtheria, pertussis, tetanus) vaccine, and a mixture of smallpox with yellow fever vaccine. More recently, a combination of live measles vaccine and live smallpox and yellow fever vaccine (*36*) has been tested, with satisfactory results, and a combined live measles and smallpox vaccine was li-

censed in 1967 (*37*). Recent studies by our group have shown the feasibility of administering combined measles-mumps and combined measles-mumps-rubella vaccines; antibody response has been good, and there have been no untoward clinical effects. We have also administered (*12*) a combined killed-virus vaccine for protection against influenza A and B; parainfluenza 1, 2, and 3; and respiratory syncytial pneumonia caused by *Mycoplasma pneumoniae* with surprisingly good antibody responses. The automatic jet gun which permits rapid injection without the need for syringe and needle and which causes little if any pain has already proved of inestimable value in mass vaccination campaigns.

Adjuvants

The multiplicity of antigens worthy of inclusion in killed virus vaccines necessitates development of some means for minimizing the volume per dose, the required number of doses, the amount of antigen per dose, and the cost. One hope for a solution lies in the development and use of a safe and effective immunologic adjuvant. The use of adjuvants should make it possible to achieve a greater and longer-lasting immunity with a smaller antigenic mass and fewer doses than would be possible if aqueous material were used instead of the adjuvant. Various substances, such as aluminum compounds, paraffinic oils, aliphatic amines, cholesterol, fatty acids, alginate, protamine, endotoxins, and acrylamide, may enhance immunologic response to antigens and so produce an adjuvant effect. The aluminum compounds have been used routinely in vaccines for man and animals. Emulsified oil adjuvants are more effective, but these have been used only experimentally in the United States. Freund's incomplete adjuvant (aqueous vaccine in mineral oil) (*38*) has proved highly effective in tests in animals and in man but has not been used routinely because of occasional rather severe local reactions, because of concern about long-term persistence of the mineral oil in the tissues, and because of a lack of the animal and other pharmacologic data that are minimal requirements for licensure for commercial distribution. Another adjuvant, called adjuvant 65 (see *7, 24, 39, 40*), which was developed in our laboratories, promises to provide the high-level, long-term antibody responses characteristic of Freund's incomplete adjuvant without its disadvantages, theoretical or real. Adjuvant 65 consists of a water-in-peanut-oil emulsion of the vaccine; Arlacel A (mannide monooleate) is used as emulsifier, and aluminum monostearate, as stabilizer. All the components of the adjuvant are readily metabolizable, and the emulsion has proved innocuous in studies in man (these include a 5-year follow-up study). The very extensive short- and long-term pathologic investigations in animals (*39, 40*) and the rigid criteria for chemical and physical control (*40*) of the components and the final product have provided a basis for general use of adjuvant 65 in vaccines in the near future. It appears that use of the adjuvant in killed respiratory virus vaccines, such as influenza vaccine, stimulates the production of circulating antibody at a concentration which is essential for protection of the respiratory tract and affords a considerable degree of immunity, which may last for more than one season.

Problems with Vaccines

The introduction of practicable cell culture technology by Enders and his associates in the late 1940's brought new knowledge about viruses and led to the development of many vaccines. With it came a sophisticated technology and a number of imponderables of real or theoretical importance. These problems fall within the general area of safety; they concern the possible presence of extraneous agents in vaccines, possible adverse effects of the vaccine virus itself, and the kinds of cell cultures that may be used in preparing vaccines. The early work with monkey renal cell cultures revealed that diverse indigenous contaminating viruses were commonly present. Cultures that contained detectable contaminating viruses were eliminated. The problem concerned the occult viruses that were undetectable by the methods then available and existed only in theory. Theory became fact when the use of grivet-monkey renal cell culture led to detection of a new virus, SV_{40} (*41*), which was found to be present in viable form in both live and formalin-treated poliovirus vaccines. The presence of this agent took on added significance when tests in newborn hamsters revealed that it was capable of producing neoplasia (*42*). Similarly, development of the RIF (resistance-inducing factor) and COFAL (complement fixation for avian leukosis) tests permitted detection of viable avian leukosis viruses (*43*) in experimental vaccines prepared from chick embryo cell culture and in yellow fever vaccine which had been widely used in man since the late 1930's. To date, these agents appear to have no real significance in man, and they have been eliminated from the vaccines. The most recent alarm with respect to extraneous viruses arose over the occurrence in grivet monkeys in Germany of an infection which was highly communicable to man and which caused seven deaths among laboratory workers or people with whom they came in contact (*44*). This agent is now readily detectable in test procedures and is no threat to the safety of the vaccines.

The problem of viruses in vaccines took on added complexity when it was shown that adenovirus types 3 and 7 used in killed vaccine were capable of inducing neoplasia when injected into newborn hamsters (*10*). The situation became still more complex when it was found that these adenoviruses were commonly hybridized with SV_{40} virus present in the cell cultures used to prepare the vaccine (*45*) and, in fact, were essential to replication of certain of the adenoviruses in monkey renal cell culture. Because of this, adenovirus vaccines are at present excluded from routine human use by the U.S. Public Health Service. This policy might have to be reconsidered if it should be shown eventually that adenoviruses cause cancer in man: the present policy of excluding all neoplasia-producing viruses, live or killed, from vaccines might rule out the very measure needed to prevent the occurrence of cancer itself.

Development of vaccines is also complicated by considerations of acceptability or nonacceptability of cell cultures for propagating the virus (*46*). At present, according to U.S. Public Health Service regulations, only primary cell cultures of animal tissues may be used for propagating viruses for preparing vaccines. Cultures propagated on serial passage—whether of neoplastic, transformed, or diploid (euploid) and, so far as is known, normal cells—are not allowed. This policy does not enjoy the full support of the scientific community. Workers oriented toward virology would argue in support of a theory of viral causation of cancer in man and would want to prevent administration to man of any oncogenic virus, oncogenic viral genetic material,

or neoplastic host genetic substance carried by a virus. They probably would prefer the use of serially propagated cells, in which the chances for introduction of a new virus by fresh tissues is excluded. Researchers oriented toward cytology might regard cancer as a state of the cell itself and would prefer that primary cultures be used, since they would wish to be sure that the cell was normal with regard to karyosome, morphology, propagability, contact inhibition in vitro, and the like. The arguments have not been resolved. The simple fact is that we do not know the cause of neoplastic disease in man and we do not know whether it is transmissible under any circumstance. We do not know whether any of the factors that have been debated are of any basic importance in the development or transmission of cancer in man. There are no guidelines.

In the considerations of safety, the practical need to prevent acute infectious diseases through the use of vaccines runs into full conflict with theories and opinions. What is real and can be found, such as an occult virus, must be eliminated. Decisions based on what exists only in theory must entail compromise, with need weighed against possible risk, and precedent and prior experience followed wherever possible. When the demands of a particular situation exceed the bounds of previous experience and when there are no clear-cut contraindications, the least radical departure seems the prudent choice, and new precedent is thereby established.

Host Resistance—Interferon

It has been known for more than three decades that infection with one virus may limit or exclude infection with a second, unrelated virus. This is called the interference phenomenon (*47–49*). Considerable interest in interference was stimulated in 1957 when Isaacs and Lindenmann (*50*) showed that such activity was mediated by a protein of low molecular weight, called interferon, which was produced by virus-infected cells and which protected new and uninfected cells from viral infection. The importance of this discovery has become increasingly evident with realization that the conventional antibody immune mechanisms may have little to do with the early stages of recovery from viral infections. Thus, interferon appears to provide the first line of defense, being produced early in infection and functioning at the intracellular level to limit or to prevent viral infection. Once infected, the fate of the individual cell—that is, its life or death—appears to depend on factors other than antibody.

Table 3. Working hypothesis for interferon induction and interference with viral synthesis.

Phase 1
Viral attachment to cell, penetration, uncoating——> release of single-stranded RNA
Viral RNA replicates, forming double strand——> alert reaction
Derepressed host DNA transcribes messenger RNA——> translation to produce interferon
*Phase 2**
Released interferon reaches new cells and derepressed host DNA transcribes messenger RNA——> translation, to produce translation inhibitory protein (TIP)
TIP attaches to ribosomes, preventing translation of viral RNA message (virus protein synthesis) but not translation of host-cell messenger RNA

* After Marcus and Salb, in part (*52*).

It was hoped originally that interferon might be produced in cells, purified, and used to prevent or to treat viral diseases. There was great hope for the substance, since it is active against essentially all viruses. The substance itself is highly species-specific with respect to utilization. Thus, interferon for human use would probably have to be prepared in human cells. Though active to some degree in experimental tests, exogenous interferon never achieved practical importance as a substance to be administered because there was no satisfactory source and because the probable costs of preparation were so out of line with the estimated dose requirement as to be prohibitive (*1, 47, 48*).

The alternative approach was that of using some safe and effective inducing substance to stimulate the body to produce and distribute its own endogenous interferon. Many kinds of substances, including bacteria, parasites, viruses, polysaccharides, mitogenic agents, endotoxin, and the like, may stimulate the formation of interferon, but none gives promise of suitability for routine use because of toxicity, antigenicity, infectiousness, and so on.

Studies in our laboratories at the Merck Institute for Therapeutic Research were directed toward finding the natural stimulus for interferon induction by viruses, in the hope that a suitable and practicable inducing chemical would be found. It was discovered in this work that certain double-stranded, but not single-stranded, ribonucleic acids were highly active in inducing interferon and host resistance in animals and in cells in culture (*51*). Double-strandedness is a necessary requirement for multiplication of viruses of the ribonucleic acid type. Such nucleic acid is not a normal component of cells, and we have theorized that the presence of the double-stranded, replicative-form ribonucleic acid of the virus provides an "alert" or "alarm" reaction in the cell, causing it to make interferon (see Table 3, phase 1). As shown by others (*52*), the released interferon (phase 2) then goes to new cells, causing them to make a new substance, translation inhibitory protein (TIP), which prevents synthesis of new viral substance but does not affect the normal synthetic processes of the host. Possibly other mechanisms may be involved in phase 2. Viruses of the deoxyribonucleic acid type might act by a mechanism, similar to that of double-stranded ribonucleic acid, involving a deoxyribonucleic acid, ribonucleic acid double strand.

Various double-stranded ribonucleic acids have been found, by our group (*51*), to induce interferon and to protect against virus infection. These include ribonucleic acids of synthetic origin [inosinic-cytidylic acid complex, also called rI:rC or poly (I-C)], of fungal (? viral) origin, of bacteriophage origin (replicative form), and of viral virion origin (one source is reovirus, a rare virus which is composed of double-stranded RNA in the virus particle).

The possibility of using these inducers in human and animal medicine is being actively explored. The interferon mechanism, with its broad spectrum of antiviral activity, gives hope for eventual prophylactic control of those viral infections in which the number of serotypes is so great as to preclude successful control by vaccines. Examples are the common cold, caused by the numerous serotypes of rhinoviruses, and the systemic and enteric diseases caused by enterovirus serotypes. Special situations might also call for use of these inducers—for example, pandemic influenza of a type for which there was no effective vaccine. Their use might also be of importance in preventing infection with lytic or oncogenic viruses in the early postnatal period of life, prior to development of full immunologic capability. Additionally, an

effective interferon inducer might function therapeutically to prevent the continuing reinfection which appears necessary to maintain the neoplastic state in RNA-virus-dependent cancer—for example, leukemia. Only time will tell to what extent such utilization is practicable. Whatever the outcome, it will be necessary to work within the limits of the relatively short-term action of interferon, and there will be need for continuing restimulation. Optimism concerning interferon induction as a means of controlling viral disease must be tempered with the realization that no system is perfect and this this nonspecific immune mechanism, like the autoallergic processes in cell- and antibody-mediated immunity, might cause adverse rather than beneficial effects. It is not impossible that in certain special circumstances, as in ordinary immune responses, it might be more beneficial to negate rather than promote the interferon effect.

It should be mentioned that some workers consider the sequential administration of live viruses of low virulence to be a feasible but limited procedure for inducing host resistance by the nonspecific interferon or interference mechanism. The Smorodintsevs have recently stated (*18*) that persons given appropriate live attenuated influenza virus vacine by mouth were rendered resistant to respiratory illness from all causes for about 2 weeks. Similarly, live attenuated mumps vaccine given parenterally was said to afford some degree of protection against respiratory illness, from all causes, for at least 2 weeks, the longest period of observation. It was recently shown (*14*) by our group that live attenuated rubella virus vaccine given to young children may have afforded broad-spectrum protection against naturally occurring respiratory illnesses—protection beginning as early as 13 days after infection and lasting as long as 3 to 4 months.

Chemical Approach

The notable successes achieved in antibacterial therapy since the beginning of World War II have stimulated an intensive search for chemical substances of comparable value for preventing or treating viral diseases (*1*, *53*). Unfortunately, the reward to date has been modest in relation to the effort expended. At present there are only three substances or classes of chemical substances which may be considered of some clinical use. These are *N*-methylisatin-β-thiosemicarbazone for prophylaxis of smallpox, adamantanamine for prophylaxis of influenza, and metabolic inhibitors (including iododeoxyuridine, cytosine arabinoside, and trifluorothymidine) for treating corneal infection with herpes simplex virus. Vaccines and interferon are prophylactic and do little to aid the cell once it is infected. The best present hope for the cure of viral infection in the individual cell seems to rest on the chemical approach; this fact encourages continued efforts in viral chemotherapy.

It is now well recognized that there are events, in viral infections in cells, which are specific to the virus and which provide points for specific antiviral attack. These include contact of cell with virus, cell penetration, decoating of viral nucleic acid, synthesis of viral nucleic acids and proteins (structural and enzymic), and assembly and release of virus particles. A principal goal in viral chemotherapy should be the inhibition of viral nucleic acid synthesis. This might be accomplished through the use of selective inhibitors of viral nucleic acid synthesis which have no structural analogy with viral nucleic acids and which are not incorporated into them, or through the use of structural analogs which are incorporated into the viral nucleic acids, giving fraudulent and nonfunctional nucleic acid. Alternatively, inhibitors might be prepared which block the formation of the early proteins (polymerases) that are essential to the synthesis of viral nucleic acid. One kind of inhibitor to be sought would be one which mocks the action of translation inhibitory protein (*52*) in preventing translation from viral but not from host-cell messenger RNA. Most important, such inhibitors ought not to be incorporated into the cell's genetic material.

Research in viral chemotherapy has not yet achieved the status of an exact science, hence random screening as well as rational approaches will probably continue to be used. In this search, studies may not be limited to compounds which are nontoxic for cells. Instead, the specific antiviral and anticellular activities of a number of substances may be defined, with a view to describing the geography of cellular and viral activities which, hopefully, may lead to synthesis of nontoxic antiviral compounds on a more rational basis.

One should bear in mind, also, the fact that the purpose of viral chemotherapy is to treat disease, and that this end might be achieved in part through attack on aspects of viral infection other than the virus itself. It is becoming clear that all or part of the pathology in certain viral infections may be due to allergic hypersensitization and to other immune phenomena. This has been emphasized in many examples, such as the deep stromatal effects of sensitization in herpes simplex infection and the role of immune phenomena in bringing about fatal disease in otherwise normal mice rendered immunologically tolerant by congenital infection with lymphocytic choriomeningitis virus.

The Long Road

The development of any measure for the control of viral disease, whether it be by immunological, chemical, or host-resistance procedure, is necessarily a slow and painstaking process for which a sophisticated technology must be evolved and a large body of information accumulated. We know most about vaccines, since they have been used longest, but all procedures must achieve the common end—safety and efficacy. The development of a vaccine begins with the discovery of a causal agent, an assessment of its clinical and public health importance, and the finding of means for its propagation which are suitable from the standpoint of both safety and economy. Live virus vaccines must be properly attenuated, by still arbitrary means, to a point where the reduction in pathogenicity is sufficient to assure clinical acceptability yet not great enough to result in inadequate immunity. Killed virus vaccines must be properly purified, concentrated, quantified, and rendered inactive by procedures which permit retention of antigenicity. Following extensive tests for safety and potency in animals and in cell cultures, cautious testing of the vaccine in informed, consenting human subjects is begun, particular attention being given to the benefits the recipient may derive from the vaccine. Efficacy is measured in terms of the vaccine's ability to evoke a significant antibody response and to protect against infection and disease caused by the corresponding virus under conditions of natural exposure, for a considerable period after vaccination. Finally, with these hurdles passed, the stage of routine manufacture is reached, in which

it must be demonstrated in laboratory assay and in tests in man that vaccine of comparable quality can be consistently produced in serial lots. The overall achievement requires the cooperative team play of a wide variety of disciplines, including, at the very least, the fields of virology, cell biology, biochemistry, biophysics, pathology, clinical medicine, epidemiology, and applied biology. The effort is doomed from the outset unless the cooperating scientists of these diverse disciplines can be brought to focus on the multifaceted problems which are involved and for whose solution the guidelines may be hazy or nonexistent. Needless to say, the remaining essential element is a fantastically large outlay of funds and facilities.

The development of the vaccine and proof of its efficacy and safety are but the first hurdle, for now the intended product must be subjected to scrutiny and review by scientists in the Federal Regulatory Agency and by the agency's review committees of outside investigators drawn largely from the academic community. If the consensus is favorable, the vaccine is licensed for general distribution. At this point, other committees come into the picture, to render judgment and advice on the extent to which, and the manner in which, the product might best be used under the existing conditions of private practice and public-health effort. At this point, state and national campaigns may be launched. Except for (i) programs to control pandemic influenza and (ii) the short-term use of killed poliovaccine, no great campaigns involving the use of killed virus vaccines have been launched. On the contrary, federal, state, and community forces have been brought into play in administering live virus vaccines, with the ultimate intent of eradicating a particular disease within the United States and sometimes in other parts of the world. The success achieved in attaining such goals is amply illustrated by the successful elimination, by vaccine and quarantine, of smallpox from the United States, and by the recent reduction of poliomyelitis and measles to all but negligible levels. Programs of major dimension for vaccinating populations against smallpox and measles are currently being pursued in many parts of the world. Present information on the degree and duration of immunity through the third year following administration of live mumps virus vaccine indicates that eradication of this disease may also be anticipated. The next likely candidate for such a program is rubella vaccine. It may well be that administration will be simplified through use of the trivalent combined live measles, mumps, and rubella vaccines.

It is difficult to predict the extent to which vaccines will eventually be used to control acute infectious diseases. This will surely be influenced by the extent of collateral development of alternative measures in the areas of chemotherapy and host resistance. The frontier is retreating as the more serious illnesses are successively conquered, and the retreat is likely to continue to the point where all those infectious diseases which pose a significant threat to life or which are important as nuisances or for economic reasons are under control. Surely, however, there must be some point at which one will pay too much. At this stage, major attention will probably be diverted away from acute infectious disease to control of the illnesses of obscure etiology for which, at present, viruses are only suspect as causative agents. The myriad possibilities of viral effect on cells, including genetic addition, genetic deletion, genetic rearrangement, neoplastic transformation, antigenic alteration, biochemical malfunction, release of sequestered antigens, cell deletion, and the like, lend some credence to the view that viruses may play a role in the vast majority of the illnesses of mankind. The degree to which viral vaccines may prevent such illnesses remains to be seen. Meantime, it seems possible that widespread use of existing and yet-to-be-developed vaccines may eliminate, or reduce the future incidence of, illnesses in which a viral role is not even suspect at present. Such is the wave of the future.

References and Notes

1. M. R. Hilleman, *Amer. J. Med.* **38**, 751 (1965).
2. Report of a WHO Scientific Group, World Health Organization Technical Report Series No. 325, Geneva, 1966; "First International Conference on Vaccines against Viral and Rickettsial Diseases of Man" (Washington, D.C., 1966), *Pan Amer. Health Organ. Sci. Pub. No. 147* (1967).
3. M. R. Hilleman, *Clin. Pharmacol. Therap.* **7**, 752 (1966).
4. K. Kodama, N. Sasaki, Y. K. Inoue, *J. Immunol.* **100**, 194 (1968).
5. M. R. Hilleman, in *Infektionskrankheiten* (Proceedings 4th International Congress for Infectious Diseases) (Schattauer, Stuttgart, 1967), pp. 119–139; M. R. Hilleman, R. E. Weibel, A. F. Woodhour, J. Stokes, Jr., C. C. Mascoli, M. B. Leagus, A. A. Tytell, P. P. Vella, in "First International Conference on Vaccines against Viral and Rickettsial Diseases of Man," *Pan Amer. Health Organ. Sci. Pub. No. 147* (1967), pp. 141–154.
6. *World Health Organ. Chron.* **22**, 528 (1968).
7. J. Stokes, Jr., R. E. Weibel, A. F. Woodhour, W. J. McAleer, L. A. Potkonski, M. R. Hilleman, *J. Amer. Med. Ass.*, in press.
8. C. B. Reimer, R. S. Baker, R. M. van Frank, T. E. Newlin, G. B. Cline, N. G. Anderson, *J. Virol.* **1**, 1207 (1967).
9. F. M. Davenport, A. V. Hennessy, F. M. Brandon, R. G. Webster, C. D. Barrett, Jr., G. O. Lease, *J. Lab. Clin. Med.* **63**, 5 (1964); A. E. Duxbury, A. W. Hampson, J. G. M. Sievers, *J. Immunol.* **101**, 62 (1968).
10. M. R. Hilleman, in *Viruses Inducing Cancer*, W. J. Burdette, Ed. (Univ. of Utah Press, Salt Lake City, 1966), pp. 377–402.
11. J. A. Kasel, M. Huber, F. Loda, P. A. Banks, V. Knight, *Proc. Soc. Exp. Biol. Med.* **117**, 186 (1964).
12. R. E. Weibel, J. Stokes, Jr., M. B. Leagus, C. C. Mascoli, M. R. Hilleman, *Amer. Rev. Respirat. Diseases* **94**, 362 (1966).
13. R. E. Weibel, J. Stokes, Jr., C. C. Mascoli, M. B. Leagus, A. F. Woodhour, A. A. Tytell, P. P. Vella, M. R. Hilleman, *ibid.* **96**, 724 (1967).
14. P. P. Vella, R. E. Weibel, A. F. Woodhour, C. C. Mascoli, M. B. Leagus, O. L. Ittensohn, J. Stokes, Jr., M. R. Hilleman, *ibid.*, in press.
15. W. J. Mogabgab, *Amer. Rev. Respirat. Diseases* **97**, 359 (1968).
16. R. H. Parrott, H. W. Kim, J. O. Arrobio, J. G. Canchola, C. D. Brandt, J. L. DeMeio, K. E. Jensen, R. M. Chanock, in "First International Conference on Vaccines against Viral and Rickettsial Diseases of Man," *Pan Amer. Health Organ. Sci. Pub. No. 147* (1967), pp. 35–41.
17. V. M. Zhdanov, *ibid.*, pp. 9–15.
18. A. A. Smorodintsev and A. Smorodintsev, personal communications.
19. W. T. Friedewald, B. R. Forsyth, C. B. Smith, M. A. Gharpure, R. M. Chanock, *J. Amer. Med. Ass.* **204**, 690 (1968).
20. R. O. Peckinpaugh, W. E. Pierce, M. J. Rosenbaum, E. A. Edwards, G. G. Jackson, *ibid.* **205**, 5 (1968).
21. C. C. Mascoli, M. B. Leagus, R. E. Weibel, J. Stokes, Jr., H. Reinhart, M. R. Hilleman, *Proc. Soc. Exp. Biol. Med.* **121**, 1264 (1966).
22. T. R. Cate, R. D. Rossen, R. G. Douglas, Jr., W. T. Butler, R. B. Couch, *Amer. J. Epidemiol.* **84**, 352 (1966); R. D. Rossen, A. L. Schade, W. T. Butler, J. A. Kasel, *J. Clin. Invest.* **45**, 768 (1966); C. B. Smith, R. H. Purcell, J. A. Bellanti, R. M. Chanock, *New Engl. J. Med.* **275**, 1145 (1966); R. H. Waldman, J. J. Mann, J. A. Kasel, *J. Immunol.* **100**, 80 (1968).
23. R. H. Waldman, J. A. Kasel, R. V. Fulk, Y. Togo, R. B. Hornick, G. G. Heiner, A. T. Dawkins, Jr., J. J. Mann, *Nature* **218**, 594 (1968).
24. A. F. Woodhour, M. R. Hilleman, J. Stokes, Jr., R. E. Weibel, in "First International Conference on Vaccines against Viral and Rickettsial Diseases of Man," *Pan Amer. Health Organ. Sci. Pub. No. 147* (1967), pp. 566–569.
25. V. V. Hamparian, F. V. Washko, A. Ketler, M. R. Hilleman, *J. Immunol.* **87**, 139 (1961).
26. *World Health Organ. Chron.* **22**, 257 (1968).
27. The true position of the rubella agent is not clear; actually it may belong to the arbovirus rather than the myxovirus family.
28. J. F. Enders, S. L. Katz, M. V. Milovanovic, A. Holloway, *New Engl. J. Med.* **263**, 153 (1960).
29. W. C. Cockburn, J. Pečenka, T. Sundaresan, *Bull. World Health Organ.* **34**, 223 (1966); M. R. Hilleman, E. B. Buynak, R. E. Weibel, J. Stokes, Jr., J. E. Whitman, Jr., M. B. Leagus, *J. Amer. Med. Ass.* **206**, 587 (1968).
30. V. A. Fulginiti, J. J. Eller, A. W. Downie, C. H. Kempe, *ibid.* **202**, 1075 (1967).
31. M. R. Hilleman, E. B. Buynak, R. E. Weibel, J. Stokes, Jr., *New Engl. J. Med.* **278**, 227 (1968).
32. R. E. Weibel, E. B. Buynak, J. E. Whitman, Jr., M. B. Leagus, J. Stokes, Jr., M. R. Hilleman, *J. Amer. Med. Ass.* **207**, 1667 (1969).
33. M. R. Hilleman, E. B. Buynak, R. E. Weibel, J. Stokes, Jr., *New Engl. J. Med.* **279**, 300 (1968).
34. G. Henle, W. Henle, V. Diehl, *Proc. Nat. Acad. Sci. U.S.* **59**, 94 (1968).
35. F. Deinhardt, A. W. Holmes, R. B. Capps, H. Popper, *J. Exp. Med.* **125**, 673 (1967).
36. H. M. Meyer, Jr., D. D. Hostetler, Jr., B. C. Bernheim, N. G. Rogers, P. Lambin, A. Chassary, R. Labusquiere, J. E. Smadel, *Bull. World Health Organ.* **30**, 783 (1964).
37. F. Kalabus, H. Sansarricq, P. Lambin, J.

Proulx, M. R. Hilleman, *Amer. J. Epidemiol.* **86**, 93 (1967).
38. F. M. Davenport, *Ann. Allergy* **26**, 288 (1968).
39. H. M. Peck, A. F. Woodhour, D. P. Metzgar, S. E. McKinney, M. R. Hilleman, *Proc. Soc. Exp. Biol. Med.* **116**, 523 (1964).
40. M. R. Hilleman, *Progr. Med. Virol.* **8**, 131 (1966).
41. B. H. Sweet and M. R. Hilleman, *Proc. Soc. Exp. Biol. Med.* **105**, 420 (1960).
42. A. J. Girardi, B. H. Sweet, V. B. Slotnick, M. R. Hilleman, *ibid.* **109**, 649 (1962).
43. P. S. Sarma, H. C. Turner, R. J. Huebner, *Virology* **23**, 313 (1964).
44. C. E. G. Smith, D. I. H. Simpson, E. T. W. Bowen, I. Zlotnik, *Lancet* **1967-II**, 1119 (1967).
45. F. Rapp, J. L. Melnick, J. S. Butel, T. Kitahara, *Proc. Nat. Acad. Sci. U.S.* **52**, 1348 (1964).
46. M. R. Hilleman, *National Cancer Institute Monograph 29* (1968), pp. 463–469; *Progr. Med. Virol.* **10**, 348 (1968).
47. ———, *J. Cell. Comp. Physiol.* **62**, 337 (1963).
48. ———, *ibid.* **71**, 43 (1968).
49. N. B. Finter, Ed., *Interferons* (Saunders, Philadelphia, 1966); M. Ho and B. Postic, in "First International Conference on Vaccines against Viral and Rickettsial Diseases of Man," *Pan Amer. Health Organ. Sci. Pub. No. 147* (1967), pp. 632–649; S. Baron and H. B. Levy, *Ann. Rev. Microbiol.* **20**, 291 (1966); R. R. Wagner and T. J. Smith, in "First International Conference on Vaccines against Viral and Rickettsial Diseases of Man," *Pan Amer. Health Organ. Sci. Pub. No. 147* (1967), pp. 616-622; R. Z. Lockart, Jr., *Progr. Med. Virol.* **9**, 451 (1967).
50. A. Isaacs and J. Lindenmann, *Proc. Roy. Soc. London Ser. B* **147**, 258 (1957).
51. G. P. Lampson, A. A. Tytell, A. K. Field, M. M. Nemes, M. R. Hilleman, *Proc. Nat. Acad. Sci. U.S.* **58**, 782 (1967); A. K. Field, A. A. Tytell, G. P. Lampson, M. R. Hilleman, *ibid.*, p. 1004; A. A. Tytell, G. P. Lampson, A. K. Field, M. R. Hilleman, *ibid.*, p. 1719; A. K. Field, G. P. Lampson, A. A. Tytell, M. M. Nemes, M. R. Hilleman, *ibid.*, p. 2102; A. K. Field, A. A. Tytell, G. P. Lampson, M. R. Hilleman, *ibid.* **61**, 340 (1968).
52. P. I. Marcus and J. M. Salb, *Virology* **30**, 502 (1966).
53. I. Tamm and H. J. Eggers, *Amer. J. Med.* **38**, 678 (1965); D. G. O'Sullivan, *Roy. Inst. Chem. London Lectures* **1965**, No. 2 (1965); H. E. Kaufman, *Progr. Med. Virol.* **7**, 116 (1965); G. Appleyard, *Brit. Med. Bull.* **23**, 114 (1967).

Transplantation Antigens

Solubilized antigens provide chemical markers of biologic individuality.

Barry D. Kahan and Ralph A. Reisfeld

The fate of tissue grafts (histocompatibility) depends upon the genetic relation of the donor to the host. The historic experiments of Loeb (*1*), Little (*2*), and Snell (*3*) demonstrated that grafts exchanged between members of the same inbred strain (isografts) survive permanently, while grafts exchanged between members of two different strains (allografts) are promptly rejected. There are at least 15 histocompatibility loci controlling transplantation in mice (*4*), eight in rats (*5*), four to six in guinea pigs (*6*), and four in Syrian hamsters (*7*). The strength of the individual genetic differences is believed to be related to the speed of graft destruction when donor and host differ solely at that locus. If the graft survives less than 14 days, donor and host are defined to be incompatible at a strong transplantation locus. On the other hand, graft rejection due to weak genetic differences does not occur until 16 to 200 days after transplantation (*8*). In each species which has been carefully investigated, there is a single strong histocompatibility locus controlling the rapid rejection of allografts: the H-2 locus of mice (*3*), the HL-A locus of man (*9*), the Ag-B (H-1) locus of rats (*10*), and the B locus of chickens (*11*).

The mechanism of the rejection phenomenon was not immediately apparent. Loeb (*1*) postulated that grafts release foreign substances which initiate primarily local, cellular reactions leading to rejection. Twelve years later Gibson and Medawar (*12*) noted that skin allografts applied to a patient who had rejected previous grafts from the same donor were destroyed in accelerated fashion—the second-set phenomenon. In a series of elegant experiments in outbred rabbits Medawar (*13*) demonstrated that the second-set phenomenon was specific for the donor of the first (sensitizing) graft, and that the resistance induced by the initial transplant was systemic, that is, grafts applied onto any site were destroyed in accelerated fashion. He concluded that local events did not determine the fate of the graft and proposed the immunologic hypothesis of rejection: after transplantation grafts release substances (antigens) which induce an immune response against themselves. Presumably it is the antigenic product (or products) of the strong transplantation locus or, in some instances, of multiple weak loci operating in concert (*14*), which are the prime movers in the rejection phenomenon and thus of the greatest biologic interest. Serologic studies have suggested that the gene product or products possess several antigenic specificities, and genetic studies suggest that these are determined by a single chromosomal region (*9*, *15*).

The gene product of this chromosomal region appears to be essential for cell function. Determinants of transplantation antigens can be demonstrated on all cells and can be detected on cells perpetuated in tissue culture (*16*). In that allografting represents a situation not known to occur in nature, it would be expected that survival pressure would have discarded these components unless they played a significant role in cell structure or function. It has been postulated that these substances mediate either transport (*17*) or, more probably, cell contact and recognition phenomena (*18*). Presumably, in the course of performing their natural function, these potentially antigenic substances are recognized as foreign by the host's immune system and become the target of his response.

Assay Systems

The products of the strong transplantation loci appear to have three biologic actions which presumably relate to histocompatibility: (i) the induction of allograft immunity, (ii) the evocation of humoral alloantibodies, and (iii) the elicitation of specific cutaneous hypersensitivity reactions. According to rigorous criteria, a substance must affect the fate of donor-specific grafts, either by hastening their

Dr. Kahan is in the Department of Surgery, Massachusetts General Hospital, Boston. Dr. Reisfeld is a senior staff scientist in the Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland.