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Continuously Cultured Tissue Cells and Viral Vaccines

Potential advantages may be realized and potential hazards obviated by careful planning and monitoring.

Report of a Committee on Tissue Culture Viruses and Vaccines

The continuing development of new viral vaccines and the widespread growing interest in their general administration to man focuses attention anew on problems accompanying their production and the assessment of their potency, purity, and safety.

During the last 15 years, the art and science of culturing animal cells and their use as growth media for viruses has developed to the point where tissue cultures provide the predominant technique for the isolation, identification, and propagation of these agents. Furthermore, mass culture of mammalian cells has become practicable for the commercial preparation of viral vaccines, such as those used for immunization against poliomyelitis. (Comparable developments of veterinary vaccines are deemed beyond the scope of this report.) We see no indication that the trend toward such applications of tissue culture will not continue.

Three general types of cell culture have been used for growing viruses. First, there are mixed populations of cells freshly explanted from normal animal tissues and cultured, for a relatively short period but not subcultured, in artificial media that usually contain serum. In this way monkey kidney cells are grown for producing polioviruses and also chick embryo cells for measles vaccine. Secondly, there are "cell lines" or "cell strains" (here used synonymously), which are derived by serial subcultures from the first type. Human skin epithelium NCTC3075, monkey kidney epithelium NCTC3526-LLCMK2, and mouse liver epithelium NCTC1469 are good examples. Lastly, there are the cell lines derived similarly from explants of neoplastic mammalian tissues, such as the HeLa line, which originated from a human cervical carcinoma. Under favorable conditions the latter two types can be subcultured indefinitely in artificial media that contain serum or some other source of protein. Some of these lines, including some cited here, have been stabilized and carried for a number of years in chemically defined protein-free media. Until now, primary cultures of the first type have been considered to be the most practicable for large-scale production of viruses. However, in anticipation of the probable need for expanded production of various new viral vaccines, the limitations which are discussed here have turned attention to the possible use of continuously cultured cells.

The procedures necessary to ensure the potency of viral vaccines are well defined and will not be considered here. Because at present the respective viruses must be propagated in living animal cells, peculiar difficulties arise in selecting criteria of purity and safety, most importantly, criteria to ensure the absence of adventitious nonbacterial infectious agents. These difficulties are particularly exigent with the more recently developed live-virus vaccines but to some degree they arise also with inactivated virus vaccines, especially those that rely on borderline conditions of inactivation, because the conditions that suffice to inactivate the virus of the vaccine may fail to inactivate unsuspected adventitious viruses. Paradoxically, smallpox vaccine, which has been in use for over a century and a half and is generally prepared from infected skin lesions of inoculated calves, has been the subject of little concern regarding the presence of adventitious agents other than bacteria and foot-and-mouth virus.

We have accordingly undertaken to evaluate pertinent aspects of current knowledge of the cultivation of animal cells which might be used in the production of viral vaccines, to consider relevant properties of the cultured cells, and to suggest guide lines for the development of a practical program for the use of continuously cultured cells in viral vaccine production.

We agreed readily that continuously cultured tissue cells afford numerous advantages in the propagation of viruses for vaccines. However, because such cells tend to develop characteristics suggestive of malignant change, and theoretically oncogenic activity might be associated with viruses propagated in them, we recognized that present knowledge permits only carefully qualified approval of their appilcation to vaccine production. On the other hand, we were mindful that too cautious an opinion would discourage the continued research needed for better definition of the permissible limits of such application. Meanwhile, continuing develop-

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This article represents the consensus of the authors on the potential advantages and hazards attending the use of continuously cultured tissue cells for the propagation of viruses to be used in vaccines. It is a report made to James A. Shannon, Director of the National Institutes of Health, at his request, by a committee whose members were Henry W. Scherp, Chairman, W. Ray Bryan, Clyde J. Dawe, Wilton R. Earle, Karl Habel, Robert J. Huebner, Karl Reinhard, and Joseph E. Smadel. The authors are staff members of the National Institutes of Health, Bethesda, Maryland.

ments in viral oncology make it advisable to maintain a tentative position. Accordingly, this report of our present consensus is submitted with the realization that at least some of the views expressed might well require revision in the near future as new information becomes available.

Cell Cultures Used in Virus Production

Primary cultures of cells freshly explanted from normal tissues. We have found no compelling advantage, for viral vaccine production, in the use of primary cultures of mixed populations of cells freshly explanted from normal tissues. On the contrary, serious limitations have been revealed by experience with cultures of monkey kidney cells in the preparation of poliovirus vaccine, which may be regarded as a prototype. At present, however, a number of viruses of public health importance, like polioviruses, cannot be cultured effectively in cells derived from nonprimate animals. Human cells are not available in sufficient quantities for the large-scale primary cultures needed. Insofar as primate tissues must be used, the supply of animals is a matter of ever greater complexity and concern, especially in the face of increasing demand, since it is controlled by international regulatory policies. Primate colonies could be established but they present formidable practical problems. Probably more serious is the emergence under cell-culture conditions of a multitude of hitherto unknown viruses. At least 40 have appeared in the case of cells from the kidneys of apparently healthy monkeys. These adventitious viruses may occur with such frequency, like foamy virus and vacuolating agent (SV40), that a large fraction of animals become unsuitable as cell donors. The pathogenicity of most of these agents for man is unknown. However, some, like SV40, can establish themselves and replicate in man. Others, like SV5 which is closely related antigenically and biologically to type 2 parainfluenza virus, resemble viruses causing human disease; and at least one simian virus, *Herpesvirus simiae* (B virus), can produce disease in man. Recent reports state that infection with SV40 induces chromosomal abnormalities in cells cultured from human adult buccal mucosa, adult skin, and fetal kidney.

Still another problem derives from the need to incorporate certain biological materials of animal or human origin, particularly serum, in the media for freshly explanted cells. This entails the risk of introducing heterologous proteins or adventitious viruses such as one of the hepatitis viruses into the culture products. Finally, cells derived from unselected animal stocks may be expected to vary genetically, nutritionally, immunologically, and in viral susceptibility from one batch to another.

Continuously cultured mixed cell populations derived from normal tissues. Continuously cultured cell systems have a number of advantages for virus production. Since a steady supply of fresh tissues is not needed, cells of human as well as animal origin can be used and the process can be carried on in almost any locale where it is convenient to establish an adequate facility, provided that the procedures and products are monitored effectively. Repeated subculture greatly increases the probability that cryptic viruses harbored by the original tissue will become manifest. Consequently, there can be greater confidence that cultures showing no cytopathic effects are free from adventitious viruses, as compared with primary explants. Also seed stocks can be accumulated and stored indefinitely in the frozen state, and mass cultures can be started from them by the techniques now used for primary explants.

Great interest has developed recently in the possible use, for virus production, of successive subcultures of primary explants which are derived from normal tissues such as human embryos, and which are maintained through a limited number of passages in media containing serum and antibiotics. Such cultures have the advantage that under certain conditions of use so far reported the cells have tended to remain diploid. However, the resultant cell populations are heterogeneous, which means that they are not precisely characterized genetically or otherwise, and may be subject to random fluctuations in properties. Also, the use of media containing serum, necessary at least until the final step of virus production, entails the aforementioned risks. Nevertheless, we judge that current considerations of practicability certainly justify continued investigation of this approach to viral vaccine preparation.

Continuously cultured stabilized cell lines. By the term "cell line" we mean the progeny of cells from as nearly a homogeneous tissue source as possible, such as tissue from a single individual and from one organ of that individual, which has descended asexually in artificial culture media long enough to have achieved reasonable stability in desired selected characteristics. When propagated from a single cell, the culture constitutes a clonal population, and is commonly referred to as a "clone" or "cloned line."

At the present time, a cell line suitable for virus production must be established in a continuously and rapidly proliferating state in one of a variety of media that usually contain serum or other biological material, the omission of which would be desirable. Subsequently, however, it is generally feasible to establish a clone, and to adapt the original and the cloned lines to a chemically defined medium. A large number of cell lines have been established, of human and animal origin, and from both normal and neoplastic tissues. Some cloned as well as uncloned lines have been cultured for a number of years in chemically defined media free from serum and protein; through many generations certain characteristics of these lines have been stable, as shown by such sensitive criteria as reproductive performance, enzyme production, and range of viral susceptibility. Such stable lines obviously have the same advantages for virus production previously noted for other types of continuously cultured cells, namely, independence of a continual supply of fresh tissue, greater assurance of freedom from adventitious viruses harbored by the primary tissue, and accumulation and storage of certified seed stock. Also, the practicability of culturing in serum-free media eliminates an important possible source for the introduction of adventitious viruses and other microbial forms, such as mycoplasmas (pleuro-pneumonia-like organisms, PPLO). Finally, especially if cloned, these lines would be expected to provide more uniform performance in virological work.

Present knowledge of the nutritional and environmental requirements indicates that these cell lines could be cultured in chemically defined media on the large scale necessary for viral vaccine production, though a number of technical problems certainly remain to be solved. In particular, much research remains to be done on definition of the most advantageous media and on the technology of large-scale growth of such cultures under exactly defined conditions.

A Potential Disadvantage of Continuously Cultured Cells

To date, the only serious impediment to the acceptance of continuously cultured cells as a source of viral or other material for administration to humans has been the incompletely resolved question of the potential oncogenicity for man of products derived from them. This is essentially the question of whether or not the cell-free products contain viruses oncogenic in man, and it arises because continuously cultured cells eventually develop characteristics suggestive of malignant change, which theoretically might be attributable to some as yet undefined viral activity. Even though no virus has been shown to be involved in these particular changes, there can be no absolute guarantee that a given strain of continuously cultured cells will never yield a previously unknown virus or some nucleic acid or nucleoprotein that is infective and pathogenic for some cells, in vitro or in vivo, under some conditions.

In evaluating the potential hazards implied by these considerations, however, it should be kept in mind that the benefits of any biological product, and particularly a live-virus vaccine, are accompanied by some degree of risk, which must be estimated as accurately as possible in each instance and weighed against the need for the particular product. The risk tends to be increased in some of the circumstances that may attend the use of live-virus vaccines, such as administration to the very young, who have greater susceptibility to some viruses, administration by routes other than the natural portal of entry, for example, parenterally rather than orally, and admistration of relatively large doses. Appropriate precautions must be observed in such cases.

During long-term culture, cells originating in normal mammalian tissue eventually undergo numerous morphologic, immunologic, and metabolic changes, some of which have been regarded by many investigators as indicators of malignancy. Some of these changes occur rapidly; others may be delayed for intervals of a year or more. For example, when mammalian kidney cells are grown in vitro under relatively anaerobic conditions, within about seven days after being freshly explanted their respiration is reduced and both aerobic and anaerobic glycolysis are increased, so that the over-all metabolism resembles that of the cancer cell. (Note that this interval approximates that used in the growth of monkey kidney cells for preparation of the Salk vaccine.) Ultimately, the chromosome number of a cell line becomes heteroploid; however, heteroploidy by itself is by no means a certain indication of oncogenic potential, for polyploidy, at least, can occur in certain cell types of some normal organs, and on the contrary heteroploidy may be absent in cells of spontaneous neoplasms. However, as a rule in rapidly growing long-term cultures even cells originating in normal tissue become capable, when transplanted under appropriate conditions into histocompatible or other suitably prepared secondary hosts, of progressive multiplication into neoplasms leading to death of the recipients. Generally this has been demonstrated only by using cells derived from highly inbred strains of animals and injecting the cells (*not* cell-free culture fluids, such as would be used in viral vaccines) either into animals of the strain of their origin, or into certain specialized sites such as the anterior chamber of the eye and the hamster cheek pouch, or into hosts of altered reactivity such as heavily irradiated or cortisone-treated animals. Experiments as yet unpublished have shown, however, that cells of a line originating in normal human skin, and long cultured successively in media containing serum and in chemically defined media, developed into local growths which had the morphological characteristics of malignancy when injected into the skin of human volunteers in the terminal stages of lymphomatous disease.

Cell-culture material for administration to humans falls naturally into two classes: material in which living tissue cells may be present, and material from which all intact cells have been removed by filtration or other means, with or without some preliminary inactivation of associated viral material. In the light of the foregoing discussion of our present knowledge, it would be definitely unwise to use any viral vaccine containing any type of living cells of human tissue origin, particularly if the vaccine is to be administered parenterally. The present report consequently concerns the use of cell-free material only. If the necessity should arise for deliberate preparation of living tissue cell material for human injection, the associated hazard should at a future time be accurately determined in animals and human volunteers, and weighed most carefully against the desirability of use.

Where all living cells have been removed by a reliable procedure such as filtration, as is customary in viral vaccine preparation, so far as we know the hazard of oncogenic action in man could come only from some as yet undefined oncogenic virus or active viral component such as an infective nucleic acid. The possibility cannot be ignored that such agents are involved where continuously cultured cells derived from normal tissues have become capable of growing into neoplasms when transplanted into suitable recipients; but at present there are no data to substantiate this possibility. Up to now, no oncogenic virus, or infective nucleic acid has been demonstrated in stabilized cell lines originating from normal tissues; however, the search for such agents should be intensified. In view of the numerous advantages of continuously cultured cells for virus production, as compared with cultures explanted from fresh tissues, every possible effort should be made to obtain definitive evidence on this point. Unfortunately, only limited trials are feasible in man and their evaluation for oncogenesis would require a lifetime, since it would be necessary to allow for a prolonged latent period. On the other hand valuable analogous information should be readily obtainable with animal systems. In particular, exhaustive tests for oncogenicity in animals should be made with cell-free filtrates of (i) culture supernatants of cell lines, including those of human origin but especially those originating in the homologous strain of animal, (ii) the material released by disruption of the cells, (iii) nucleic acids extracted from the cells, and (iv) ordinarily non-oncogenic viruses grown in the cells.

In this connection, we believe it is pertinent to note that recent developments have begun to broaden our concepts of the relationships of viruses to oncogenesis. For example, certain viruses such as polyoma, SV40, and type 12 adenovirus widely infect their natural hosts but no oncogenic manifestations have been reported; yet polyoma virus induces tumors when injected in sufficient dosage into newborns of its natural host (mouse), or certain alien hosts (hamster, rat, guinea pig, ferret, mastomys). Similarly, SV40, of simian origin, and type 12 adenovirus, of human origin, have been reported to induce tumors when injected into an

alien host, the newborn hamster. Recent reports state that infection with such common viruses as vaccinia, ECHO, Coxsackie, poliomyelitis, and influenza synergistically activates or aggravates carcinogenesis by certain chemicals in experimental animals. An increasing number of investigators consider seriously the concept that common acute virus infections may initiate cellular alterations that are expressed later as neoplasia, without necessity for continued presence of virus.

In many laboratory investigations, and in some clinical studies where living virulent viruses were administered to humans for the determination of etiology, immunological response, and oncolysis, stabilized cell lines derived from neoplastic tissues (mainly human) have been favored for the propagation of viruses because these lines were less difficult to culture, grew much faster, and had a more favorable viral susceptibility. The information available from these investigations is not unfavorable to the use of viruses grown in such cell lines. (In this connection, it must not be forgotten that if viruses oncogenic in man should be discovered, vaccines against them would be derived perforce from cancerous or other tissues or cell lines infected with the respective oncogenic viruses.) No evidence has been presented of oncogenicity in products from these cell cultures when they have been free from cells and known viruses. None of the stabilized cell lines used, such as HeLa, KB, or NCTC929-(L), has ever been shown to harbor an indigenous virus. Since 1954, adenoviruses and a number of related viruses have been grown in the HeLa and KB cell lines and administered to over 400 volunteers without evident untoward effect. Although these explorations provide at the moment many more relevant data than are available for any other continuously cultured cell system, they can not be regarded as definitive. In the first place, much more testing of the culture products in animals is needed, of the kind discussed previously in relation to cell lines derived from normal tissue. Secondly, the normal recipients of viruses grown in HeLa or KB cells have been adult, whereas an increasing number of studies in animals emphasize that viral tumors often do not develop unless the viral infection is acquired early in life. Lastly, judging by the generally long latent period in experimental viral oncogenesis, many more years of careful examination of these subjects will be required to determine whether the incidence of neoplasms is unusually high in this group.

Considerations for Practical Programs

Four principal stages in the development of a practical program using continuously cultured cells for the production of viral vaccines intended for human use would be selection of optimal cell types, development of criteria for stability of the cell cultures and for their freedom from contaminating viruses and other microbial forms, exhaustive laboratory tests of trial batches of the vaccines for potency, purity, and safety, and progressive controlled trials in humans.

Selection of cell types. The use of a given cell type for virus production presupposes that it can be grown on a sufficiently large scale, that it is appropriately susceptible to virus infection, and that it yields sufficient quantities of virus.

Cells of human origin might be preferable to those of animal origin for three reasons. They would be susceptible to a broader range of viruses infective for man; for example, some stabilized cell lines cultivable in chemically defined media can support the replication of any one of at least 15 viruses. The adventitious viruses infective for man, which they might harbor, are better defined. The use of human cells would diminish the possibility of including sensitizing antigens; this feature is particularly desirable in vaccines intended for parenteral administration. The possibility of sensitization to human isoantigens and organ-specific antigens, however, would not be eliminated.

On the other hand, cells of animal origin might be advantageous for two reasons. In the unlikely event of accidental carry-over of living cells, those of animal origin would be expected to undergo heterograft rejection by the human recipient, whereas a comparable accident with human cells might in rare instances result in an overt tumor. A hypothetical human cancer virus, which might conceivably be present in an initial explant of human tissue, might be carried in a cryptic state or become manifest in subsequent generations of the culture; this would be less likely to occur in animal cells. However, the aforementioned tumorigenic effects of polyoma, SV40, and type 12 adenovirus in alien hosts, and analogous examples, suggest that the hazards for man from cryptic viruses of animal origin might equal or exceed the hazards from cryptic viruses in human tissue cells. Conversely, in the case of attenuated viruses intended for oral administration, unknown indigenous viruses of animal origin might be of less concern than those that might be encountered in human cell lines, for much evidence indicates that man frequently consumes the former in the animal products in his diet, without apparent ill effects.

As primary sources of cell lines, embryonic or neonatal tissues might be preferable, since they are likely to have experienced fewer viral infections than adult tissues and consequently might be expected to harbor fewer cryptic viruses. Because of considerations discussed previously, except for investigational purposes it seems wise for the present not to use as primary tissue any cellular material known to have originated from a recognized human or animal tumor, regardless of clinical and histopathological characteristics, whether encapsulated or invasive. Advances in our knowledge may modify this restriction at a later date, but at present it seems to us valid as a conservatively precautionary and wise means of removing causes for possible public criticism or alarm. An exception could perhaps be justified if the need for immunization against a particular viral disease should be deemed sufficiently urgent and if no practicable alternative were available. In these circumstances, serious consideration might be given to the use of a killed-virus vaccine prepared from virus propagated in such a selected stabilized cell line as HeLa. Alternatively, one might use an established line that originated in normal tissue, such as human skin epithelium NCTC3075, even though it had diverged in some characteristics. The conditions of preparation would have to be such as would inactivate the most resistant known viruses and infective nucleic acids with a generous margin of safety, for example, by the synergistic action of chemical means such as formaldehyde or beta-propiolactone and physical means such as ultraviolet radiation.

Criteria for control. Before it is used in virus production, a cell line should pass through a sufficient number of cultures to achieve reasonable stability in key characteristics and to provide

adequate opportunity for the manifestation of cryptic viruses.

Among the useful operational indicators of deviations from stability in the characteristics of a cell line would be changes in its spectrum of viral susceptibility, the yields of viruses from it, its chromosomal pattern and other intrinsic cellular and clonal morphological characteristics, and its capacity to grow as invasive neoplasms when transplanted into isologous, immunologically defective, or other suitable hosts.

Axiomatically, any cell culture to be used for the purpose under discussion must be shown to be free of known viral and other microbial forms. Since the methodology and criteria to accomplish this are generally well known, they will not be discussed here. With particular relevance to the question of possible oncogenic viruses, however, thorough initial examination and subsequent periodic spot checks of the cultured cells should be made (i) for the presence of virus-like particles by electron microscopy, (ii) for infectivity and oncogenicity of their extracted nucleic acids by tests in appropriate cell systems and animals, and (iii) for oncogenicity when transplanted into suitable hosts. It should be emphasized that the manifestation of these phenomena by cells does not necessarily signify that a virus oncogenic for man would be present in a cell-free viral vaccine propagated in such cells. Pending favorable developments in our knowledge of the significance of these phenomena, however, it seems wise at present to avoid the use of such cells for the general preparation of live attenuated virus vaccines. This requirement might be modified or waived for the preparation of inactivated virus vaccines and for investigational purposes.

In laboratories where numerous cell types are maintained under continuous cultivation, unintentional and undetected mixing is a common error. Consequently, we recommend that only one type of cell culture be permitted in a given production facility. As we have indicated previously, certified seed stocks should be accumulated and stored in the frozen state, and rigorously monitored.

Laboratory tests of pilot production. The general methodology and criteria of laboratory testing of vaccines for potency, purity, and safety are well defined and will not be discussed here. We do recommend that viral preparations for human use be processed by filtration or other appropriate means to eliminate intact cells, and cell particles larger than 0.5 to 1.0 micron in diameter. In addition, vaccines made from viral materials propagated in continuously cultured cells should meet all available laboratory criteria of nononcogenicity; for example, they should produce no tumors when injected into appropriate animals by appropriate routes in large doses, and they should induce no malignant transformations when inoculated into cultures of suitable cells.

Progressive controlled trials in humans. After all appropriate criteria have been applied to select a given continuously cultured cell system for the preparation of a cell-free viral vaccine and after it has been adequately demonstrated by laboratory tests or equivalent measures with experimental batches that the vaccine meets specifications of potency and safety for use in man, steps leading to large-scale human immunization may be taken.

Initially, one or two adult volunteers should be inoculated with what is expected to be a standard dose of vaccine given by the route which is expected to be used. These volunteers should be under careful observation, preferably institutionalized, for a period of several weeks. Their immune response should be determined. If the cell system used to prepare the vaccine is of human origin then the surveillance period should continue for at least six months in order to be assured that the cell system has not harbored one of the human hepatitis viruses or some other covert agent which may have a long incubation period.

After the accumulated information has indicated that the vaccine has produced no untoward effects in the original volunteers and that the immune response was satisfactory, the number of recipients can be progressively expanded. A second group of 6 to 12 volunteers likewise should receive adequate clinical surveillance for at least several months. The next group, of 25 to 100 persons, should include at least some in the age group for which the vaccine is intended, and all should be observed with similar care. If all has gone well, the fourth step might consist of a field trial of the vaccine involving several thousand persons. If the results continue to be favorable, the next step might be a larger-scale field trial as a prerequisite to approval for general production and use.

Suggested Areas of Research Emphasis

Present knowledge does not suffice to answer unequivocally the focal question of whether or not viruses oncogenic for man occur in cell-free viral vaccines prepared from viruses propagated in continuously cultured cells, particularly in those that have developed characteristics suggestive of malignant change. Though crucial, this question is nevertheless only part of the paramount problem of determining the causes, mechanisms, and significance of the transformation of the normal cell into the malignant cell. The immediate question is whether or not viral activity is associated with this transformation in circumstances where no virus has as yet been demonstrated, that is, in the development of "spontaneous" tumors and in the development of oncogenic potential in cells under continuous cultivation.

Because of the inherent limitations of experimentation in man and because of the necessity to allow for an extended latent period, it seems at present unlikely that an explicit answer for human beings can be obtained within a reasonable time. However, the same question can and should be investigated intensively in readily available animal systems by (i) using genetically heterogeneous as well as inbred strains of animals; (ii) testing cells cultured from both normal and neoplastic tissues, at various intervals after primary explantation; (iii) testing for oncogenic or other viral activity associated with the cultured cells (iii*a*) in cell-free culture supernatants, (iii*b*) in cell-free extracts of the cells (iii*c*) in cell-free preparations of nucleic acids extracted from the cells, (iii*d*) in cell-free preparations of ordinarily nononcogenic viruses propagated in the cells; and (iv) using a variety of indicator systems, such as cells in culture, isologous and heterologous strains of the donor animal, alien species, newborn animals, irradiated animals, and cortisone-treated animals.

Concurrently, basic and developmental research on all aspects of cell culture should be augmented. Particular attention should be directed to the determination of more favorable environmental conditions and the development of improved chemically defined media that would support continuous culture of cells from primary explants, with little or no alteration of intrinsic cellu-

lar characteristics. Closely related is the urgent need for better understanding and control of factors responsible for the "spontaneous" transformation, in long-term cultures, of normal cells into cells having oncogenic capacities, manifested on transplantation. Techniques for large-scale cultivation of cells need expedited research and development, with particular attention to such goals as growth without antibiotics, sterilization of culture media to inactivate adventitious viruses without degradation of the media, and physical conditions favoring improved growth and virus production.

Pending developments in the aforementioned areas, pertinent problems of viral growth and immunochemistry should be attacked vigorously. Particularly necessary in relation to viral oncogenesis is a better understanding of viral latency in host cells, and of the factors that induce proliferation of a complete virus from a latent system.

Since many of the potential hazards discussed would be greatly reduced or even negligible if inactivated viruses were used, the question should be examined thoroughly in each instance as to whether the comparative safety of an inactivated virulent virus vaccine is more advantageous than the potentially superior immunizing capacity of a live attenuated virus vaccine. To this end, energetic search should be made for improved methods of inactivating viruses without impairing their antigenicity. To the same end, investigation of the molecular structure and immunochemistry of viruses should be emphasized, with the aim of producing potent highly purified noninfective viral antigens. Conceivably, viral proteins free of nucleic acid could be made available as essential immunizing antigens. Accordingly, refinements in the isolation and biological testing of viral proteins and nucleic acids are needed.

Summary

1) Continuously cultured tissue cells afford numerous potential advantages for the propagation of viruses to be used in vaccines.

2) Because continuously cultured tissue cells sooner or later become capable of growing into neoplasms when transplanted into a suitable host, every possible precaution should be taken to ensure that viral vaccines grown in cell cultures are free from living cells and cell particles larger than 0.5 to 1.0 micron.

3) The radical abnormalities that occur in cell lines derived from neoplasms and those that develop sooner or later in cell lines derived from normal tissue cannot be ignored. However, no evidence has been recorded (i) that untoward consequences follow administration of cell-free preparations from such cultures to humans or (ii) that oncogenic or other viral activity is associated with the ability of cells of these lines to grow into neoplasms when transplanted into a suitable host. It seems very unlikely, nevertheless, that acceptance could be won at present for the general use of a live-virus vaccine prepared from a virus grown in cells showing evidences of malignancy. This conclusion is based more on psychological and public relations considerations than on the available scientific information, which, however, needs considerable augmentation. In this connection, careful consideration should be given to the question whether the absence of the cited kinds of abnormalities from a continuously cultured cell system is a sufficient indicator of freedom from oncogenic potential. In the absence of unfavorable data, we judge that present knowledge does not preclude judicious extension of clinical trials, in volunteers, of appropriately filtered and otherwise controlled experimental live-virus vaccines grown in carefully selected continuously cultured cell systems. Only in this way can sufficient data be collected, and adequate criteria be developed, to define eventually the conditions for acceptability of such preparations for general administration to humans.

4) Every possible effort should be devoted to the development of non-oncogenic and otherwise acceptable cell lines from normal tissues for use in viral vaccine production. It is suggested that exploratory studies begin with continuously cultured mixed-cell populations in the diploid state and stabilized cloned cultures. Criteria for the selection and monitoring of cell lines, and progressive steps leading to large-scale application are outlined.

5) If the need for immunization against a particular viral disease should be deemed sufficiently urgent, and if no practicable alternative were available, serious consideration might be given to a vaccine prepared by inactivating the virus, grown in such a selected stabilized cell line as HeLa or human skin epithelium. The conditions of preparation would have to be such as would inactivate the most resistant known viruses and infective nucleic acids with a generous margin of safety.

6) Principal areas needing intensified research emphasis are indicated.

Note

The authors were appointed by the Director, National Institutes of Health (NIH), in January 1962, as a Task Force on Tissue Culture Viruses and Vaccines, to review current knowledge of problems and trends relating to the use of continuously cultivated cells in the production of viruses to be used in manufacturing vaccines and similar preparations, and to identify areas requiring additional research emphasis. This report was submitted to the Director, NIH, on 4 September 1962. Since it is necessarily a compendium of generally known information, personal communications of unpublished data, and individual judgments based on specialized knowledge, the customary bibliography was deemed to be impracticable. After completion of this report, an independent discussion of some of the same problems was made available to us as a Report (MHO/PA/140.62) by the Scientific Group on the Human Diploid Cell, to the Director General, World Health Organization, Geneva.