Transformation of Cells in vitro by Viruses

Investigation of such transformations provides an experimental approach to the study of cancer.

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Transformed cells differ from normal cells in a number of other properties. Paramount among them is the ability of many kinds of transformed cells to grow like cancer cells when injected into an animal of isologous origin. Thus, transformation and cancerization often, but not always, occur simultaneously. It is likely that when the two changes do not occur together this is because of complicating circumstances. A common complication derives from the presence of a new surface antigen in virus-transformed cells. I shall not take time to discuss the nature of this antigen here and merely point out that it causes an immunological rejection of the cells in the isologous host; sometimes the cells grow in x-rayed animals, where the immunological responsiveness has been lowered (*3*).

Normal cells can be transformed into cancer cells experimentally by a variety of agents which are diverse in nature and properties. For instance cancer can be induced by radiations, which are known to alter the cellular DNA; by chemicals which strongly bind to proteins; by hormones; by viruses; or even by completely inert substances, such as plastic films inserted under the skin. The diversity of these agents suggests that they induce cancer by altering, through different pathways, the same cellular function.

Viruses are interesting objects for an experimental study of cancer because many aspects of their interaction with the cells are fairly well understood. Furthermore, viruses are the only agents with which it has been possible so far to induce cancer in cultures of dispersed cells maintained in vitro, an obvious advantage from a methodological point of view. This article is mainly concerned with the effects of cancer-producing viruses on cells cultivated in vitro; some relevant results obtained in the animal are also discussed.

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Transformation

When normal cells are infected by a cancer-producing virus, some of them undergo, in one or more steps, a characteristic change called transformation (*1*). A similar—so-called spontaneous—transformation can occur, in the absence of virus infection, in cell cultures kept under growing conditions in vitro for a long time (*2*, *3*).

An understanding of the nature of the changes involved in the transformation can be gained from comparative in vitro studies of normal cells and of cells which have reached an advanced stage of transformation. For these studies the cells are grown in glass or plastic dishes in contact with the solid substrate and immersed in a liquid nutrient medium. Under these conditions normal cells grow rapidly until the solid substrate is covered by a continuous layer of cells, often of the thickness of a single cell (monolayer); then growth stops. It is evident that the cessation of growth is not caused by exhaustion of the nutrients in the medium, since growth does not begin again if more nutrients are added. Growth reoccurs if the cells are detached and transferred in more diluted concentration to a new dish, where they are not in contact with each other; or, simply, if part of the monolayer is mechanically removed. Therefore, it appears that cessation of growth is caused by regulatory mechanisms which are sensitive to the establishment of reciprocal contacts between cells.

The cell surface appears to be the sensor that detects the contact. This is suggested by the phenomenon of contact inhibition (*4*), revealed by microcinematographic studies of cultures of normal cells. As shown by these studies, the cells move around and their contours have a continuous undulating motion. The undulating motion is immediately arrested when the margin of one cell touches another cell. The exact relationship between contact inhibition—that is, cessation of movement on contact—and cessation of growth when the monolayer is formed is not known; however, there is reason to believe that the two phenomena are intimately interrelated. These phenomena demonstrate the existence of regulatory mechanisms based on cell-to-cell contact.

The behavior of transformed cells under the same conditions is totally different. Such cells continue to grow after they have constituted a monolayer, and they form thick multilayered sheets. In microcinematographs they show little or no contact inhibition. Therefore, in the transformed cells, either there is no regulation of cell multiplication and of cell movement or the regulatory mechanism is ineffective.

Transformed cells differ from normal cells in a number of other properties. Paramount among them is the ability of many kinds of transformed cells to grow like cancer cells when injected into an animal of isologous origin. Thus, transformation and cancerization often, but not always, occur simultaneously. It is likely that when the two changes do not occur together this is because of complicating circumstances. A common complication derives from the presence of a new surface antigen in virus-transformed cells. I shall not take time to discuss the nature of this antigen here and merely point out that it causes an immunological rejection of the cells in the isologous host, sometimes the cells grow in x-rayed animals, where the immunological responsiveness has been lowered (*3*).

The decreased response to regulatory influences that is characteristic of

transformed cells has made it possible to develop methods for assaying the transforming titer of virus preparations. In fact, in a monolayer culture the transformed cells that arise after virus infection grow to form easily recognizable colonies, called foci. Statistical considerations show that a focus is produced by a single virus particle (*5, 6*); thus, the number of foci is a measure of the transforming titer of the virus.

Charactertistics of Cancer-Producing Viruses

There are two main types of cancer-producing viruses, differing profoundly in composition, structure, and biological properties. The viruses of one group contain RNA as the genetic material, the viruses of the other group contain DNA. The RNA-containing viruses produce leukemias and solid tumors in chickens and rodents. They have a complex structure that also characterizes certain viruses, such as the influenza virus, which do not produce cancer. In these RNA-containing viruses the RNA is surrounded by protein units arranged in the form of a helix, which is folded and included in a membranous, lipid-containing envelope. The molecular weight of the RNA in each particle is about 10^7 (see *7*) for the viruses of the chicken leukosis group. Thus, every virus particle may contain about 50 genes, many more than are required for governing the synthesis of the component proteins of either the envelope or the helix of the virus. This shows that these viruses perform many unknown functions in the infected cells. In all viruses of this structure the outer envelope is synthesized as a part of a normal cellular membrane, usually the cell surface. When the virus is released from the cell, the viral envelope is pinched off and separated from the cell membrane without destroying its continuity. Thus, progeny virus can be released without killing the cell.

The DNA-containing viruses produce solid tumors, mainly in rodents; one of them produces warts in man. These viruses are made up of an outer icosahedral shell of protein units, which surround the packed DNA; they have no membranous envelope. For the smallest of these viruses the molecular weight of the DNA of one particle is 3.5×10^6 (see *8*); thus, DNA-containing viruses also have several genes and functions of unknown nature. The DNA of polyoma virus, which belongs to this group, has a ring structure (*9*), a property whose significance I discuss later. These viruses multiply in the nucleus of the cell and are released when the cell lyses; therefore, release of progeny virus appears to be incompatible with survival of the cell. When a cell population is exposed to a DNA-containing tumor-producing virus, some cells die and produce virus, others are transformed. The proportions of cells that show one or the other type of response differ greatly in different cell systems. For instance, for polyoma virus, in cultures of mouse embryo cells the lytic titer is about a million times higher than the transforming titer, but in cultures of cells derived from hamster or rat the two titers may be comparable; under some conditions there may be no lytic response of the cells (*6*). The large variability in the ratio of the two titers in different cell systems is caused by variability in the proportion of cells that show a lytic response; the proportion of cells that undergo transformation is, on the contrary, much less variable.

Characteristics of the Transformed Cells

An interesting generalization can be made about the state of differentiation of the transformed cells in relation to the type of the transforming virus. Cells transformed by DNA-containing viruses in the last stage of transformation are in a low state of differentiation and sometimes essentially undifferentiated. For instance, the cells which arise in vitro after transformation by polyoma virus or SV40 virus are usually fusiform or star shaped; the cells present in the tumors induced in hamsters by these viruses, as well as by adenovirus, are similar in shape. On the other hand, cells transformed by RNA-containing viruses are in most instances highly differentiated (*10*). For example, the transformed cells produced by viruses of the chicken leukosis group include many different types of blood cells, antibody-forming cells, and cells of various tissues, as in kidney tumors. An important exception are cells transformed by the Rous sarcoma virus, which are not differentiated but are either fusiform or round.

The relationship of virus type to the state of differentiation of the transformed cells reflects the morphogenetic power, either positive or negative, of the virus, since the state of differentiation changes *after* the cells are infected by the virus. For example, the differentiation of myeloblasts from embryonic cells infected by the myeloblastosis virus in vitro is well documented (*11*). Equally clear is the loss of differentiation of the polygonal, pigmented cells of the iris epithelium infected by the Rous sarcoma virus (*12*); they become transformed into pigment-free elongated or round cells. There is clear evidence in the latter case that the loss of differentiation is due to the morphogenetic action of the virus, since one mutant type of the virus causes the formation of round cells and another mutant type causes the formation of fusiform cells (*13*).

Significance of Differentiation

The significance of the relationship between transformation and differentiation can be evaluated by examining some current ideas about differentiation. During the development of an organism from the fertilized egg, more and more specialized cells appear as a function of the time after fertilization. The specialization of the cells is caused by specific molecules, essentially proteins, which are synthesized in the cells on specification of certain structural genes. Thus, differentiation is the expression, at a certain biological time, of structural genes which were previously inactive.

The time-dependent expression of gene function is the consequence of the operation of regulatory mechanisms. The operation of regulatory mechanisms affecting gene expression is now well understood, on the basis of studies carried out in bacteria (*14*). In these organisms regulation occurs through the synthesis of specific intracellular repressors, which are made by regulator genes and which block the expression of the structural genes. Only when a repressor is not made can the corresponding structural gene be expressed. In bacteria, the production, or interruption of the production, of repressors is under the control of specific controller substances, of small molecular weight and of many kinds, which come from the outside.

The expression of structural genes in animal cells is believed to be also reg-

ulated by specific intracellular repressors. However, in these cells the mechanism for the control of repression may differ in an important way from the mechanism in bacteria. The controller substances for a limited number of functions of animal cells have been found to be of small molecular weight and diverse nature, like those of bacteria. But for many other functions, and perhaps the most significant ones for differentiation, the controller substances are fairly large molecules, belonging to a few classes. Among them we find hormones, of which many are steroids and some are proteins, we find growth factors, such as the protein which controls the growth and survival of certain cells of the nervous system (15); we find the protein antigens, which elicit a specific antibody response.

The site of action of these controlling substances is not known. An interesting possibility is that they act on the cell surface. In all instances the cell surface is responsible for selecting and accumulating molecules and ions in the cell, through the action of specific permeases. Controller substances could act by altering in a specific way the permeability of the cell surface and by thus altering the regulator genes through changes in the composition of the intracellular milieu. Cell-to-cell contacts could have a regulatory influence of similar nature.

Another important aspect of differentiation is the fact that the controlling substances can induce a certain type of differentiation only by acting on cells which have already reached another, well-determined, stage of differentiation. Thus, differentiation is a sequential process which occurs according to instructions that are coded in the genetic material of the cells.

In view of these considerations the ability of most RNA-containing viruses to cause transformation of cells to cancer cells and, at the same time, to induce a well-defined stage of differentiation is a remarkable event; it shows that these viruses alter the programming of the instructions coded in the genetic material of the cells without disrupting the coordination of the cells. Thus, these viruses must cause a specific and functionally well circumscribed change in the cell. On the other hand, the production of undifferentiated cancers by DNA-containing viruses and by the Rous sarcoma virus implies that these viruses cause more profound, or less circumscribed, functional disturbances.

Mechanism of Action of Cancer-Producing Viruses

RNA-containing viruses. In cells transformed by RNA-containing viruses, the viral RNA is present all the time. In most cases this is shown by the continuing production of progeny of the transforming virus. Cells transformed by the Rous sarcoma virus are exceptional in this respect, since under certain conditions they totally fail to produce infectious virus progeny. This is because the Rous sarcoma virus is defective: by itself it is unable to give rise to infectious virus because of failure to synthesize some component of the viral envelope (*16*). The presence of the viral RNA in the transformed cells can, however, be demonstrated by infecting them with a related "helper" virus, which supplies the missing component and thus permits synthesis of infectious Rous sarcoma virus particles (*16, 17*).

The state of the viral RNA in the transformed cells is not known. Since at every cell division many RNA copies are made, the RNA must multiply at a higher rate than the cells, and therefore autonomously. On this basis it is tempting to conclude that the association between the viral RNA and the cell is determined statistically. If this were the case, the transformed cells should occasionally segregate cells that did not contain the viral RNA. In many cases the existence of such segregants would be difficult to demonstrate because the cells could be reinfected by virus produced by other cells; however, they could be detected with certainty among the progeny of cells transformed by the Rous sarcoma virus in the absence of helper virus. These cells cannot become secondarily infected because infectious Rous sarcoma virus is not produced in the cultures. Under these conditions, no virus-free segregants have been observed (*18*). Since these investigations have been of limited scope, the result does not invalidate the statistical hypothesis. It does, however, raise the question of the possible existence of a more stable association of the viral RNA with the cells.

Owing to the continued presence and reproduction of the viral RNA, viral components are continuously produced in cells infected by RNA-containing viruses. Some of these components become incorporated in the cell surface; we know that this includes not only constituents of the viral envelope but other viral components as well, since cells transformed by the Rous sarcoma virus have a new antigen in their surface, irrespective of the presence of a helper virus (*19*). These modifications of the cell surface are likely to affect its function; in view of the significance of the surface for the state of regulation of the cell, it seems likely that both the growth and the state of differentiation of the cell may be affected.

It is possible, therefore, to attribute the cancer-producing action of certain RNA-containing viruses, at least in part, to the special constitution of the components that these viruses introduce in the surface of the cell.

Two characteristics of the differentiation which takes place in cells transformed by these RNA-containing viruses can be explained along these lines. (i) The state of differentiation induced by the virus corresponds to the normal developmental capabilities of the cells which are infected. For instance, with the myeloblastosis virus, myeloblasts are produced in vitro from embryonic cells which normally give rise, in later development, to cells of the granulocitic hemopoietic series; plasma cells are induced from cells of the bursa of Fabricius, which are their regular precursors (*20*); also the development from the kidney of tumors composed of many tissues can be attributed to latent differentiative properties of some cells of the kidney (*21*). Thus, the differentiation induced by the virus is abnormal only because it takes place in the absence of the normal controlling substances. This result could be explained by assuming that a cell which has viral components in its surface can, under certain conditions, recognize as specific controllers of differentiation substances which normally do not have that function. (ii) The type of virus-induced differentiation is dependent to some extent on the type of virus. This influence of the virus can be explained by assuming that the incorporation in the cell surface of viral components of different structure gives rise to different types of functional alterations.

The production of undifferentiated cells by the Rous sarcoma virus may depend on the defectiveness of the virus. It is conceivable that components of this virus cannot be incorporated regularly in the cell surface; they may therefore give rise to unusually large functional changes. These would destroy the fine balance required

for maintaining a state of advanced differentiation.

DNA-containing viruses. The DNA-containing viruses cause transformation of the cells in two or more steps; in each step the transformed cells have different characteristics (the succession of steps is called a progression). In the initial step the cells retain many of the properties they had before infection, but they are partially released from the regulatory constraints which limit growth; they are not yet cancerous. In the second step they acquire cancerous properties. An example of this progression is the formation of papillomas in the skin of rabbits infected with the Shope papilloma virus. The papillomas are benign warts made up of a basal and a keratinized layer similar to those of normal epidermis, but thicker. The cells of the papillomas show some abnormalities, such as a new virus-induced antigen (*22*) and a new arginase (*23*). Cancers arise from most of these papillomas after a sufficient length of time.

The transformation that follows infection with DNA-containing viruses has been followed in a more direct way by studying the transformation of hamster embryo cells by polyoma virus in vitro (*24*). These studies show that in the first step, which immediately follows the infection, the transformed cells have increased growth ability but are not greatly altered in morphology. It is evident that these cells do not have cancerous properties, since they do not grow as cancer cells in the animals. The cancer cells arise later, after a number of cell generations. It is interesting to note that many chromatid breaks occur in the cells during this intervening period, and that the cancer cells often show alterations in the structure or number of their chromosomes. From the association of these phenomena it appears likely that the chromosomal alterations are the consequences of the chromatid breaks. There may be chromosomal alterations in all the transformed cells which reach the final, cancerous stage, although in some cases they may not be noticeable.

Thus, it seems that with the DNA-containing viruses the formation of cancer cells is a process in which structural alterations of the cellular DNA take place, perhaps through the activity of virus-induced enzymes. It is understandable that the permanent and often gross alterations of the genetic material of the cells in the later step of transformation disarrange the coordination of the cellular genes, and that these cells are undifferentiated.

An important problem concerning the transformation by DNA-containing viruses is the relationship of the transformed cells to the virus that causes the transformation. The transformed cells in later steps usually do not contain the transforming virus in infectious form, nor do they produce it after infection with a helper virus (*25*). In some cases, however, synthesis of infectious virus occurs in a small proportion of the cells, as in cultures of cells transformed by SV40 virus (*26*), or in some rabbit cancers which derive from virus-induced papillomas (*27*). This finding supports the hypothesis that the genome of the transforming virus is present in a repressed state in all the cells transformed by DNA-containing viruses; the state of repression would, however, vary for different viruses, being complete for polyoma virus and incomplete for SV40 or papilloma virus. In all cases, however, some genes of the virus must escape repression, since the transformed cells show novel properties, such as viral-specific antigens (*28*)—not necessarily identical to those of the virus particle—and, in some cases, the persistent occurrence of chromatid breaks (*24*). Thus, the transformation caused by these viruses bears a strong resemblance to the phenomenon of lysogeny which occurs in bacteria infected by temperate bacteriophages.

In an effort to define more precisely the role of the virus in transformation, attempts to directly detect the viral DNA in the transformed cells and possibly to count the number of molecules per cell have been initiated (*29*). This was done by studying the hybridization of the DNA of the transformed cells with a specific RNA synthesized from polyoma DNA in vitro. The results of the experiments carried out so far show that there are less than five viral genomes per cell; it may turn out that there is only one, or that there are very few, per cell, as in lysogenic bacteria.

Circularity of the DNA

The DNA of polyoma virus has a circular configuration, as already mentioned. The DNA of the papilloma virus and of related viruses has peculiarities of sedimentation (*30*) which may also derive from circularization. This raises the question of the possible significance of circularity in the process of integration of the viral DNA in the cell. This question is also raised by two results of considerable interest recently obtained with bacteriophages. One result is the demonstration that the DNA of bacteriophage lambda, which can become integrated in the cells, circularizes (*31*), whereas the DNAs of several lytic bacteriophages do not; this finding suggests that circularization may be important for integration. The other result is the formation of a circular, double-stranded DNA in bacteria infected by a bacteriophage which contains a single-stranded DNA (*32*). The interest of the latter observation is enhanced by the special properties of this double-stranded DNA: it does not participate in the multiplication of the viral DNA but, presumably, gives rise to the synthesis of the RNA, which is the primary product of the viral genes.

These observations suggest that circular, double-stranded DNA has important specific properties: stability in the cells, failure to multiply, as such, and functionality. Stability and failure to reproduce may be the consequence of the lack of free ends, which are essential for the action of many enzymes acting on DNA.

Thus, the circularity of the DNA of tumor-producing viruses may be a requirement for its integration in the cells. It is conceivable that a molecule of DNA which remains circular can survive unchanged until integration occurs and, at the same time, cause the synthesis of the gene products essential for its integration. The integration itself would then occur when the DNA molecule breaks at one point and immediately becomes incorporated in the cellular DNA. A process of this nature has been proposed for the integration of the DNA of bacteriophage lambda (*33*). In contrast, linear DNA molecules could either multiply autonomously and kill the cells or be destroyed by cellular enzymes.

Frequency of Transformation

For both the RNA-containing and the DNA-containing viruses the efficiency of transformation, expressed as the ratio of the number of transformed cells to the number of infecting virus particles, is usually very low, of the order of 10^{-5} or less. An exception is the Rous sarcoma virus, which has a much higher efficiency. The gener-

ally low probability of transformation of cells infected by RNA-containing viruses shows that the incorporation of viral components in the cell surface is not sufficient to cause the transformation; some other event of rare occurrence must be superimposed. The nature of this event is not known. One can speculate that it is a mutation either of the virus or of the cell. Examples of a mutation of the first type would be mutations which make the virus defective but not as defective as the Rous sarcoma virus. No such mutations have as yet been observed; this may be because they are difficult to observe, since the transformed cells would be infected with the nonmutated virus as well. Examples of a mutation of the second type would be mutations which change the structure of a normal surface component of the cell; the changed surface, in conjunction with the viral component, could then cause the alterations of function required for transformation. The low frequency of transformation with DNA-containing viruses can be attributed to the low probability that the virus will establish a noncytocidal complex with the cell and enter into the state of integration required for transformation.

Conclusions

As the foregoing discussion shows, the problem of viral carcinogenesis is a very complex one. Considerable progress has, however, been made in several areas. The development of systems by which the transformation of normal cells to cancer cells can be studied in vitro, at the level of the individual cells, constitutes an important accomplishment from the point of view of methodology. As for the transformation process itself, the recognition that transformation affects in a characteristic way the state of differentiation of the cells may be of considerable significance. This phenomenon suggests that transformation involves cellular systems which are responsible for differentiation; this in turn permits the formulation of hypotheses, such as that in which the cell surface is considered the link between the two processes. Even if the link is a different one, the evidence that a link exists facilitates the study of the site of action of tumor-producing viruses, especially of those containing RNA.

Another point of considerable interest is the special position among RNA-containing viruses of the Rous sarcoma virus, in view of its defectiveness. Defectiveness may have a special role in transformation, either directly, because of the lack of some viral function, or indirectly, because of enhancement of other functions not affected by the defect. Thus, the study of transformation caused by the Rous sarcoma virus may also lead to a better understanding of the coordination of the viral functions among themselves and with the cellular functions.

Significant evidence has now been obtained of a similarity between the state of the viral DNA in cells transformed by DNA-containing viruses and the state of prophage in lysogenic bacteria. This evidence brings support to a theory which has been repeatedly advanced on purely theoretical grounds to explain viral carcinogenesis. Some characteristics of the cell transformation brought about by DNA-containing viruses suggest that some of the viral genes present in the cells continue to function whereas others do not. The problem of the regulation of these genes thus arises. In cells transformed by RNA-containing virus, the state of the viral RNA remains unknown; the main question here is whether the viral RNA can enter into some kind of integrated state in the cell.

Finally, it should be pointed out that a major task faces the investigators interested in the mechanisms of viral carcinogenesis: that of assigning functions to the many genes present in tumor-producing viruses containing either DNA or RNA. Undoubtedly some of these functions have a direct bearing on the mechanism of transformation. The identification of these functions is important, not only for solving the problem of the mechanism of viral carcinogensis but in a more general way. It seems probable that this knowledge would also improve our understanding of phenomena involved in regulating cell growth and function.

References

1. M. Vogt and R. Dulbecco, *Proc. Natl. Acad. Sci. U.S.* **46**, 365 (1960); H. M. Shein and J. F. Enders, *ibid.* **48**, 1164 (1962).
2. K. K. Sanford, W. R. Earle, E. Shelton, E. L. Schilling, E. M. Duchesne, G. D. Likely, M. M. Becker, *J. Natl. Cancer Inst.* **11**, 351 (1950).
3. R. Weisberg, thesis, California Institute of Technology (1963).
4. M. Abercrombie, *Cold Spring Harbor Symp. Quant. Biol.* **27**, 427 (1962).
5. H. M. Temin and H. Rubin, *Virology* **6**, 669 (1958); M. Stoker and P. Abel, *Cold Spring Harbor Symp. Quant. Biol.* **27**, 375 (1962).
6. K. Bayreuther, personal communication.
7. L. V. Crawford and E. M. Crawford, *Virology* **13**, 227 (1961).
8. R. Weil and J. Vinograd, *Proc. Natl. Acad. Sci. U.S.*, in press.
9. R. Dulbecco and M. Vogt, *ibid.*, in press.
10. U. Heine, G. de The, H. Ishiguro, J. R. Sommer, D. Beard, J. W. Beard, *J. Natl. Cancer Inst.* **29**, 41 (1962).
11. M. Baluda, *Cold Spring Harbor Symp. Quant. Biol.* **27**, 415 (1962).
12. B. Ephrussi and H. M. Temin, *Virology* **11**, 547 (1960).
13. H. M. Temin, *ibid.* **10**, 182 (1960).
14. F. Jacob and J. Monod, *J. Mol. Biol.* **3**, 318 (1961).
15. R. Levi-Montalcini and S. Cohen, *Proc. Natl. Acad. Sci. U.S.* **42**, 695 (1956).
16. H. Hanafusa, T. Hanafusa, H. Rubin, *ibid.* **49**, 572 (1963).
17. H. M. Temin, *Cold Spring Harbor Symp. Quant. Biol.* **27**, 407 (1962).
18. H. Rubin, personal communication.
19. ———, *Cold Spring Harbor Symp. Quant. Biol.* **27**, 441 (1962).
20. M. A. Baluda, I. E. Goetz, S. Ohno, in *Proceedings M. D. Anderson Hospital Symposium, 1963*, in press.
21. J. G. Carr, *Brit. J. Cancer* **10**, 379 (1956).
22. C. A. Evans, R. S. Weiser, Y. Ito, *Cold Spring Harbor Symp. Quant. Biol.* **27**, 453 (1962).
23. S. Roger and M. Moore, *J. Exptl. Med.* **117**, 521 (1963).
24. M. Vogt and R. Dulbecco, *Proc. Natl. Acad. Sci. U.S.* **49**, 171 (1963).
25. ———, *Virology* **16**, 41 (1962).
26. A. B. Sabin and M. A. Koch, *Proc. Natl. Acad. Sci. U.S.* **49**, 304 (1963).
27. P. Rous, J. G. Kidd, W. E. Smith, *J. Exptl. Med.* **96**, 159 (1952).
28. H. O. Sjogren, I. Hellstrom, G. Klein, *Cancer Res.* **21**, 329 (1961); K. Habel, *J. Exptl. Med.* **115**, 181 (1962); R. J. Huebner, W. P. Rowe, H. C. Turner, W. T. Lane, *Proc. Natl. Acad. Sci. U.S.*, in press.
29. E. Winocour and R. Dulbecco, in preparation.
30. J. D. Watson and J. W. Littlefield, *J. Mol. Biol.* **2**, 161 (1960).
31. A. D. Hershey, E. Burgi, L. Ingraham, *Proc. Natl. Acad. Sci. U.S.* **49**, 748 (1963).
32. R. L. Sinsheimer, personal communication.
33. A. M. Campbell, *Virology* **20**, 344 (1963).