The discovery of the polyoma virus (80) provides the strongest incentive yet for continued serious efforts to find a human cancer virus. According to previous experience in the field of animal viruses, the polyoma virus should prove to be one member of a group of related viruses with different species specificities, causing similar diseases. When attempts to isolate human viruses have failed, we have reason to question the suitability of the materials and methods used. This attitude served as a basis for the present discussion of virus detection by biochemical and biological means. An attempt was made to envisage situations in which a virus would not be readily detected and to analyze the reasons for this, rather than summing up the various current techniques for virus detection and their limitations. For this purpose the discussion was grouped under three headings, corresponding to the known stages in the developmental cycle of viruses: provirus, vegetative virus, and mature virus.1

I. PROVIRUS

The evidence of the existence of a provirus stage is conclusive only for bacteriophages. In this case, however, prophage may be regarded as the "natural" form of the virus and lysogeny as a mechanism safeguarding the survival of both host and virus. For a complete review of the lysogeny problems reference is made to Lwoff (50). In this connection only some salient points will be summarized.

The fate of an infecting temperate phage seems to be determined at the moment of penetration or very soon thereafter. The factor deciding between the alternatives reduction to prophage or production of vegetative phage appears to be the metabolic state of the host cell. Reduction does not automatically lead to establishment of lysogeny; the prophage first has to become attached to and apparently interact with a specific intracellular site, to all appearances a specific chromosomal locus. By this interaction both the respective locus and the prophage seem to be stabilized.

The infection represented by the lysogenic state seems, in most cases, to remain completely silent, with no morphologic or metabolic characteristics to distinguish a lysogenic from an uninfected cell. The two important features revealing the true status of the lysogenic bacterium are (a) "immunity," i.e., refractoriness to lysis by related (incompatible) virulent phages, and (b) susceptibility to spontaneous or sometimes also to artificial "induction."

The phenomenon called immunity suggests that a specific chromosomal locus is also involved in infection with virulent phages. If a lysogenic bacterium is exposed to a related virulent phage, the latter is adsorbed and its nucleic acid injected. However, as long as the prophage blocks the specific locus, no multiplication of virulent phage takes place, although its nucleic acid may remain potentially active for considerable periods of time. If the prophage is activated by induction, synthesis of both the temperate and the virulent phage is initiated.

In the situation as here described the genetic information contained in the prophage DNA seems not to find any demonstrable expression. This is not always the case, however, the most notable example being the toxogenic C. diphtheriae. The capacity of toxin production is invariably associated with lysogeny, and lysogenization by the temperate phage from a toxogenic bacterium invariably confers toxigenic capacity to a nontoxogenic variant (26). Thus, if the bacterial gene determining toxigenicity is not identical with the prophage DNA it must be linked to it with an unusual firmness. It would seem a more plausible explanation that the same DNA molecule which in a "resting" state controls the synthesis of toxin, when activated is commanding synthesis of phage. A quantitative and qualitative analysis of the phage DNA might provide

1 The term mature virus, referring only to properties of the virus particle itself, is preferred to infective virus with its implication of host-virus interaction.
an answer to this question. As another example of “phenotypic” prophage manifestation the mucoid growth of certain lysogenic enteric bacteria may be mentioned.

Whether or not animal proviruses exist is a matter of discussion. The majority of the inapparent, persistent infections with animal viruses have been clearly shown to represent a carrier state as principally distinct from virogeny. In a tissue culture system a carrier state is established if the rate of growth of the cell population and the rate of spread of infection are in balance. This can be artificially achieved with susceptible cells and virulent virus by addition to the medium of neutralizing antibodies in the proper concentration, as first described by Ackermann and Kurtz (1). A similar equilibrium may exist in a relatively resistant cell population even without antibodies in the medium (15, 60). In a typical carrier culture only a fraction of the total cell population is infected at any given time. Persistence of infection depends upon extracellular transfer of virus as shown by the facts that the culture can be “cured” by addition to the medium of antibody in high concentrations as well as by cloning.

In some Rous virus-carrying tissue cultures each individual cell may actually be infected (83). However, since virus seems to be continuously released, the situation can hardly be analogous to lysogeny in bacteria. Most probably the intracellular virus is in a vegetative phase throughout, although the rate of virus synthesis is sufficiently low not to interfere significantly with the growth of the host cell. The same explanation seems to fit in the persistently infected, resistant cell line, described by Puck and Cieciura (60).

Persistent infections in the macro-organism are usually more difficult to analyze. Inapparent infections in higher animals, with one exception, seem to be associated with continuous production of antibodies, an indication that mature virus is released at least intermittently. The exception is LCM virus-carrying mice which, according to Traub (86), do not develop serologic immunity. As the infection in this case is initiated in utero, Burnet (14) suggested that immunologic tolerance might be involved, which indeed seems to be the case (34).

A slow or intermittent release of mature virus is by no means inconsistent with the assumption of virogeny. As a matter of fact the most characteristic property of a lysogenic culture is the continuous, spontaneous release of mature phage. Therefore, the demonstration in persistently infected tissue of small amounts of virus cannot serve to distinguish between carrier state and virogeny. If, on the other hand, systematic attempts consistently fail to demonstrate the presence of mature virus during clinically silent periods, the assumption of a true virogenic state gains in probability. For such reasons the recurring herpes simplex and the swine influenza virus in the intermediate lungworm host (72) appear to be likely candidates, even if positive evidence is lacking.

The only definitely established provirus, the prophage, is of the DNA type and somehow associated with the genome of the host cell. A priori there is no obvious reason not to expect a similar pattern of infection within other groups of viruses as well, and then primarily among intranuclear DNA agents, i.e., a category to which the herpes virus belongs. No RNA viruses have yet been found in a provirus stage, and the whole question must here be left wide open.

Detection of a provirus may be extremely difficult. The most promising approach should be attempts to induce formation of vegetative and mature virus, for instance, by application of ionizing irradiation or carcinogenic and mutagenic chemicals. Some prophages, however, have resisted all attempts at artificial induction. It is possible that systematic studies of such systems will reveal additional physical or chemical agents with inducing capacity. Prolonged cultivation in vitro to improve the chances of spontaneous induction should also be tried.

The only possibility to detect and identify a resting provirus would seem to be by chemical means. In all probability provirus consists of nucleic acid only and can hardly be expected to carry any immunological markers. Thus, the formidable task of the biochemist would be to single out and identify as foreign a nucleic acid that can be expected to appear in the pool in a ratio of one molecule per cell. This will obviously not be theoretically possible, unless the provirus has some unique constituent.

Until recently nucleic acids were supposed to contain no more than four bases: in DNA, adenine, guanine, cytosine, and thymine; in RNA the same set, except that uracil replaces thymine. The structural specificity is presumably accounted for entirely by the sequential order of the base pairs or bases in the chain. Virus nucleic acids generally seem to have no particular chemical characteristics setting them apart from those of the host cell. However, in 1953 Wyatt and Cohen (95) isolated from DNA of T-even phages a previously unknown base, 5-hydroxymethyl-
cytosine (HMC), so far not found anywhere else in nature. These phages contain no cytosine, which is thus completely replaced by HMC. As another unique feature glucose enters into the molecule, probably in glucosidic linkage with the methyl group of HMC (76, 85). It might also be mentioned that Tessman et al. (84, 85) recently reported an abnormally high irradiation sensitivity of the small phages S 13 and φ X 174, which was interpreted as an indication of a single-stranded DNA instead of the double helix of the Watson-Crick model (89).

In recent years new bases, primarily methylated purines and pyrimidines, have been identified as minor constituents of nucleic acids (3, 18, 48). The significance of these observations is still obscure; sometimes the appearance of such bases or an increase in their concentration is observed under abnormal metabolic conditions together with deviations from the base ratios required by the Watson-Crick theory. It would thus seem that the chemical composition of nucleic acids may be less monotonous than hitherto assumed, which means that chemical analysis might become a practicable means of identification of individual nucleic acids.

II. VEGETATIVE VIRUS

Methods devised for the detection of vegetative virus will assume particular practical importance in cases of slowly growing viruses where relatively small amounts of mature virus are released and extracellular virus is continually neutralized by specific antibodies or inactivated by other, nonspecific agents present in the tissues. This type of infection should be expected principally in species and tissues with a high degree of resistance to the infectious agent, which, in turn, would mean that demonstration of small amounts of mature virus by means of infection experiments would be almost impossible if no other more sensitive test systems were available.

A complete analysis of the vegetative phase should include both viral and nonviral substances produced in the course of the infection. To be considered are (a) virus nucleic acid or nucleoprotein, (b) one or more proteins forming integral parts of the mature virus particle, (c) specific byproducts of the virus synthesis, regularly appearing as "soluble antigens," etc., but apparently not incorporated in the virus particle, and, finally (d) substances of a nonspecific nature.

A. NUCLEIC ACIDS

Synthesis of virus DNA may take place in the nucleus as seems to be true of papilloma, herpes, adenoviruses, and several insect viruses, or in the cytoplasm. So far only two groups of cytoplasmic DNA viruses are recognized, the pox viruses and the capsular insect viruses. The members of these groups can be characterized as structurally complex viruses, probably more autonomous than others. Whether or not they should be classified as true viruses is debatable. As pointed out by Burnet (14) the replication pattern of the pox viruses resembles that of the psittacosis group. The fact that the fibroma-myxoma virus apparently belongs among the pox viruses indicates that cytoplasmic DNA viruses cannot be disregarded as possible tumor agents. However, the size and morphological peculiarities to be expected from viruses of this category makes it seem unlikely that they should remain unnoticed and escape identification even if attempts to demonstrate infectivity were unsuccessful. For this reason cytoplasmic DNA agents shall not be made the subject of further deliberations in the present connection.

The intranuclear replication of animal DNA viruses shows some features resembling those observed in the synthesis of the T-even phages: margination—"precipitation"—of the chromatin, appearance of a structureless "pool" of viral DNA, and subsequent "crystallization" with formation of spatially oriented virus "nuclei" (43, 44, 57). To this writer's knowledge no attempts have been made to clarify the processes leading to the breakdown of the chromosomes of the host cell. It would be of interest to know what chemical relations, if any, exist between such extremely radical alterations of the structure and the disturbances of the nuclear activity observed in malignant tumor cells.

Also, RNA virus replication seems to be possible both in the nucleus and in the cytoplasm. Intranuclear synthesis of fowl plague G-antigen (9) has been definitely established; electron micrographic evidence of formation of poliovirus "nuclei" in the nucleolar and perinucleolar region has been presented (64). The most convincing evidence to date of cytoplasmic virus RNA synthesis is the recent report of crystalline structures in the cytoplasm of cells infected with one insect virus (94) and Coxsackie B 5 virus (56), respectively.

The problem of detection by chemical means of vegetative virus nucleic acid is less formidable than the task of finding a provirus. At present, the success of such attempts will depend entirely upon the presence of specific constituents, primarily purines and pyrimidines, possibly sugars. The base ratios determined on the pooled nucleic acid of vegetative virus produced in the course of the infection. To be demonstrated in the cytoplasm of cells infected with one insect virus (94) and Coxsackie B 5 virus (56), respectively.

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acid extracted from a tissue represent, of course, averages. Supposedly such mixtures are made up of thousands of individual molecular species with quite possibly widely differing ratios. The virus particles, on the other hand, contain only one or very few molecules of nucleic acid, and the purified preparations, therefore, possess a chemical homogeneity entirely lacking in the first type of preparation. It is hardly justifiable to base conclusions concerning structural characteristics on a comparison of two such incongruous materials. For a further elucidation of this question methods will have to be developed by which a separation of individual nucleic acids in a mixture is feasible. It does not seem entirely Utopian to hope that structural specificity might be made to work for such purposes. Even methods by which the degree of homogeneity could be estimated without a complete separation of molecular species would be of great help.

It is usually assumed that the pure nucleic acid is devoid of antigenic activity, and immune sera prepared against whole virus are reported to have no neutralizing effect on the infectivity of phenol-extracted virus (30). However, in studies on poliovirus-infected cells with the fluorescent antibody technic Buckley (11) found a very faint but apparently specific fluorescence of the nucleolus and the perinucleolar region, i.e., that part of the nucleus which probably serves as the site of virus RNA synthesis. The nucleus remained otherwise unstained, while the cytoplasm was gradually filled with staining material. The significance of this observation is somewhat obscure. Two interpretations are possible: the faint fluorescence of the nucleolus may indicate either the presence of minute amounts of a specific protein or else that the RNA as such has a certain immunologic specificity. A further study of the phenomenon appears justifiable.

The vegetative phase corresponds largely to the “eclipse” in the cycle of infection, characterized by a loss of infectivity which was reported not to return until morphologically typical virus particles reappeared during the maturation phase. In view of the now established fact that the pure nucleic acid of many viruses is by itself infective, the current explanation of the eclipse phenomenon is hardly satisfactory. Virus nucleic acid is being synthesized throughout the whole period, and failure to demonstrate its presence in infection experiments can apparently not be attributed solely to the disintegrated state of the virus. As perhaps the most likely explanation of the failures it may be assumed that earlier methods used for extraction of nucleic acid from the cells were unsuitable. In particular, it should be essential during preparation to protect the nucleic acid from tissue nucleases in order to prevent inactivation.

There is some evidence to indicate that these assumptions might be correct. Wecker and Schäfer (91) isolated infectious EEE and WEE RNA from infected cells by treatment with cold phenol, whereas extraction of purified virus with the same technic yielded negative results. Wecker (90) later found that the virus protein was soluble in hot phenol and that treatment of the virus at temperatures of 40°–60° C. gave nucleic acids of an activity comparable to that previously found in RNA prepared from cells. He concluded that the cellular RNA most probably represented vegetative virus. Phenol treatment undoubtedly inactivates all tissue enzymes, which might account for the positive results.

There are thus good reasons to hope that detection of vegetative virus with biological methods will prove to have a more general applicability. The low yields of activity obtained with the phenol technic as it is now practiced are an obvious disadvantage. The reason may be partial inactivation in the process of extraction, or a low avidity of the pure nucleic acid for the cell, or both. However, improvements in the techniques of preparation as well as infectivity testing can probably be achieved. Eventually tests on pure nucleic acid rather than mature virus may prove to offer great advantages. It was recently reported (52, 58) that poliovirus RNA could cause infection in cells completely resistant to mature virus. Infection was followed by one cycle of virus replication only. Apparently the mature virus released from the first infected cells was incapable of initiating a second cycle. Similar results were obtained with phage when the receptor mechanism was sidetracked by simultaneous removal of the bacterial membrane (protoplast formation) and liberation of the phage DNA core by osmotic shock (77). Even if self-propagating infections could not be established in these experiments, the possibility by such means to widen the host range of a virus must be considered as a valuable addition to the diagnostic arsenal.

B. Virus Proteins

The chemical properties of a number of plant virus proteins have been studied in detail, including amino acid analyses and preliminary sequence determinations (46). In general the protein of the virus particle seems to be made up of a number of identical subunits linked together
by relatively weak secondary bonds. Dissociation and reassociation of the subunits of rod-shaped plant viruses are easily achieved, whereas spherical viruses are more resistant. The number of subunits may vary considerably, from about 20 to more than 2000 per virus particle, with molecular weights from 18,000 and upwards.

So far no exotic or unusual amino acids have been found in plant virus proteins. Although the amino acid composition is specific enough to distinguish between variants of the same virus, it does not show any peculiarities, setting virus proteins clearly apart from those of the host cell. Some enzyme-resistant proteins are supposed to have a hemicyclic structure without free N-terminal groups (25).

The highly specialized proteins of phages shall be mentioned only in passing. The anatomy of the bacterial cell calls for a particular functional differentiation of the virus particle which can hardly be expected to be copied in any animal viruses.

In comparison, information on animal virus proteins is meager. Among the best studied are the relatively complex myxoviruses. As shown by Hoyle (37) the virus particle can be disrupted by treatment with ether with release of two distinct types of subunits: hemagglutinin and group antigen. Schäfer and co-workers (9, 35, 66–68, 92), studying fowl plague virus, describe the hemagglutinin as a spherical particle, 30 mₜ in diameter, and composed of protein and polysaccharide. It carries the hemagglutinating, enzymic, and antigenic capacity (type specific V antigen) of the intact virus. The group antigen (S-antigen, or G-antigen in Schäfer’s terminology) appears in the shape of 12-mₜ particles made up of nucleoprotein. The hexagonal discs obtained by Valentine and Isaacs (57) by tryptic digestion of the virus represent presumably the intact nucleoprotein subunit. In the virus particle it is supposed to be arranged in one turn of a spiral; when liberated by enzyme treatment this structure collapses, forming the hexagonal discs; in the course of ether treatment it apparently breaks down into smaller fragments. Each virus particle is supposed to contain six hemagglutinin units in close packing, the nucleoprotein occupying the central space between them and lipides filling out the rest. The lipides are presumably derived mainly from the host cell surface.

So far the chemical characteristics of animal virus proteins have hardly been systematically studied. Ion exchange chromatography used for purification purposes has revealed certain interesting adsorption-elution patterns (36), which presumably must be attributed to the protein component. Present data do not permit any general conclusions and very little speculation beyond the fact that the technic itself looks promising.

The enzyme activity of certain virus proteins, notably those found in the myxovirus group, might appear to offer possibilities of detection and identification of such proteins by purely chemical methods. However, even after many years of systematic studies the chemistry of influenza virus enzyme-substrate reactions is not sufficiently clarified to make a chemical identification feasible. The highly specific affinities to be expected would also make a search for a substrate for an unknown virus an extremely hazardous undertaking. Less specific enzymes like the ATPase reported to enter into some fowl leukosis viruses (54) cannot be distinguished from normal tissue components and would thus have no diagnostic potentialities.

Therefore semibiologic methods—hemagglutination-elution, hemadsorption, and immunologic analysis—are the most valuable of those at present available. The capacity of certain viruses or purified proteins of agglutinating red cells is based on the presence in the cell surface of a receptor substance of sufficient specificity. As an equivalent of this phenomenon the in vitro reaction between purified influenza virus and purified receptor substance leads to formation of a precipitate (82). Poliovirus, with which no hemagglutination has been obtained, nevertheless seems to react specifically with a cellular lipoprotein (33), presumably lacking in erythrocytes. This observation, further supported by the apparently specific capacity of susceptible cells to adsorb virus (42), indicates that virus-receptor mechanisms may have to be expected in most virus-host cell systems. Detection by direct precipitation of virus and receptor substance requires a preparatory purification of both components which also have to be used in high concentrations. A possibility that might be worth trying, when naturally agglutinable cells cannot be found, is coating of native or tannic acid-treated red cells with receptor substance in an attempt to develop a more sensitive and less exacting test system. The receptors so far identified seem to belong in the class of surface lipoglycoprotein antigens.

The simplest and by far most sensitive method of detecting the protein component in the cell is with the aid of specific antibodies, particularly by means of Coons’s fluorescent antibody technic.
Applying specific antibodies against the G-antigen and the hemagglutinin, respectively, Schäfer and his group found that the former first appeared in the nucleus of cells infected with fowl plague virus, later probably migrating out into the cytoplasm. The synthesis of the hemagglutinin seemed to be initiated later than the G-antigen, and it was found only in the cytoplasm. The synthesis of several other virus proteins has been followed by the same technic (11, 49, 59, 93). Generally the amount of stainable material in the cells is remarkable. In the later stages of infection the entire cytoplasm appears fluorescent, an indication that much more specific protein is synthesized than is needed for incorporation in the comparatively small amount of mature virus that is eventually released (representing a fraction of about $10^{-5}$ of the cell mass).

C. Nonviral, Specific Substances

Some of the nonviral material produced in and released from infected cells might simply be regarded as components of the virus particle, synthesized in excess of what is finally incorporated into the mature virus. These substances differ in no respect from the counterparts that can be extracted from the virus particles. The intracellular myxovirus hemagglutinin and soluble antigens exemplify the principle.

Often, however, other nonviral substances appear, specific in the sense that they are foreign to the host cell and are synthesized only in the course of a specific infection. A particularly interesting example is provided by the so-called incomplete influenza virus (51). The incomplete virus forms particles of a somewhat varying size but of the same order as that of the classical virus. They seem to contain normal hemagglutinin and large amounts of lipides. According to Ada (2) they may be free of nucleic acid or contain reduced amounts of this constituent. In view of the fact that the mature particle is supposed to contain one single RNA molecule, Ada's interpretation of the analytical data would imply that only a fraction of a molecule were incorporated in the incomplete virus particle, which in turn would indicate aberrations in the RNA synthesis. However, the results obtained by Ada could be equally well explained by the assumption that his preparations represented mixtures of complete and incomplete virus, a likely explanation considering the technical difficulties associated with the preparative separation of the two. Incomplete virus has the capacity of interfering with mature virus in a peculiar way. Thus, after exposure of the cells to a mixture of mature and a large proportion of incomplete virus at high multiplicities, the amount of hemagglutinin released from the cells remains at normal levels but is accounted for mainly by incomplete virus. The proportion of RNA-containing mature virus is reduced, sometimes to $10^{-6}$ of a normal yield. The incomplete virus alone seems not to be able to incite any infection or induce the cells to production of hemagglutinin.

It is tempting to speculate on the mechanism underlying these phenomena. The RNA content of the virus particle corresponds, as already mentioned, to a single molecule of $2 \times 10^6$ mol. wt. One molecule is supposed to carry sufficient coded information for synthesis of one specific protein only. If this is so, the myxovirus RNA could provide the pattern for the synthesis of either the G-antigen protein or the hemagglutinin but not both. The information needed for production of one of the proteins would have to be supplied from some other source. Most likely, the protein itself would serve this purpose. According to Schäfer the complete G-antigen enters the cell, whereas the hemagglutinin is supposed to be largely retained on the cell surface. However, it would be difficult to explain the interference phenomenon without assuming penetration into the cell of the interfering agent. It seems reasonable, therefore, to assume that the hemagglutinin is capable of entering and that this is indeed a sine qua non for the synthesis of virus.

According to this line of thought, then, at least one of the six hemagglutinin subunits of the virus particle would have to enter the cell together with the nucleoprotein. The RNA of the latter is presumably self-replicating, provides the information necessary for synthesis of the G-antigen protein, and somehow activates the cell in such a way that the hemagglutinin can serve as a primer for the synthesis of the identical enzyme in the cytoplasm. If more than one hemagglutinin unit enters, presumably inducing an excessive hemagglutinin synthesis, the RNA synthesis would seem to be inhibited, and the end-product consists largely of incomplete virus.

This phenomenon, infection ending in dissolution and destruction of the cell with release of considerable amounts of a specific, noninfective material (but none or only insignificant quantities of mature virus), resembles the production of bactericines, lytic but noninfective substances, identical with or closely related to phage lysin (75). Both phenomena provide examples of situations in which the true nature of the processes as induced by a virus infection might be almost impossible to assess.
In the formation of incomplete virus the synthesis of the basic components of the virus may be considered to proceed in a regular fashion—unless Ada is right, in which case an aberration of the nucleoprotein synthesis would have to be postulated. The main abnormality, however, is in the maturation process. To what extent this situation is replicated in other virus-host systems is at present not known. Obviously, the above reasoning is applicable only to more complex viruses. In the small viruses, containing a single protein, all information needed for the virus synthesis can be carried by the nucleic acid. Nevertheless, material morphologically slightly resembling incomplete virus seems to appear regularly in poliovirus-infected cells. Schwerdt and Schaffer (70) reported the presence in purified virus preparations of a nucleic acid-free protein component. Immunologically, this component is distinct from the virus particles (47), in electron micrographs it looks like an empty sphere of approximately the same size as the virus particle (69). The significance of these observations is obscure. The non-infective soluble antigen might represent a regular byproduct of the virus synthesis or the result of an erratic synthesis.

Substances discussed in this section, being proteinaceous, are antigenic. For their detection in the interior of the cell the fluorescent antibody technics should be particularly suitable. Cell extracts may preferably be analyzed with the aid of any of the gel precipitation technics or immunoelectrophoresis.

D. Nonspecific Substances

The phenomenon of interference has generally been regarded as an expression of a competition between viruses for certain key enzyme systems in the cell. However, in a series of papers Isaacs and co-workers (12, 13, 38-40) have presented evidence of an alternative mechanism of a presumably entirely different nature. Cells exposed to inactivated myxoviruses or to active virus of low or moderate virulence were found to produce a substance capable of conferring resistance to other cells against virulent strains of a variety of related as well as unrelated viruses. This substance, presumably a protein, was called interferon.

Interferon does not interact with virus in vitro; it does not prevent adsorption of virus on the cell surface, but it does inhibit synthesis of (myxovirus) nucleoprotein. Resistance induced by treatment of the cells with interferon requires 12–24 hours to reach maximum levels. Induced cells do not themselves release interferon, unless they are challenged with active virus. In the chick embryo chorioallantoic membrane infected with influenza virus, no interferon seems to be released as long as virus is produced at a high rate. When virus synthesis has passed its peak, however, interferon appears in the allantoic fluid (39).

Isaacs has hazarded a guess that interferon might represent an aberration of the normal virus synthesis at some intermediate stage and that the presence of interferon in the cell would serve to redirect the synthesis to production of more interferon instead of the normal intermediate. Considering the proteinaceous nature of interferon this could hardly explain the inhibition of nucleic acid synthesis that is apparently established. As another possibility it might be suggested that interferon has a place in a normal regulatory mechanism in the cell, serving the purpose of controlling the nucleic acid synthesis. If so, interferon would be expected to show a certain degree of species specificity, which indeed seems to be the case (39).

Apparently interferon might become an important means of detection of infections with moderate viruses in which histologic lesions are absent and the amounts of virus or nonviral specific substances are below the threshold of demonstrability. The chemistry of interferon is not yet known to such an extent as to permit a suggestion of chemical diagnostic methods. For the time being only biological demonstration is possible, including determination of resistance of infected cells to challenge with selected indicator viruses and induction of resistance with material released from infected cells.

III. MATURE VIRUS

The mature virus would by definition possess infectivity as its most characteristic marker. If, for various reasons to be discussed below, infection tests are unsuccessful, other methods of detection might be tried. In vitro demonstration by chemical or immunological methods would—in most cases—require a concentration and partial purification of the test material. Occasionally, as found by Beard's group (20, 21, 55) with certain strains of fowl leukemia virus, enormous amounts of the agent may appear free in the blood or other body fluids, from which it is easily obtained simply by centrifugation. More often virus will be scarce and found only in tissues. Without methods by which results of fractionation experiments can be evaluated, purification will be extremely difficult. Beyond a statement to the effect...
that differential centrifugation probably has a wider applicability than any other method tested, few general recommendations can be made.

Whatever the outcome of other attempts to demonstrate the existence of a virus, the crucial test will always be infection experiments in volunteers, in test animals, or in tissue cultures. Provided a virus exists, infection tests may yet yield negative results for any one or more of the following reasons.

A. VIRUS CONCENTRATION

The virus concentration in the test material may be too low for detection by biological means. This again may be accounted for in several ways.

1. Only small quantities of virus may be released from the cells and at a low rate. In such cases a concentration of the test material might produce positive results. It is, of course, important to select material of initially highest possible virus content.

2. The virus may be continuously inactivated as it is released from the cell. A very labile virus may simply undergo spontaneous inactivation; it may be sensitive to tissue enzymes; or it may interact with specific or nonspecific inhibitors, including antibodies. Accordingly, attention must be paid to the methods for preparation of inocula. Rapid preparation at low temperatures may be essential; the use of stabilizers may be tried, such as cysteine, reducing the oxidation rate. It has been shown that some viruses are inactivated by enzymes and that the enzyme sensitivity spectrum to a certain extent serves to characterize individual viruses (53). The effect of tissue enzymes on the yield of active virus seems not to have been studied, however, and enzyme inhibitors have not been used in attempts to improve yields. Antibodies and inhibitors can be removed by perfusion, extensive washing, electrophoresis, etc., before the cells are disrupted and intracellular virus is set free.

B. VIRULENCE OF THE VIRUS

Viruses of very low virulence may fail to produce manifest infections. In principle, two different situations may be envisaged: the virus may be regularly infective but the infection established remain inapparent; or the infection rate may be low. The latter case will be discussed in a following section in connection with host resistance.

At least theoretically a state of virogeny might account for an inapparent infection. As already mentioned, no unequivocal evidence of the existence of this state in the field of animal virology has been presented. Since lack of evidence proves nothing, it would be unwise to disregard the possibility, however. Detection of virogeny might be possible either by means of induction and identification of subsequently appearing vegetative or mature virus, or it may express itself as a change in some particular property of the host cell.

Artificial induction of prophage can be achieved with agents apparently affecting the chromosomal structures in a manner as yet unknown. According to Lwoff (50) induction is possible only under certain conditions; the cells have to be metabolically preconditioned. In some cases all attempts at artificial induction have failed. What is known about provirus and its induction refers exclusively to DNA viruses, presumably attached to or incorporated in the chromosomes. It would be futile now to discuss the hypothetical case of an RNA provirus, its possible point of attachment in the cell, and the mechanisms of its induction.

So far no successful attempts to induce cyologic manifestations in inapparently infected tissue cultures have been reported. The enhanced susceptibility of irradiated cells reported by Puck and co-workers (61) obviously is a phenomenon of a different nature. Systematic studies of the effects of conditioning and induction on such carrier cultures as those described by Puck and Cieciura (60) and by Deinhardt et al. (15) would seem to be of great interest.

Even in host-prophage systems where attempts at artificial induction have failed to produce results, spontaneous induction occurs. This would have to be expected also of an animal provirus. Spontaneous induction is a comparatively rare event even in inducible prophage systems. Evidence of an activation, therefore, can be expected only in a small fraction of a population of infected host cells. A recent report by Dmochowski et al. (17) is of a certain interest in this connection. The authors describe virus-like structures in human leukemic tissues as well as in cells of such origin cultivated in vitro. Rounded particles of a uniform size in a double-contoured membrane appeared either in cytoplasmic inclusions or extracellularly. A characteristic feature was the sporadic occurrence of this phenomenon. In diseased tissues large areas showed no reaction at all of this type; in limited areas affected cells were observed in perhaps one out of 30 sections. In tissue cultures 2–12 weeks of culturing and up to ten subcultures were needed to bring out the
phenomenon. This observation thus conforms with what could be expected from a virogenic cell population. Needless to say, many other interpretations are possible. In any event, the technique used was exactly what can be generally recommended for detection of a provirus, i.e., prolonged cultivation of presumably infected cells in vitro.

It was already pointed out that prophage at least sometimes confers an easily demonstrable new hereditary property to the host cell, e.g., toxicogenic capacity. In cases of suspected virogeny it may therefore be worth while to look for characteristics distinguishing supposedly infected from normal cells. Tumor biologists have of course paid much attention to the genetic constitution of tumor cells and listed large numbers of characteristics, distinguishing them from normal tissue cells as well as one tumor from the other, the tendency being to regard each individual tumor as an equivalent of a separate biological species. Such an analysis includes all the innumerable genes stemming from the parent cell from which the tumor took its origin, whereas in the present connection the interest should be centered around one single “gene” that would probably be the same in a large proportion of the various tumors observed. In other words, we should look for common, not for distinguishing, characteristics of tumors. One such common feature of human cancers has indeed been reported. Björklund (7), using immune cytolysis, and Zilber (96), using active anaphylaxis as an aid in immunologic analysis of cancer cells, have found a common antigen in all tumors examined which seems to be absent in normal tissues. According to Björklund et al. (8) the antigen is a phospholipopo-protein localized to the cell surface.

Even with virus in the vegetative phase, continuously evolving into maturation and being released, an infection may remain inapparent, as in the system described by Puck and Cieciura (60). In that case infected cells could be cloned and give rise to infected populations. Whether or not an infected cell will be able to grow and divide probably depends upon the rate at which virus is being synthesized. In Jacob's (41) elegant experiments on phage it was shown that virus synthesis had a priority over the synthesis of host material. In bacteria kept on a strict metabolic regimen only phage was synthesized, if the rate of assimilation was kept below a certain threshold value. With assimilation in excess of what was needed for virus synthesis at maximum rates, metabolites and energy for synthesis of host material became available; a residual growth of the

host cell, at a rate proportionate to the rate of excess assimilation, was observed. Eagle (19) and others have also shown that certain metabolites may be indispensable for the production of virus. Together these observations suggest certain approaches to the study of inapparent infections in tissue cultures.

In a stable system the establishment of an inapparent infection could be expected to cause some changes in growth and metabolism of the tissue. Deinhardt et al. (15) observed that their NDV-carrying culture had a higher rate of respiration but a lower rate of growth than an uninfected culture of the same cell line. In the present writer's laboratory another system is currently being studied where the only manifestation of a presumed virus infection is a consistent reduction in the metabolic rate of the tissue cultures.

As the “masked” state of infection probably is a function of the ratio virus/host synthesis, it should be possible to affect the manifestations of infection by selective nutritional regimentation. Thus, for unmasking, optimal conditions for virus synthesis should be established, while at the same time the normal metabolic activity of the cell would have to be restricted. There are already many indications that conditions of nutrition of tissue cultures may have a decisive influence upon virus yields and incubation periods. Systematic studies of this problem on inapparently infected cultures should yield much valuable information.

C. HOST RESISTANCE

Failure to produce infection after transfer of virus-containing material to a new host may simply be a failure to infect, accounted for by resistance of the intended host organism or host cell. In principle, host resistance may be either constitutional or conditioned.

1. It is hardly to be doubted that resistance and susceptibility to infection may be genetically determined. This is true for the macro-organism as well as at the cellular level. As examples may be mentioned that Gross's agent of mouse leuke mia (31) goes in the inbred mouse lines C3H and C57BL but not in DBA or the heterozygous Swiss, whereas the reverse is true of Friend's virus (28). Hardly any two lines of HeLa cells are identical with regard to susceptibility to viruses; Puck and Cieciura (60) described a method for selection of resistant lines. As illustrated by the mouse leukemia viruses, resistance and susceptibility may be strictly specific and therefore largely unpredictable characters. For such reasons it
is important that a sufficient number of genetically distinct host organisms be used in tests for unknown viruses.

Resistance is seldom absolute; it can often be overcome by massive infection. Papilloma virus in domestic rabbits may serve as an illustration; one ID$_{50}$ corresponds in this case to about 80 million physical particles (5). Thus, in many situations the problem of detection can be reduced to that discussed on page 735 of this section: positive results might be obtained, if a sufficient virus concentration in the test material can be established.

As a particular type of constitutional resistance the examples mentioned on page 731 should be remembered. In those systems—chick embryo or chick tissue culture cells/poxvirus RNA and bacterial protoplasts/osmotically shocked phage—resistance depends upon the barriers against penetration of virus into the cell. If the process can be shunted around these barriers, a manifest infection might follow.

2. A naturally susceptible cell may be conditioned into resistance by interference with the receptor mechanism. Thus, the lining of the chorioallantoic cavity of the chick embryo acquires a temporary resistance to influenza virus infection after treatment with receptor destroying enzyme or periodate (23, 81).

In most cases the mechanism of conditioning is less obvious. A possible effect of nutrional factors upon the relative rates of synthesis of virus and host material was discussed in a previous section. Similar mechanisms operating on the macro-organism level might be responsible for changes in resistance observed in connection with avitaminosis or amino acid deficiencies. In the present connection the effect of hormones is of special interest. Shwartzman (73, 74), studying poliovirus infection in the hamster, found that cortisone treatment enhanced the susceptibility of the animals, whereas testosterone reduced it. In this case the hormones probably act on the brown fat tissue. Cortisone stimulates the brown fat, the cells grow in size, and assimilation of lipides increases. Under these conditions virus multiplies in the tissue to high concentration levels. Testosterone has the opposite effect. The analogy to the well known, less well understood conditioning effect of hormonal stimulation in the development of the Bittner tumor in mice is apparent.

Another example of growth stimulation as a conditioning factor has been described by Friedewald (27). Methylcholanthrene applied on the rabbit epidermis acts as an irritant, producing proliferation and hyperkeratinization. A skin area so treated may be found as much as 10,000 times more susceptible to papilloma virus infection than is normal skin.

In the bacteriophage field, lysogeny is one of the most common and most important causes of resistance to infection. In metazoans immunity after a previous exposure probably plays a greater role. However, inapparent infection can also produce resistance through interference (cf. p. 734). This possibility may be particularly important to keep in mind in a discussion of cancer and viruses. Apparently, some of the known tumor viruses are widely disseminated, almost ubiquitous; the animals seem to become infected very soon after birth or maybe already in utero or in ovo; they carry the infection in a clinically inapparent form for considerable periods of time. If the etiology of human cancer is a virus (or viruses), the epidemiology of the disease can hardly be explained without assumption of a similar near ubiquity of the causative agent. Therefore, inapparent infection might be expected to be of common occurrence, causing resistance of the macro-organism as well as of cells in tissue culture.

In this connection a phenomenon, familiar to most workers handling tissue cultures, assumes special significance. Trypsinized tissue usually grows rapidly during a period of about a week after explantation. Sooner or later growth slows down, and after three to five subcultures the cell population remains stationary or decreases. Sometimes one or more islands of morphologically changed elements appear in the sheet of cells. This metamorphosis is usually associated with increased growth capacity, and the cells now can be established in permanent culture. The morphology of such cells resembles that of established tumor cells; capacity of giving rise to malignant tumors has been observed; and the immunological character of such cells has been found indistinguishable from that of cancer cells. Obviously, such observations have to be evaluated with great caution. This is not the place for an exhaustive discussion of the various possible interpretations; it shall only be emphasized that the possibility of an induction of inapparent virus infections should not be neglected. If that is the explanation of the phenomenon, resistance because of inapparent infection might make detection of tumor viruses by means of tissue cultures difficult, depending upon how widely virus is disseminated. Dmochowski et al. (17) reported that the agent found in human leukemic tissue produced suggestive changes in monkey but not in human tissue culture. Without access to more
detailed data it is difficult to evaluate this piece of information; it may prove to be significant, however.

IV. SOME CHEMICAL AND BIOLOGICAL CHARACTERISTICS OF KNOWN TUMOR VIRUSES

By a rough classification four groups of tumor-inducing viruses may be distinguished, producing papillomas, leukosis, sarcomas, and cancerous tumors, respectively. Each one of these groups contains several members with different host specificities or immunological properties. It must be emphasized, however, that a classification at present can be only tentative, as the true inter-relationships between the various tumor viruses are unknown.

Only two tumor viruses have been prepared in a sufficiently pure state to permit conclusions concerning their chemical characteristics. Rabbit papilloma virus (10, 45, 65) contains DNA and protein and is synthesized in the cell nucleus. One fowl leukemia virus (4) was found to contain RNA; the site of replication is apparently in the cytoplasm. The fact that ATPase activity was found to be invariably associated with this virus (55) suggests that virus synthesis takes place in or in the immediate vicinity of mitochondria.

Most tumor viruses can be regarded as moderate. The incubation periods are often remarkably long, and manifestation of the infection sometimes requires some conditioning stimulus. Rous sarcoma virus retains its moderate character in tissue cultures; it does modify the morphology of the host cell (83), but has so far not acquired cytocidal properties on subcultivation. Others, like avian lymphomatosis virus (24, 71) and polyoma virus (79), produce degenerative changes in tissue cultures and may become adapted in the course of serial passages. The viruses so far isolated have a narrow host range in vivo. They may be less specific in tissue culture.

Polyoma virus is of special interest because of its versatility. It propagates and produces symptoms in several breeds of mice and in hamsters. Large inocula produce degenerative lesions in mice, particularly in the kidneys; otherwise tumor formation is the main symptom, involving many different organs and tissues (78). The virus is antigenic, and both neutralizing and CF antibodies are produced (62, 63). It possesses hemagglutinating capacity (22). With fluorescent antibody, stainable material appears first in the nuclei of infected cells; later a migration to the cytoplasm occurs, and the nuclei lose the capacity to fix antibody. At this stage the cells begin to show signs of degeneration, and hemagglutinin appears (32). The similarities with myxovirus infection are considerable. Early in the course of infection the cells develop resistance to super-infection with vesicular stomatitis virus, often used as an indicator virus in studies on interference.

Some tumor viruses appear as physical, apparently mature particles in the interior of the cell—papilloma virus in the nucleus, the milk factor in the cytoplasm (16). Avian leukemia virus is found in cytoplasmic inclusions (6). No intracellular particles are demonstrable in Rous virus-infected cells; maturation in this case seems to take place at the surface of the cell as in myxovirus infections (29).

It would appear from this brief summary that tumor viruses by no means form a uniform group. So far there are few indications of any characteristics setting them apart from other animal viruses. However, the behavior of Rous virus in tissue culture is somewhat unusual. The infection is apparent, the manifestation being a distinct change in the morphology of the host cell. Virus seems to be continuously synthesized and released. Yet the viability of the host cell seems not to be impaired.

The strict host specificity of the mouse leukemia viruses is another unusual feature. Genetically determined resistance to infection is usually not an all-or-none phenomenon; the term resistance has to be applied in a relative sense. It is possible that future studies with improved technic will prove this to be true also of resistance to the agents causing leukemia. On the basis of present evidence, however, the possibility of an “immunity” analogous to that found in lysogenic bacteria should be seriously considered.

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Detection of Viruses by Chemical and Biological Means

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