Viruses and Evolution

D. C. REANNEY

Biochemistry Department
Lincoln College
Canterbury, New Zealand

I. Introduction

Molecular biology has focused attention on viruses as simple models for basic biochemical and genetic processes. However, its very success in this context has tended to obscure the fundamental question, "What is the role played by viruses in Nature?" This article is an attempt to examine this issue. Various factors make the task a difficult one: (1) viral taxonomy is still a subject of controversy (see Lwoff and Tournier, 1971); (2) the amount of available data is now so great that one scientist cannot critically assess the validity of every claim; (3) much of the most pertinent information is so recent that certain key principles must still be regarded as suggestive rather than established; and (4) a further major factor concerning the available information relates to the origins of much of the data. Because of their small size, their haploid state, and their rapid replication rate, the genomes of viruses are in very intimate equilibrium with environmental stress. The act of *isolation* immediately removes a virus from many of the influences that hitherto determined the character of its genome.

After several thousand passages on indicator cells (themselves often the products of frequent subculturing *in vitro*) in the "unnatural" universe of the laboratory, the entity available to the experimenter may possibly have lost the genetic features most relevant to its original ecology. For example, much modern knowledge concerning lysogeny has been gained using laboratory stocks of λ coliphage "constructed" by isolation of mutants and outcrossings with other stocks so as to make them more amenable tools for genetic analysis. It is worth remembering that the *original* λ isolate (Lederberg, 1951) was considered "a rather poor object for genetic studies" because "the plaques were small and differences in plaque morphology difficult to discern" (Campbell, 1969).

Taken together, factors (1) through (4) mean that any attempt to unify the available data into a novel framework is bound to attract criticism from specialists in various areas. However, it is hoped that the theory developed in this article will serve a useful function in stimulating discussion, and will force a re-evaluation of the role played by viruses.

Before the main postulates of this article are presented, it is useful to condense certain data concerning viruses into perspectives. The points made below are not novel, and they are intended as conclusions derivable from available data rather than as postulates.

Although the topics discussed below overlap to some extent, to simplify treatment they have been presented separately.

II. Most Species Have Associated Viruses

Cytolytic viruses are known for most prokaryotes that have been the subject of intensive investigation. The number of distinct virus types per species is considerable. There are 50 known phages able to infect the enteric bacterium *Escherichia coli* (see Appendix, Part A). Barnet (1972) found 28 distinguishable phages for various strains of the soil organism *Rhizobium trifolii*. Teh and Reanney (1973) found over 15 morphologically distinct phage types lytic for one strain of another soil microbe, *Bacillus pumilus*.

As one progresses from unicells to higher organisms, the number of different viruses associated with any one species becomes very large. The best researched vertebrate is man himself. Depending on the criteria used for classification, a figure of about 200 to 500 "different" viruses are known to be associated with human disease (see Appendix, Part A) (Jawetz *et al.*, 1970). This figure ignores passenger viruses with no established pathological effects.

It is difficult to gain meaningful estimates for virus numbers for other eukaryote species, but it is likely that the figures are considerable. Rueckert (1971) states that "it is probably safe to venture that thousands of picornaviral serotypes exist in nature in non-human hosts."

A survey of the major taxonomic groupings shows that, for the main orders, viruses are known in many cases (see Appendix). It is true that no viruses have been reported for certain major phyla such as the Bryophyta (Matthews, 1970). However, where no viruses are documented, it is more likely that the relevant research has not been done or that the particular biology of the group makes it refractory to virus assay than that viruses are completely absent.

These observations lead to the conclusion: *Most cellular species, which are themselves not obligately parasitic, have associated with them one or more types of virus.*

III. Viruses Are the Most Numerous Genetic Objects in the Biosphere

Few studies have been made on the population biology of viruses in natural environments. Reanney and Marsh (1973) examined the population structure of a phage—D5—attacking the soil organism *Bacillus stearothermophilus*; in enriched soil where conditions favor virus multiplication, D5 populations often exceeded 10^8 pfu/gm dry weight of soil. In untreated soil moistened with rainwater, titers were of the order of 10^3–10^4 pfu/gm. In other untreated environments, such as compost, D5 titers of 10^5–10^6 pfu/gm were obtained.

Some ecological studies have been carried out on the phages that parasitize the bacteria that ripen milk into cheese, since these phages are of economic importance. When phage was added to *Streptococcus lactis* in skim milk, the phage titer rose to 10^9 pfu/ml (Babel, 1962). However, in the *natural* situation in raw milk containing diverse microbial populations, such wholesale lysis has not been observed. Collins (1952) found that the production of lactate by lactic acid bacteria was little affected by phage, even when susceptible bacteria made up 50% of the total microflora. In a later study, Collins demonstrated that the production of acid by a mixture of three different strains of *S. lactis* in skim milk was not reduced when any one of the three corresponding phages was added to the culture (Collins, 1955).

To the extent that these results are a true reflection of the natural situation, it follows that the lytic patterns and very high virus titers that fill texts on virology are artificial products of human manipulation. However, it must be remembered that laboratory conditions optimize virus multiplication by providing uniquely favorable conditions for host cell growth. In a natural environment such as soil, when the number of plaque-forming units scored for a given phage in a given sample is small, the metabolizing cell population sensitive to that phage is also likely to be small; phage titer has in fact been used to monitor bacterial activity in soil because of this presumed proportionality (Robinson and Corke, 1959; Reanney, 1968). In view of the fact (Section II) that there can be a large number of virus types lytic for any given species, it becomes apparent that the

number of virus particles in (most) natural environments probably exceeds the
number of host organisms. The likelihood that virus populations outnumber those
of their hosts is enhanced by the existence of temperate viruses which may only
occasionally manifest an infectious character. Few studies on the frequency of
occurrence of latent viruses in natural contexts have been made, but it is sug-
gestive that MacPhee *et al.* (1969) found temperate phages for 5 of 10 strains
of *Klebsiella* tested. It should be noted that failure to isolate a temperate virus
for a given organism does not prove that no such virus exists.

Data presented in the next section are compatible with the inference that
most prokaryote and eukaryote species harbor temperate or cryptic virus genes.

If it is admissible to consider episomal DNA "potential virus," then it is
clear that in many (most?) natural environments *viruses are the most numerous
genetic objects.*

While the size of a viral genome is small in relation to that of the cell it in-
fects, the rate of replication of the viral genome is almost always very much faster
than that of the host. The chromosome of *E. coli* is about 1.00 mm long. The
DNA of T4 is about 0.06 mm long; as a T4-infected *E. coli* cell produces about
200 progeny particles, the total DNA production of an *E. coli* cell is 12 times
greater for the virus DNA than for the host DNA generated by the normal
division of one cell into two.

When one considers (1) the enormous numbers of viruses present under
favorable conditions; (2) the variety of viruses often present for a given species;
(3) the rapid multiplication rate of viruses; and (4) as shown in Section IV,
the fact that certain genes which act as a part of cellular chromosomes can re-
enter an independent existence as virus particles; then a second conclusion seems
justified: *For a given environment at any given moment, virus genes constitute
a sizable fraction of the **replicating** nucleic acid in that environment.*

IV. Virus Genes Can Enter and Leave Cell Chromosomes

The phenomena of lysogeny and transduction are well documented in pro-
karyotes (e.g., see, Stent, 1963). It is not yet possible to generalize these pro-
cesses throughout the rest of biology, but there are now suggestive indications
that very similar processes occur in vertebrates.

The oncogene theory (Huebner and Todaro, 1969; Todaro and Huebner,
1972) envisages genes of viral origin as more-or-less normal components of
vertebrate chromosomes. A substantial amount of evidence is consistent with
this general notion. C-Type RNA viruses have been isolated from cats, rats,
hamsters, cows, pigs, monkeys, humans(?), and in addition from chickens and
snakes (Todaro and Huebner, 1972). Using hybridization techniques, Baluda
(1972) demonstrated that DNA complementary to the RNA of avian leukosis

virus occurs widely in chickens. Similarly, Varmus *et al.* (1972) found avian virus-specific nucleotide sequences in normal chick cells, as well as transformed cells; quail DNA also contained virus-specific sequences. The virus "marker" gs antigen has been found in cells that show no trace of virus particles (Huebner *et al.*, 1970). The most convincing support comes from the fact that apparently "virus-free" cells can be induced to produce C-type virus by treatment with halogenated pyrimidines (Aaronson, 1971a). Rowe *et al.* (1972) found that all their clonal and subclonal lines could be induced to produce murine leukemia virus (MuLV) by treatment with 5-iododeoxyuridine, indicating that the viral genome was present in unexpressed form in *all* AKR cells. They also noted that one mouse seemed to carry more than one MuLV strain. Weiss *et al.* (1971) showed that even cells that lack the gs antigen can be induced to make leukosis-type virus.

Evidence is accumulating which suggests that the herpes virus, Epstein–Barr virus, occurs in latent form in human chromosomes (Hampar *et al.*, 1972; Zur Hausen *et al.*, 1972; Gerber, 1972). At the moment it seems possible that many "normal" cell clones harbor several latent virus genomes.

The analogy with lysogeny is particularly apt in the case of the papova viruses, polyoma and SV40. It seems likely (Watson, 1970) that the circular polyoma DNA integrates with host DNA by a mechanism similar to that proposed by Campbell (1962). The opposite process—encapsidation of host genes by viral capsomeres—has been demonstrated for polyoma. Michel *et al.* (1967) showed that polyoma pseudovirions contain fragments of mouse genome. Subsequently, Qasba and Aposhian (1971) demonstrated that polyoma pseudovirions could deliver mouse DNA to human cells. Further, Merril *et al.* (1971) found that phage λ carrying a functional bacterial Gal transferase gene could initiate transferase activity in human fibroblast cells taken from a patient with a congenital lack of α-D-Gal-1-phosphate uridyl transferase. λ-Specific RNA and transferase activity were distinguished at undiminished levels 41 days after infection, suggesting that stable incorporation of the transduced DNA into the human chromosome(s) had occurred. In these "transductions" the DNAs of donor and recipient were separated by many millions of years of evolutionary divergence, so that such interchanges may not occur in nature; but between closely related species or between individuals of the same species, it seems reasonable to assume that such genetic exchanges may occur frequently.

Smith *et al.* (1972) examined abortively transformed cells for SV40 and found that two out of three clones tested contained virus-specific material, despite the fact that they were phenotypically normal. They suggested that cryptic transformants might represent the most common cell type harboring the viral genome, and implicated virus sequences as a possible source of the new gene families present in eukaryote DNA (cf. Britten and Kohne, 1968).

Conclusion three is perhaps more contentious than the previous two. However, the data just presented are consistent with the inferences (1) that virus genes can and do enter and leave cellular chromosomes, but that (2) *at any one time more "virus" genetic material exists in cryptic form integrated with cellular chromosomes than is to be found in the capsids of "infectious" virus particles.*

V. Integrated Virus Genomes Are Often Reiterated

One of the facts that has emerged from recent data is that integrated viral genomes in eukaryote cells are seldom present in single copies. Baluda (1972) calculated that cells of leukemic chickens contained 4.95 avian leukosis virus DNA equivalents per cell. Normal cells contained 3.2 DNA equivalents per cell. Gs-negative cells contained as much viral DNA as their gs-positive siblings. Varmus *et al.* (1972) found multiple copies of avian tumor virus genes in chick and quail cells but not in HeLa cells or salmon sperm. Cells transformed with Rous Sarcoma virus had 15 copies of rapidly reassociating sequences (Varmus *et al.,* 1972). Interestingly, a similar number of viral genes in chick cells was observed regardless of the degree of expression of viral information. Smith *et al* (1972) found an SV40 abortively transformed line to contain about five virus genome equivalents per diploid cell. Subclones of this abortively transformed line had 2.7 to 10 viral copies per diploid cell. A similar situation obtains for polyoma, but the number of viral copies has been estimated to be as high as 60 (Watson, 1970).

The presence of reiterated sequences in eukaryote DNA is well-known (Britten and Kohne, 1968). Models such as the master-slave hypothesis (Callan, 1967) emphasize that the tandem alignment of identical (or similar) genes permits frequent crossing-over between the extensive regions of homology thus created, with the result that *excision of DNA is facilitated* (see Callan, 1967). Thus *reiterated DNA favors episome formation* (Edelman and Gall, 1969).

By the nature of the crossover events involved, the DNAs excised from repetitive sequences are often *circular* (see Callan, 1967). Thomas *et al.* (1970) sheared salmon sperm DNA by passage through fine needles; when these preparations were denatured and annealed and examined by electron microscopy, many circular molecules were observed; similar experiments with prokaryote DNA revealed essentially no circles under similar conditions. Thomas (1970) concluded that the ability to form circles is a general property of all eukaryote chromosomal DNA.

It seems very likely that at least for some viruses, such as adenovirus, the integrated virus genes lie adjacent to reiterated cellular DNA sequences. Thus, in addition to the virus-specific genes, RNA transcripts contain repetitive cell sequences (Tsuei *et al.,* 1972). Wall and Darnell (1971) found that cells transformed with SV40 contain RNA with both viral and cellular sequences. In

cells transformed with SV40 heterogeneous nuclear RNA (HnRNA), molecules of very high molecular weight were shown to contain virus-specific RNA sequences, while polysomal virus-specific sequences were much smaller (see Darnell *et al.*, 1971). HnRNA is thought to be transcribed from the reiterated DNA sequences implicated in regulation (see Darnell *et al.*, 1971).

It also seems likely that such integrated virus DNA can undergo genetic exchanges with adjacent cell sequences. Aaronson (1971b) found that, when serologically distinct murine RNA viruses were grown in human cells, the various viruses acquired common surface antigens. He interpreted these data as signifying genetic exchange between human and viral genomes.

The information summarized in this section seems adequate to sustain the following conclusion: in eukaryotes "episome"-type (circular) DNA containing cellular and/or viral sequences can arise from reiterated DNA not only as a result of "infection" *but also as a normal consequence of the processes of DNA replication-recombination-repair.*

VI. Cytopathic Viruses May Be Atypical

Cytolytic viruses "broadcast" their presence because of their drastic effects on host cells. It was inevitable that such viruses would be the first to be discovered and investigated, and this has led to a widespread tendency to think of viruses only as destructive agents responsible for disease. However, the fact that cytopathic viruses are often of medical or economic importance to man does not justify the conclusion that such viruses are the norm when the whole ecology of viruses in the biosphere is taken into account.

The points discussed above raise the possibility that the reverse is true, that is, that cytopathic viruses—the ones most familiar to us—are atypical *as viruses.* This concept seems less drastic when one remembers the accepted dictum that "many infectious agents may remain latent in their natural hosts . . . but . . . may become pathogenic when transferred to other species" (Gross, 1970). For example, SV40 remains latent in its natural carrier *Macaca mulatta* but can induce a high incidence of sarcomas following inoculation into newborn hamsters (Gross, 1970).

This suggestion calls into question current views on the basic nature of (certain) viruses. The intention of this article is to reinterpret the role of (certain) viruses in terms of evolution.

VII. Some Evolutionary Considerations

There are certain aspects of evolution that have never been fully explained. The dilemma can be illustrated by quoting from Francis Crick (1970): "All biologists believe essentially that evolution is driven by natural selection. But someone from the more exact sciences could well point out that it has yet to

be explained that the rate of evolution can be adequately explained by the processes which are familiar to us. It would not surprise me if nature has evolved rather special and ingenious mechanisms so that evolution can proceed at an extremely rapid rate...."

The possibility that gene exchange mediated by viruses might fit one requirement for a "special and ingenious mechanism" demanded by Crick is argued below. However, before this can be discussed it is necessary to examine carefully some of the accepted axioms of evolutionary theory.

The basic issue is the problem of variation. There was initially some doubt as to whether the inferred rates of mutation in certain species were enough to sustain the observed rates of evolution. But *expressed* rates of mutation are much lower than *actual* rates of mutation, for reasons summarized by Holmquist (1972).

The premise is accepted in this article that enough variation is provided by spontaneous mutation to account for the rates of evolution of all species in all periods.

However, in considering the dissemination of variation, it is necessary to distinguish carefully between the roles of processes such as recombination and sexual transfer. Significant (from the evolutionary standpoint) recombination can occur only when chromosomes or chromosome fragments of different genetic pedigrees are united in one cell. Thus while molecular recombination plays a basic role in enhancing variability, it is dependent for its effectiveness on the prior existence of mechanisms of gene transfer from cell to cell.

It should also be remembered that, especially in prokaryotes, the gene material transferred has already passed the test of natural selection insofar as strongly deleterious mutations usually cause the death of the cell harboring them before any transfer can occur. Thus to a certain extent the numerical value of a given mutation rate is *irrelevant* to the rate and direction of evolution of a given species. The only "variability" of direct significance to an evolving clone is that which is not immediately selected out in the prevailing environment.

This statement does not presuppose that the variation introduced by mutation and accepted by the environment permits evolution to preceed at the observed rates. The complex enzymology of molecular recombination in all cells and the finely organized cytology of mitosis and meiosis in eukaryotes, underscore the fact that an enormous selective advantage accrues to any biological system able to shuffle preexisting variations into novel genetic mosaics for environmental "editing." This point is fundamental to the rest of the discussion.

VIII. The Role of Viruses in Evolution

There exist three known mechanisms by which gene material can be transferred from cell to cell: (1) transformation, (2) transduction, and (3) sexual

conjugation. It seems logical to assume that the order in which these have been listed reflects the order in which they have been exploited by evolution. Transformation is the most random and inefficient process, but it requires nothing more complex than the laws of diffusion and the existing chemistry of the cell membrane, modified in contemporary cells by the development of transport systems which facilitate membrane penetration (Tomaz, 1969). Transduction requires the development of genes for capsomere proteins able to encapsidate nucleic acid and/or a sophistication of the process of membrane evagination so as to package nucleic acid into free particles. These are relatively modest genetic adaptations. However, true sexual union as it occurs in modern eukaryotes requires such a high degree of cytological organization that it is inconceivable that it could have operated efficiently during the first billion or so years of cell evolution.

It is characteristic of evolution that the development of a system that more efficiently fulfills a strongly advantageous evolutionary need seldom entirely eliminates the less efficient system that preceded it in time. For example, nerve cells transmit messages from one part of a biological system to another far more quickly than the more "ancient" mechanism of transmission by chemicals, but chemical messengers (hormones) are still of basic importance in physiology.

It may be that the first postulated mechanism of gene interchange between cells—transfer of naked DNA (transformation)—has now been so effectively displaced by the second two that it modifies cell genotypes only in a few rare cases among prokaryotes (this may in fact not be true, as we know very little of the processes of genetic interchange among cells in natural environments). However, the postulate is advanced here (1) that the mechanism of gene transfer by virus vectors played a predominant role in spreading acceptable variation in the long period prior to the effective emergence of eukaryote life, and (2) that because of the advantages discussed below this mechanism continues to influence evolution among most species and in certain situations may still be the dominant "driving force" for adaptive change.

In any sexual union there is always sedentary genetic material (female) and actively transferred genetic material (male). The male "gametes" transferred vary from virtually naked DNA in the case of mating male E. coli to the complex structures of vertebrate sperm which possess specialized organs of motility. However, in essence sperm are simply packets of DNA encapsidated in lipoprotein covers. The accessory structures (tail, and so on) are related almost entirely to the problem of transfer. The similarity to viruses is obvious; virus infection involves the transfer from one individual to another of packets of nucleic acid encapsidated in protein shells, for example, icosahedra, or in lipoprotein envelopes, for example, C-type viruses. While the *quantity* of information transferred by an individual virus is small, when the huge *numbers* of

viruses obtainable (in infection) are considered along with the *rapid* replication rate, then the potential effectiveness of viruses as gene vectors is considerable.

The sexual mechanism has the very great advantage that it achieves a full diploid condition. This has made possible the allelic shielding of mutant traits which might otherwise cause the immediate death of the cell harboring them. Sex allows a vast amount of variation to be *stored* in diploid cells and trans- mitted without loss or detriment to the cell from generation to generation.

There are many situations, however, in which the more random but more rapid mechanism of viral transduction may have advantages as a means of spreading variation. This postulate can be illustrated by the following example. The genetic map of *E. coli* can be transferred from one cell to another by mating. But the total genome of *E. coli* also exists in a lysate of cells infected with a generalized transducing phage such as PI (Ozeki and Ikeda, 1968). Now if it is of selective advantage to spread variation as effectively as possible, then clearly gene markers from one total *E. coli* genome can be disseminated among a far greater *number* of cells from the virus lysate than from the mating donor cell which, although it can potentially transfer the entire chromosome, can do so to only *one* recipient.

Further, it may be possible by transduction to spread genetic variations among species barred from interbreeding by species infertility. While viruses in gen- eral are highly specific for particular hosts, several are distributed throughout a surprisingly wide taxonomic range. Alfalfa mosaic virus, for example, is able to infect 222 different species of plants (Crill *et al.*, 1970) (see Table I).

The ability to transfer genetic material is a property of the virus coat. In small, rapidly adapting systems such as viruses with (often) regular repetitious coat structures, it is far simpler to change cell substrate specificity than it is to break down the barriers that prevent two organisms from interbreeding, as a vastly greater number of mutations would be required to effect the latter change than the former.

Anderson (1970), in formulating a theory similar in part to that discussed in this article, postulated that a major evolutionary role of viruses was to carry genes across species and phylum barriers. We dispute this. Irrespective of how genes are transferred from cell to cell, the ability of the recipient to incorporate donor genes effectively into its chromosome depends upon the degree of base homology between donor and recipient DNAs. Clearly, the more closely related any two DNAs from different taxonomic sources, the greater the probability of effective assimilation. This may explain the usually strict specificity shown by viruses for particular cells. What is claimed here is that the more rapid adaptive capacity of viral transductants provides a greater *flexibility* which has surely been exploited by evolution at certain times in certain contexts.

Specifically then, this article envisages (certain) "viruses" as *primarily* agents

TABLE I

RANGE OF HOSTS NATURALLY INFECTED WITH ALFALFA MOSAIC VIRUS[a]

Scientific name	Common name
Apium graveolens	Celery
Ballota nigra	—
Beta saccharifera	Sugar beet
Capsicum spp.	Pepper
Carthamus tinctorius	Safflower
Chenopodium album	Lambs quarters
Cicer arietum	Garbanzo bean
Cichorium endivia	Endive
Crotalaria juncea	Sunn hemp
Crotalaria spectabilis	—
Daphne odora	—
Dianthus caryophyllus	Carnation
Dolichos lablab	Hyacinth bean
Glycine javanica	—
Glycine max	Soybean
Lathyrus odoratus	Sweet pea
Lupinus spp.	Lupin
Majorana hortensis	—
Medicago lupulina	Hop clover
Medicago sativa	Alfalfa
Mentha piperita	Peppermint
Nicotiana tabacum	Tobacco
Ocimum basilicum	—
Phaseolus vulgaris	Bean
Physalis sp.	Groundcherry
Pisum sativum	Pea
Portulaca oleraceae	—
Solanum melongena	Eggplant
Solanum nigrum	—
Solanum tuberosum	Potato
Sonchus arvensis	—
Sonchus cornutus	—
Sonchus olearaceus	—
Stachys annua	—
Trifolium pratense	Red clover
Trifolium repens	White clover
Viburnum opulus	—
Vicia faba	Broad bean or horse bean
Vitis vinifera	Grape

[a] From Crill *et al.* (1970).

of gene exchange between cells. On this basis (certain) viruses and cells have coevolved from a "point" close to the origin of the phylogenetic tree and/or from branch points in the tree. Gilden and Oroszlan (1972) note that, for the gs antigen of the C-type viruses, three of the first four amino acids at the N terminus of cat and mouse gs antigen are the same, while, except for the terminal Pro, the chick sequence is distinct. This is the first experimental evidence for the coevolution hypothesis.

What is the detailed evidence for this postulated role of viruses? The discussion falls into two parts, the first dealing with DNA viruses, and the second with RNA viruses. The reason for this division becomes apparent as the discussion proceeds.

IX. DNA Viruses

The ability of DNA phages such as λ to integrate with cell genomes with the necessary base homology, and conversely to act as vectors of host genes, is well documented. Virtually no ecological work has been done to investigate the extent to which these processes operate in nature.

As a model of a DNA virus for higher cells, which may fulfill some of the requirements of the theory, consider polyoma.

Early experiments seemed to indicate extensive base homology between mouse and polyoma DNA (Axelrod *et al.,* 1964). This homology was subsequently shown to result from the presence of host DNA encapsidated within polyoma virions (Winocour, 1969).

Further experiments have indicated that *any* host genetic marker can become encapsidated in polyoma protein (Winocour, 1968, 1969). The host DNAs so incorporated have a buoyant density indistinguishable from that of the uninfected cell (Winocour, 1968). Ben-Porat and Kaplan (1967) have concluded *that all mouse embryo cell DNA that replicates after infection is eventually degraded to a size similar to that of polyoma DNA.* Winocour (1969) concludes that "the breakdown of mouse cellular DNA before encapsidation must be a *specific* [author's italics] process in view of the relatively restricted size of the fragments that are produced."

These data suggest that the polyoma genome encodes a nuclease specific for the host DNA. To account for the uniform size of the fragments produced, it *is tempting to speculate that the five methylcytosine residues that occur in mouse DNA but not in polyoma DNA* act as signals for the enzyme, and that these are spaced at "polyoma DNA unit lengths" throughout the mouse genome. It is difficult to see the biological advantage of this specific fragmentation of host DNA if the virus is considered solely as a lytic virus. However, the phenomenon

is quite intelligible if we consider the mouse cell–polyoma genomes part of one general and interchangeable genetic system.

An alternative hypothesis to explain the data is that the size of the mouse cell DNA fragments is determined by the capacity of the polyoma capsid to accomodate them physically (cf. Streisinger's "headfull" hypothesis for T4). If this is true, it means that polyoma capsid subunits must have far less preferential affinity for polyoma DNA than do the capsomeres of another "temperate" virus such as λ which normally efficiently packages only homologous DNA. Either possibility leads rather easily to the concept that the encapsidation of host genes by polyoma protein is more a normal function of the system than an atypical "mistake" in the assembly of the virus.

If polyoma does fulfill the requirements of the theory, we might expect that its own DNA could integrate into mouse cell chromosomes. It is known that multiple copies of polyoma DNA can lie in tandem in cell DNAs (Watson, 1970). The presence of these cryptic copies of virus genetic material has been detected only because cells harboring it are *transformed* from normal into cancerous. However, if no such medically obvious effect occurred, it is possible that the virus genes could lie latent in the cell chromosomes without ever being detected.

One obvious objection is this. The very act of encapsidating an *essential* host gene presupposes that this gene will be *lost* from the host chromosome. Does it not follow that when such transductions occur the donor chromosome will inevitably be damaged or destroyed and the cell killed?

It does follow that such "silent" transducing viruses must preserve the integrity of cell chromosomes. One way to do this would be for the virus to stimulate compensating host cell DNA synthesis. In accord with this requirement, polyoma infection has been shown to overcome the blockages of DNA synthesis arising from a number of causes (Winocour, 1969). It may be significant that every cell induced by polyoma to make DNA approximately doubles its DNA content (Gershon et al., 1965). The DNA made after infection is methylated normally (Winocour et al., 1965), a point that may be important if five methylcytosine residues serve as recognition signals for DNA degradation by a virus-encoded enzyme, as suggested earlier.

It should not be forgotten that polyoma may not be and probably is not a true representative of the type of silent transducing virus envisaged here. A truly cryptic virus would have no adverse effect on the cell it enters. However, it would have to stimulate DNA synthesis to prevent damage to the cell chromosome from which genes were being transduced. In a truly cryptic type of virus, stimulation of DNA synthesis would be a controlled process and may in fact be coupled to normal replication of the host chromosome. In the case of polyoma,

the usual controls have apparently ceased to operate; from this it follows that laboratory strains of polyoma can cause cancer which arises from *uncontrolled* DNA synthesis.

X. RNA Viruses

ACTIVATOR RNA

Before beginning to discuss the possible role of RNA viruses in evolution, it is necessary to digress.

It is now well established that many DNA sequences in higher organisms are repetitive (Britten and Kohne, 1968). This has led to a widely accepted model of eukaryote cell regulation (Britten and Davidson, 1969). The main features of this model are:

1. Much DNA in the cells of higher organisms serves a regulatory function.

2. Gene sets are switched on by activator RNA, this RNA being produced by a special class of regulatory genes called *integrator* genes.

3. The regulatory genes are members of families of repeated DNA sequences.

From the Britten–Davidson model, it follows that, during the evolution of higher organisms, few *new proteins* need enter into the structure and/or physiology of organisms. Rather large morphological changes result from slight changes in the integrative function, by modulating preexisting gene sets. We stress that a key molecule in this scheme is the product of the integrator gene— activator RNA whose function it is to switch on a given gene battery, depending upon the particular signal fed into the system either by the environment or by the product of a previously activated structural gene.

Let us examine RNA viruses in the light of this theory. If one tabulates the viruses that contain RNA as opposed to DNA and lists the taxonomic groups for which these are specific (see Appendix), one very striking fact emerges. Most of the viruses affecting differentiated organisms contain RNA. Almost all plant viruses contain RNA and, according to currently available evidence about 70% of known animal viruses contain RNA. By contrast, in prokaryotes, the vast majority of phages contain duplex DNA. The only RNA viruses that occur in bacteria are specific for cells that may perhaps be considered "differentiated" in that they possess sex factors.

We suggest that there is a direct correlation between the distribution of RNA and DNA viruses and the degree to which a given host genome contains repetitive DNA. In prokaryotes, in which most genes are unique, selection has favored *DNA*-transducing viruses which act as vectors for *structural* genes. In differentiated cells, however, as Britten and Davidson have stressed, the probability of utilization of new DNA for *regulation* is far greater than the likelihood of inven-

tion of a new and useful amino acid sequence. Since a key element in their regulatory model is activator RNA, it becomes possible to see *why*—if viruses fulfill an essential role in spreading variability through gene pools—viruses specific for differentiated cells normally contain RNA.

Note that this "rule" may not apply to very small viruses whose RNA genomes are the size of cellular mRNAs, for example, among plants TYMV, and so on, and among animals polio, foot and mouth, and so on. In these cases a different and plausible explanation for the RNA character of their genomes is possible, namely, that once selection has simplified a virus genome to the extent that all relevant information can be contained in one polycistronic message a DNA equivalent of this message is uneconomical in terms of packaging and expensive in terms of replication/transcription. We are specifically thinking of viruses whose RNA is relatively large and whose structure seems to be derived in large part from cell membranes. The "budding" process so characteristic of C-type viruses strongly resembles a normal cellular function such as membrane evagination in superficial appearance. However, it is possible that the only host components to enter virus envelopes are lipids, and that virus maturation involves a marked restructuring of the cell membranes to accommodate virus-specified proteins. The status of this question is still open, but it is interesting to note that viruslike particles are known that bud from the membranes of the endoplasmic reticulum but which have no demonstrable infectious ability. These intracisternal A-type particles (Kuff *et al.*, 1972) may be regarded as immature virions, or they may be viewed as mistakes in the process of information flow—for some reason they are not encapsidating activator RNA.

The most striking feature of Part B of the Appendix is that with the exception of the herpesvirus group, no virus whose essential envelope structure is formed by budding from "cell" membranes contains DNA. (Viruses of the herpes group bud from membranes, but the particles are already encapsidated in icosahedral shells.)

Thus the "infectious" viruses that most closely fit the postulates of this article are those like lettuce necrotic yellows and wheat striate mosaic, and so on in plants, and rabies, and so on in animals. Even more relevant are the temperate B- and C-type cancer viruses of animals. However, such viruses have been detected only because of their ability to bring about cell transformation and/or to produce "infectious" virus on treatment with inductive chemicals. The possibility exists that "virus" particles may be found that are not infectious, because they act in part or in whole as vectors of host genetic material (see Section XII).

The efficiency of such a pathway of information flow would be greatly enhanced if the transduced host material could be *amplified* either before leaving the donor cell or on entry into the recipient cell. This could be achieved (a) by enzymes associated with the structure of the transducing virus particle, (b) by

enzymes encoded in further polynucleotide contained in the transducing particle, (c) by enzymes in the donor or recipient cells.

XI. The Genomes of (Certain) RNA Viruses Can Be Considered Activator RNA

Specifically, it is suggested here that the single-stranded RNA virus genomes *are* in part activator RNA. While there is no evidence yet that such RNA genomes bind to reiterated cell DNA, it may be significant that the RNA transcript of adenovirus DNA contains reiterated cell sequences, suggesting that the DNA of this virus integrates at such repetitive parts of the chromosome (Tsuei *et al.,* 1972). Sivolap and Bonner (1971) found that chromosomal RNA (cRNA), which may be the homolog of activator RNA, binds chiefly to repetitive DNA. In his model of the eukaryote chromosome, Crick (1971) postulated that *unpaired* regions of DNA consisting of repeated sequences serve as control elements. Crick noted that the unpaired state of control DNA facilitates selective binding of activator RNA.

One possible experimental prop for the concept of "viral" activator RNA is the existence of viroids such as potato spindle tuber virus (PSTV). Diener (1971) showed that the infectious entity in the disease is a low-molecular-weight RNA of sufficient size to code for only about 25 amino acids. The difficulty of envisaging how such a small molecule could effect its own reproduction led Diener to the postulate that the necessary biosynthetic information preexists in susceptible hosts, and that PSTV RNA functions not as mRNA but as "an abnormal regulatory RNA" (Diener, 1971). In this context one notes that two of the nucleus-confined RNAs put forward by Britten and Davidson (1969) as possible candidates for activator RNA (e.g., the RNAs from HeLa cell nuclei and pea seedling nuclei) were only about 40 to 180 nucleotides in length.

The chances that the introduction of alien RNA into cells will result in gross malfunction are negligible, because virus RNA can act as activator RNA only if it possesses adequate base homology with (unpaired) receptor DNA.

It is especially easy to see how C-type RNA tumor viruses might act in this context. As Schlom *et al.* (1971) showed, these particles all contain the enzyme reverse transcriptase as part of the physical structure of their cores. Thus not only could they activate gene batteries, but the change could—if advantageous—become hereditary because the DNA transcript of the RNA could itself become integrator DNA from which modulated activator RNA might be produced. The theory predicts either that reverse transcriptase will be found in association with any RNA virus that fulfils the role of spreading gene variation, or that normal cells will be found to contain reverse transcriptase, especially during embryogenesis.

Since hereditary stabilization of any variation introduced by viruses seems to

require reverse transcriptase activity, it might at first sight seem more economical to introduce such variation in DNA form; on this basis most eukaryote viruses should contain DNA. Such DNA, however, if integrated into receptor or integrator gene sets, would still be switched off. The result would be an increment in the quantity of available information and a slight change in quality if the introduced sequences differed from the original, but no immediate change in biochemical patterns of activation. (This point is further discussed in Section XII.

The status of the function of reverse transcriptase at the moment is still ambiguous. Goodman and Spiegelman (1971) have defined criteria for distinguishing "true "reverse transcriptase from other cell polymerases. Fridlender *et al.* (1972) found a novel enzyme in human tissue cells which efficiently copies the ribo strand of synthetic oligonucleotide-homopolymer complexes but, unlike the reverse transcriptase in C-type viral capsids, this enzyme seems unable to utilize efficiently natural RNA as substrate. Temin (1972) has promulgated the view that reverse transcriptase plays an important role in normal embryogenesis. Todaro and Huebner (1972) have noted the occurrence of virus-specific gs antigen early in embryonic life, and the possibility must be entertained that this "viral" element may play a role in differentiation. In this context it is interesting to note that Stavrianopoulos *et al.* (1971) found a predominant enzymic activity in the developing (4-day) chick embryo, which prefers a DNA–RNA hybrid as template; the importance of this enzyme seems to decline in later stages of embryogenesis.

It is possible from the above arguments that erroneous activation of cell gene sets by RNA viruses, while unlikely, may sometimes occur; one such case is the activation of the gene set for DNA synthesis under conditions in which synthesis is not needed. This might result in a cancerous condition. The probability of tumor generation seems to be higher for the specifically regulatory RNA viruses than for a general DNA- transducing virus such as polyoma, and this may account for the fact that RNA viruses cause cancer in nature, whereas lethal oncogenesis by polyoma is thought to be a laboratory artifact (Watson, 1970).

One fascinating extension of the theory is that activator RNA introduced into differentiated cells by viruses may activate repressed gene sets which are themselves of viral origin. This would complete the feedback circuit and may provide an explanation for the low-frequency stochastic release of C-type RNA virus particles from many vertebrate cells (see Weiss *et al.*, 1971).

XII. Nongenetic Influence of Transducing Viruses

One of the most serious criticisms of the theory presented in this article is that viruses (apparently) infect predominantly *somatic* cells, hence any variation introduced by them would not be *hereditary*.

This contradiction may be more apparent than real. Bentvelsen *et al.* (1970)

found that murine oncogenes were transmitted vertically, and it is basic to the oncogene hypothesis that virus information is part of the vertically transmitted gene complement. The oncogene hypothesis thus presupposes that virus genes entered cellular germ lines at past points in *evolutionary* time [conversely, one can accept the postulate that such "viruses" arose (arise?) from normal cell chromosomes by acquiring independent replicative potential.] How far such processes operate in the lifetimes of *individual organisms* must be speculative until more data are available. C-Type viruses are known to occur in germinal cells. Virus budding was found by Dougherty and Di Stefano (1967) to take place in cells derived from all three embryonic germ layers. Avian leukosis virus multiplied well in the adult female reproductive system. Electron microscope data showed the presence of type-B viruses in the genital organs of R 111 and DBA male mice (Moore, 1963).

The propagation of a virus in the germinal cells is well documented in the case of of sigma virus of *Drosophila*. The carbon dioxide sensitivity caused by sigma is hereditary but is not transmitted according to Mendelian laws (Howatson, 1970). Berkaloff *et al.* (1965) found budding particles in sections of ovarian cysts of female *Drosophila* stabilized in their possession of the carbon dioxide-sensitive phenotype. Teninges (1968) saw rod-shaped particles budding from *early* spermatids but not from somatic cells.

In this context it may be significant that Szakal and Hanna (1968) found budding particles in the *germinal* centers of mouse spleens, compared to their relative absence from mature lymphocytes.

In point of fact it is not strictly necessary to postulate stabilization of variation in the germ cells to obtain the same overall result. Consider a simplified case. Organism X has associated with it 10 virus types. These interact with each other and with X such that continual but slight *somatic* variations exist between the individuals of the X population over and above those introduced by sexual transfer. Now selection acts not upon the genome of X per se but upon the "symbiotic" X-virus genetic interchange. If a particular virus (e.g., virus 5) confers upon an individual of X (or more probably an interbreeding group) a slight advantage such that X1 can reproduce more often and/or more effectively than the rest of the population, then there will be a slow selection for X1, provided the relevant virus continues to transduce advantageous information; the process will have a snowball effect, for the more rapidly X1 reproduces relative to the rest of the population, the greater the availability of the relevant virus 5 in the environment.

In one sense virus 5 is an environmental influence. Since it is a *replicating* genetic system, however, an evolutionary feedback is possible which is inconceivable with any other "nonhereditary" factor.

XIII. Noninfectious Viruslike Particles

If the chief postulate(s) of this article is correct, then there should exist in many cells at many times viruslike particles with no "infectious" character to which no pathological state can be attributed.

The existence of such "passenger" viruses has been documented for a very long time. It is exceedingly difficult, however, to prove that a virus which is a passenger in one species is not an infectious agent in another. The listing given below simply indicates that many reports are consistent with the postulate that viruslike particles transduce information without autonomous replicative potential.

The number of species in which such observations have been made is striking. Viruslike particles have been noted in the ameboflagellate *Nagleria gruberi* (Schuster and Dunnebacke, 1971); in *Paramecium aurelia* (Preer and Jurand, 1968); in *Cephalosporium acremonium* (Day and Ellis, 1971); in various strains of *Penicillum* (Banks *et al.*, 1969); in *Drosophila melanogaster* (Philpott *et al.*, 1969; Akai *et al.*, 1967); in the wood ant (Steiger *et al.*, 1969); in the beetle *Tenebrio molitor* (Zeikus and Steinhaus, 1969); in mice (Szakal and Hanna, 1968); and in guinea pigs (Ma *et al.*, 1969). These are only a few of the many references that could be quoted.

Viruslike particles have often been noted in higher plants; Anton-Lamprecht (1965, 1966) observed in healthy *Epilobium* hybrids with the "irregular" plasma type large numbers of double-membrane particles with a core which was assumed to be RNA.

A large number of references describe the occasional appearance of particles resembling B- and C-type viruses in healthy animals (Dmochowski *et al.*, 1963; Feldman and Gross, 1966; Feldman *et al.*, 1967; Benedetti, 1957; among others).

XIV. History of the Theory

Several scientists have from time to time implicated viruses as positive agents in various aspects of evolution (e.g., Danielli, 1953). A short article embodying the central thesis of the present theory was presented by Anderson (1970). However, we disagree with the interpretation placed by Anderson on certain data, for example, the transducing ability of viruses between distantly related species. Anderson's views have also been criticized by West (1971). What is novel in this article is the unification of a considerable amount of data, much of it very recent, into what purports to be a coherent framework in which the existence of cancer viruses and the distribution of DNA and RNA viruses through taxonomy finds a logical basis.

XV. Conclusion

Viral transduction of both structural and regulatory genes provides a means for information to leave the body of an organism other than through the germ cells.

Thus if the theory is correct natural selection does not—and perhaps never has—act upon cell genomes as has commonly been believed. Natural selection acts upon the cell-virus nucleic acid coupling, and the rate and direction of the evolution of any species depends upon the number of associated viruses and the extent to which and speed with which they allow information to be cycled through the total gene pool of that population.

It is to be expected that nature has exploited this fundamental mechanism in a variety of ways, so that to look for a uniform process today may be unrealistic.

NOTE ADDED IN PROOF

A large amount of pertinent data has accumulated since acceptance of this article on November 3, 1972. Among the relevant papers, that by Koch deserves mention [J. Koch, *FEBS Lett.* **32**, 22 (1973)]. Koch found (a) that in hamster cells, small DNA molecules (cDNAs) were migrating from nucleus to cytoplasm, (b) that such cDNA molecules appeared to contain unique base sequences, and (c) that the quantity of cDNA was greatest in embryo cells or in polyoma transformed cells. These observations are consistent with the notion that nuclear genes are regularly passaged from nucleus to environment: Koch's suggestion that polyoma activates a mechanism for nuclear gene amplification normally repressed in fully differentiated cells can be interpreted in terms of the role postulated for such viruses in this article.

If viral RNA does serve, in part, as regulatory RNA as suggested in this article, it may seem odd that about 99% of known plant viruses contain RNA since plants, which lack complex nervous and muscular systems, are generally considered *less* complex than animals. But to an extraordinary degree the variety of plant species can be explained on the basis of regulatory changes: modulations in overall size/shape/leaf and stem morphology, etc., do not usually require the introduction of novel proteins, rather alterations in *rates* and *patterns* of growth and differentiation. The existence of a pool of regulatory RNAs able to pass from individual to individual or species to species makes good sense in plants as it would maximize rapid adaptability to environmental changes. The essence of this argument is that it is precisely because the structure of higher plants is *less* inflexibly organized than that of higher animals that virus-transduced regulatory genes are *more* likely to be effective in producing adaptive change. For example, the introduction of a regulatory RNA that doubles the quantity of tissue produced at a specific stage of differentiation might matter little in a plant whereas to the finely coordinated biology of a vertebrate such a change would be intolerable.

Also, being sessile, many higher plants have to rely for fertilization on random processes (wind, passive carriage by insects)—the very processes which disseminate viruses! A final point is that in many plants the difficulty raised by the oncogene hypothesis of having to introduce virus information into the germ cells may not occur. An advantageous change stimulated in a local part of a plant by a viral RNA could be passed on to progeny by vegetative propagation of that section. If the viral RNA genes were transformed into DNA by reverse transcriptase, the vegetative product could regenerate a plant able to engage in sexual conjugation. It must be remembered that unlike the situation with animals an entire plant can often be regenerated from a differentiated cell.

We wish to take this opportunity to stress that the validity of the hypothesis proposed in this article does not depend too heavily on the existence or otherwise of activator RNA as such. As Britten and Davidson (1969) noted in their paper the structure of their model would not be changed if the regulatory function was carried out by some translation product of "activator" RNA.

REFERENCES

Aaronson, S. A. (1971a). *Proc. Nat. Acad. Sci. U.S.* **68**, 3069.

Aaronson, S. A. (1971b). *Nature (London)* **230**, 445.

Akai, H., Gateff, E., Davis, L. E., and Schneiderman, H. A. (1967). *Science* **157**, 810.

Anderson, N. G. (1970). *Nature (London)* **227**, 1346.

Anton-Lamprecht, I. (1965). *J. Ultrastruct. Res.* **12**, 624.

Anton-Lamprecht, I. (1966). *Z. Vererbungsl.* **98**, 257.

Axelrod, D., Bolton, E. T., and Habel, K. (1964). *Science* **146**, 1466.

Babel, F. J. (1962). *Advan. Appl. Microbiol.* **4**, 51.

Baluda, M. A. (1972). *Proc. Nat. Acad. Sci. U.S.* **69**, 576.

Banks, G. T., Buck, K. W., Chain, E. B., Darbyshire, J. E., and Himmelweit, F. (1969). *Nature (London)* **222**, 89.

Barnet, Y. M. (1972). *J. Gen. Virol.* **15**, 1.

Benedetti, E. L. (1957). *Bull. Cancer* **44**, 473.

Bendis, I., and Shapiro, L. (1970). *J. Virol.* **6**, 847.

Ben-Porat, T., and Kaplan, A.S. (1967). *Virology* **32**, 457.

Bentvelsen, P., Daams, J. H., Hageman, P., and Calafat, J. (1970). *Proc. Nat. Acad. Sci. U.S.* **67**, 377.

Berkaloff, A., Bregliano, J. C., and Ohanessian, A. (1965). *C. R. Acad. Sci.* **260**, 5956.

Bradley, D. E. (1967). *Bacteriol. Rev.* **31**, 230.

Bradley, D. E. (1971). *In* "Comparative Virology" (K. Maramorosch and E. Kurstak, eds.), p. 208. Academic Press, New York.

Britten, R. J., and Davidson, E. H. (1969). *Science* **165**, 349.

Britten, R. J., and Kohne, D. E. (1968). *Science* **161**, 529.

Brunt, A. A. (1971). *Ann. Appl. Biol.* **67**, 357.

Callan, H. G. (1967). *J. Cell Sci.* **2**, 1.

Campbell, A. (1962). *Advan. Genet.* **11**, 101.

Campbell, A. (1969). "Episomes." Harper and Row, New York.

Collins, E. B. (1952). *J. Dairy Sci.* **35**, 381.

Collins, E. B. (1955). *Appl. Microbiol.* **3**, 137.

Crick, F. H. C. (1970). *Nature (London)* **228**, 613.

Crick, F. H. C. (1971). *Nature (London)* **234**, 25.

Crill, P., Hagedorn, D. J., and Hanson, E. W. (1970). *Wis., Agr. Exp. Sta., Res. Bull.* No 280.

Danielli, J. F. (1953). *Symp. Soc. Exp. Biol.* **7**, 440.

Darnell, J. E., Wall, R., and Tushinski, R. J. (1971). *Proc. Nat. Acad. Sci. U.S.* **68**, 1321.

Day, L. E., and Ellis, L. F. (1971). *Appl. Microbiol.* **22**, 919.

de Bokx, J. A., ed. (1972). "Viruses of Potatoes and Seed-Potato Production." Center for Agricultural Publishing and Documentation, Wageningen.

Diener, T. O. (1971). *Virology* **45**, 411.

Dmochowski, L., Grey, C., Padgett, F., and Sykes, J. A. (1963). *In* "Viruses, Nucleic Acids and Cancer," Symp. Fundam. Cancer Res., Vol. 17, pp. 85 Williams Wilkins, Baltimore, Maryland.

Dougherty, R. M., and Di Stefano, H. S. (1967). *Cancer Res.* **27**, 322.

Edelman, G. M., and Gall, W. E. (1969). *Annu. Rev. Biochem.* **38**, 415.

Feldman, D. G., and Gross, L. (1966). *Cancer Res.* **26**, 412.

Feldman, D. G., Dreyfuss, Y., and Gross, L. (1967). *Cancer Res.* **27**, 1792.

Fridlender, B., Fry, M., Bolden, A., and Weissbach, A. (1972). *Proc. Nat. Acad. Sci. U.S.* **69**, 452.

Gerber, P. (1972). *Proc. Nat. Acad. Sci. U.S.* **69**, 83.

Gershon, D., Hausen, P., Sachs, L., and Winocour, E. (1965). *Proc. Nat. Acad. Sci. U.S.* **54**, 1584.

Gilden, R. V., and Oroszlan, S. (1972). *Proc. Nat. Acad. Sci. U.S.* **69**, 1021.

Goodman, N. C., and Spiegelman, S. (1971). *Proc. Nat. Acad. Sci.* **68**, 2203.

Gross, L. (1970). "Oncogenic Viruses," 2nd Ed. Pergamon, Oxford.

Hampar, B., Derge, J. G., Martos, L. M., and Walker, J. L. (1972). *Proc. Nat. Acad. Sci. U.S.* **69**, 78

Holmquist, R. (1972). *J. Mol. Evol.* **1**, 115.

Howatson, A. F. (1970). *Advan. Virus Res.* **16**, 195.

Huebner, R. J., and Todaro, G. J. (1969). *Proc. Nat. Acad. Sci. U.S.* **64**, 1087.

Huebner, R. J., Kelloff, G. J., Sarma, P. S., Lane, W. T., Turner, H. C., Gilden, R. V., Oroszlan, S., Meier, H., Myers, D. D., and Peters, R. L. (1970). *Proc. Nat. Acad. Sci. U.S.* **67**, 366.

Hunt, D., Saito, Y., and Watanabe, M. (1971). *J. Biol. Chem.* **246**, 4151.

Jann, K., Schmidt, G., Wallenfels, B., and Freundmolbert E. (1971). *J. Gen. Microbiol.* **67**, 289.

Jawetz, E., Melnick, J. L., and Adelberg, E. A. (1970). "Review of Medical Microbiology," 9th Ed. Lange Med. Publ., Los Altos, California.

Kuff, E. L., Lueders, K. K., Ozer, H. L., and Wivel, N. A. (1972). *Proc. Nat. Acad. Sci. U.S.* **69**, 218.

Lederberg, E. M. (1951). *Genetics* **36**, 560.

Lwoff, A., and Tournier, P. (1971). *In* "Comparative Virology" (K. Maramorosch and E. Kurstak, eds.), p. 2. Academic Press, New York.

MacPhee, D. G., Sutherland, I. W., and Wilkinson, J. F. (1969). *Nature (London)* **221**, 475.

Ma, B. I., Swartzendruber, D. C., and Murphy, W. H. (1969). *Proc. Soc. Exp. Biol. Med.* **130**, 586.

Maramorosch, K., and E. Kurstak, eds. (1971). "Comparative Virology." Academic Press, New York.

Matthews, R. E. F. (1970). "Plant Virology." Academic Press, New York.

Miyake, T., Haruna, I., Shiba, T., Itoh, Y., Yamane, K., and Watanabe, I. (1971). *Proc. Nat. Acad. Sci. U.S.* **68**, 2022.

Merril, C. R., Geier, M. R., and Petricciani, J. C. (1971). *Nature (London)* **233**, 398.

Michel, M. R., Hirt, B., and Weil, R. (1967). *Proc. Nat. Acad. Sci. U.S.* **58**, 1381.

Mise K. (1971). *J. Virol.* **7**, 168.

Moore, D. H. (1963). *Nature (London)* **198**, 429.

Nowinski, R., Edynak, E., and Sarkar, N. (1971). *Proc. Nat. Acad. Sci. U.S.* **68**, 1608.

Ozeki, H., and Ikeda, I. (1968). *Annu. Rev. Genet.* **2**, 245.

Philpott, D. E., Weibel, J., Atlan, H., and Miquel, J. (1969). *J. Invert. Pathol.* **14**, 31.

Preer, J. R., and Jurand, A. (1968). *Genet. Res.* **12**, 331.

Qasba, P. K., and Aposhian, H. V. (1971). *Proc. Nat. Acad. Sci. U.S.* **68**, 2345.

Reanney, D. C. (1968). *N.Z.J. Agr. Res.* **11**, 763.

Reanney, D. C., and Marsh, S. C. (1973). *Soil Biol. Biochem.* **5**, 399.

Robinson, J. B., and Corke, C. T. (1959). *Can. J. Microbiol.* **5**, 479.

Rowe, W. P., Lowy, D. R., Teich, N., and Hartley, J. W. (1972). *Proc. Nat. Acad. Sci. U.S.* **69**, 1033

Rueckert, R. R. (1971). *In* "Comparative Virology" (K. Maramorosch and E. Kurstak, eds.), p. 256. Academic Press, New York.

Schlom, J., Harter, D. H., Burny, A., and Spiegelman, S. (1971). *Proc. Nat. Acad. Sci. U.S.* **68**, 182.

Schuster, F. L., and Dunnebacke, T. H. (1971). *J. Ultrastruct. Res.* **36**, 659.

Shephard, R. J., Wakeman, R. J., and Romanoko, R. R. (1968). *Virology* **36**, 150.

Sivolap, Y. M., and Bonner, J. (1971). *Proc. Nat. Acad. Sci. U.S.* **68**, 387.

Smith, H. S., Gelb, L. D., and Martin, M. A. (1972). *Proc. Nat. Acad. Sci. U.S.* **69**, 152.

Sober, H. A. (1968). "Handbook of Biochemistry," 2nd Ed. Chem. Rubber Publ. Co., Cleveland, Ohio.

Stavrianopoulos, J. G., Karkas, J. D., and Chargaff, E. (1971). *Proc. Nat. Acad. Sci. U.S.* **68**, 2207.

Steiger, U., Lamparter, H. E., Sandri, C., and Akert, K. (1969). *Arch. Gesamte Virusforsch.* **26**, 271.

Stent, G. (1963). "Molecular Biology of Bacterial Viruses," Freeman, San Francisco, California.

Szakal, A. K., and Hanna, M. G. (1968). *Exp. Mol. Pathol.* **8**, 75.

Teh, P., and Reanney, D. C. (1973). *Soil. Biol. Biochem.* Submitted for publication.

Temin, H. M. (1972). *Proc. Nat. Acad. Sci. U.S.* **69**, 1016.

Teninges, D. (1968). *Arch. Gesamte Virusforsch.* **23**, 378.

Thomas, C. A. (1970). *In* "The Neurosciences. A Study Program." (F. O. Schmitt, ed), p. 973. Rockfeller Univ. Press, New York.

Thomas, C. A., Hamkalo, B. A., Misra, D. N., and Lee, C. S. (1970). *J. Mol. Biol.* **51**, 621.

Todaro, G. J., and Huebner, R. G. (1972). *Proc. Nat. Acad. Sci. U.S.* **69**, 1009.

Tomaz, A. (1969). *Annu. Rev Genet.* **3**, 217.

Tsuchida, N., Noboyama, M., and Ikeda, Y. (1971). *J. Gen. Appl. Microbiol.* **17**, 63.

Tsuei, D., Fujinaga, K., and Green, M. (1972). *Proc. Nat. Acad. Sci. U.S.* **69**, 427.

Varmus, H. E., Weiss, R. A., Friis, R. R., Levinson, W., and Bishop, J. M. (1972). *Proc. Nat. Acad. Sci. U.S.* **69**, 20.

Wall, R., and Darnell, J. (1971). *Nature (London), New Biol.* **232**, 73.

Watson, J. D. (1970). "The Molecular Biology of the Gene." 2nd Ed. Benjamin, New York.
Weiss, R. A., Friis, R. R., Katz, E., and Vogt, P. K. (1971). *Virology* **46**, 920.
Weppelman, R., and Brinton, C. (1971). *Virology* **44**, 1.
West, D. A. (1971). *Nature (London)* **229**, 637.
Wildy, P (1971). "Classification and Nomenclature of Viruses." Monogr. Virol. vol. 5. Karger, Basel.
Winocour, E. (1968). *Virology* **34**, 571.
Winocour, E. (1969). *Advan. Virus Res.* **14**, 153.
Winocour, E., Kaye, A. M., and Stollar, V. (1965). *Virology* **27**, 156.
Wolf, K. (1966). *Advan. Virus Res.* **12**, 36.
Zeikus, R. D., and Steinhaus, E. A. (1969). *J. Invert. Pathol.* **14**, 115.
Zur Hausen, H., Diehl, V., Wolf, H., Schulte-Holthausen, H., and Scheider, V. (1972). *Nature (London), New Biol.* **237**, 189.

APPENDIX

Part A. The Numbers of Different Viruses/Species

Bacteria

While phages are known for many bacterial species, in the majority of cases the numbers of characterized phages are gross *underestimates* of the "correct" figures. Hence only the known phages for two intensively researched bacteria, *E. coli* and *Bacillus subtilis*, are given here. There is no reason to doubt that the number of phages for other bacterial species will prove to be much less when the pertinent research is done.

DNA-CONTAINING VIRUSES
FOR *E. coli*

Name	Reference	Name	Reference
T1	Sober (1968)	T2	Sober (1968)
T3	Sober (1968)	T4	Sober (1968)
T5	Sober (1968)	T6	Sober (1968)
T7	Sober (1968)	λ	Sober (1968)
Ω8	Jann *et al.* (1971)	ΦX174	Sober (1968)
WAK(2)	Bradley (1967)	ZG/3A	Bradley (1967)
P1	Mise (1971)	D108	Mise (1971)
N4	Sober (1968)	M13	Sober (1968)
fd	Sober (1968)	E1	Bradley (1967)
		DD7	Sober (1968)
		Y2	Bradley (1967)
		Φ15	Sober (1968)
P2	Sober (1968)	α3	Bradley (1971)
ΦR	Sober (1968)	Φ80	Sober (1968)
χ	Sober (1968)	Sd	Sober (1968)
C16	Sober (1968)	fr	Sober (1968)
AE2	Sober (1968)	f1	Sober (1968)
P2	Sober (1968)	P1kc	Sober (1968)
15	Sober (1968)	Total	34

DNA-CONTAINING VIRUSES
FOR *B. subtilis*[a]

Name	Name	Name
Φ3	F	NT
Φ15	SP10	SP8
Φ29	PBS1	SP82
SP3	PBS2	SPO-1
pKc	SP90	Φe
SPα	SP100	25
SPX	SP70	SP60
SP50	SP80	SP6
Φ1	IKT	7
Φ2	6a	9
Φ14	6b	13
Vx	4P	SPP-1
		Total = 36

[a] From Sober (1968).

Apart from the 18 phages listed below, *all* remaining characterized phages, to the best of our knowledge, contain DNA.

RNA-CONTAINING VIRUSES

Name	Host	Reference
f2	*E. coli*	Mikaye *et al.* (1971)
r17	*E. coli*	Mikaye *et al.* (1971)
MS2	*E. coli*	Mikaye *et al.* (1971)
ZR	*E. coli*	Mikaye *et al.* (1971)
GA	*E. coli*	Mikaye *et al.* (1971)
SD	*E. coli*	Mikaye *et al.* (1971)
SP	*E. coli*	Mikaye *et al.* (1971)
F1	*E. coli*	Mikaye *et al.* (1971)
Qβ	*E. coli*	Mikaye *et al.* (1971)
VK	*E. coli*	Mikaye *et al.* (1971)
ST	*E. coli*	Mikaye *et al.* (1971)
ZIK/1	*E. coli*	Bradley (1971)
ZJ/1	*E. coli*	Bradley (1971)
R23	*E. coli*	Hunt *et al.* (1971)
Y	*E. coli*	Tsuchida *et al.* (1971)
Z	*E. coli*	Tsuchida *et al.* (1971)
Total = 16		
PP7	*Pseudomonas aeruginosa*	Weppelman and Brinton (1971)
ΦCb5	*Caulibacter*[a] *crescentus*	Bendis and Shapiro (1970)

[a] It is not yet firmly established whether this organism possesses a sex factor analogous to the F factor of *E. coli* and the FP factor of *P. aeruginosa*.

VIRUSES INFECTING HIGHER ANIMALS—*Homo sapiens*[a,b]

Virus genus	Number of viruses per genus that infect humans	Ether susceptibility	Budding
RNA-containing viruses			
Rhinovirus	90	—	—
Enterovirus	64	—	—
Reovirus	3	—	—
Arbovirus	84	+	+
Rhabdovirus	3	+	+
Orthomyxovirus	3	+	+
Paramyxovirus	7	+	+
Leukovirus	2	+	+
Coronavirus	1	+	+
Arenavirus	3	+	+
Total	260		
DNA-containing viruses			
Poxvirus	3	—	—
Adenovirus	32	—	—
Papillomavirus	2	—	—
Herpesvirus	6	+	+
Total	43		
Combined total	303		

[a] The classification recommended by the ICNV, 1971 has been adopted except in the case of the arboviruses.

[b] From Jawetz *et al.* (1970) and Wildy (1971).

VIRUSES INFECTING HIGHER PLANTS—ALFALFA[a]

Alfalfa dwarf virus
Alfalfa mosaic virus
Alfalfa witches broom virus
Alsike clover mosaic virus
Bean yellow mosaic virus
Beet curly top virus
Broad bean mottle virus
Clover yellow mosaic virus
Cranberry false-blossom virus
Datura rugose leaf curl virus
Little leaf disease virus
Pea enation mosaic virus
Pea leaf roll virus
Pea streak virus
Tobacco ringspot virus
White clover mosaic virus
Total = 16

[a] From Crill *et al.* (1970).

VIRUSES INFECTING HIGHER PLANTS—POTATO[a]

Potato virus X
Potato virus S
Potato tuber spindle virus
Tobacco mosaic virus
Potato virus A
Potato virus Y
Potato virus M
Potato aucuba mosaic virus
Alfalfa mosaic virus
Potato leaf roll virus
Cucumber mosaic virus
Tomato spotted wilt virus
Andean potato latent virus
Tobacco rattle virus
Potato mop-top virus
Tobacco necrosis virus
Tomato black ring virus
Potato yellow dwarf virus

Total = 18

[a] From de Bokx (1972).

With the two exceptions specified below, *all* plant viruses characterized to date contain RNA.

DNA Containing Plant Viruses	Reference
Cauliflower mosaic virus	Shephard *et al.* (1968)
Dahlia mosaic virus	Brunt (1971)

Part B. Distribution of RNA and DNA Viruses throughout a Major Taxonomic Grouping
—Vertebrates

A simple list of viruses pathogenic for a given organism, e.g. *H. sapiens,* cannot be taken as a statistically significant reflection of the distribution of viruses throughout a wider taxonomic grouping. The list given below is *not* intended as a tabulation of all viruses known for various vertebrates, but rather as a sample, hopefully sufficiently large to have a reasonable chance of reflecting the actual pattern of distribution of RNA and DNA viruses which could be seen to exist in nature if sufficient data were available. It must be remembered that this tabulation probably only represents the "tip of the iceberg" with respect to the number of vertebrate viruses which actually exist. Possibly the *largest* group of viruses—those of a silent or temperate character—are those *least* represented in this table (see text). Except in the case of the arboviruses, the classification used is that recommended by the ICNV, 1971. Host(s) for any given virus represent those thought to be "natural" hosts. However, as viruses are often identified with the species from which they were originally isolated, the natural host range is extremely difficult to assess.

RNA-Containing Viruses

Common name of virus	Host(s)	Susceptible to ether	Budding
Mammalia and aves[a]			
Genus calicivirus			
Feline picornavirus	Cat	No	No
Genus rhinovirus			
Human, 90 serotypes	Human	No	No
Equine, 2 serotypes	Horse	No	No
Bovine, 1 serotype	Cattle	No	No
Foot and mouth disease, 7 serotypes	Cattle	No	No
Genus enterovirus			
Poliomyelitis, 3 serotypes	Human	No	No
Coxsackie, 29 serotypes	Human	No	No
Echo, 32 serotypes	Human	No	No
SA-1	Cattle	No	No
Teschen	Pig	No	No
Mengo	Chimpanzee Baboon Monkey Mongoose Cotton rat	No	No
Murine encephalomyelitis	Mouse	No	No
Duck hepatitis	Birds	No	No
Genus reovirus			
Human reoviruses, 3 serotypes	Human	No	No
Colorado tick fever	Human, rodents	No	No
Monkey reovirus	Monkey	No	No
Canine reovirus	Dog	No	No
Avian reoviruses, 5 serotypes	Birds	No	No
African horse sickness, 9 serotypes	Horse	No	No
Blue tongue, 12 serotypes	Sheep and other artiodactyls	No	No
Epizootic hemorrhagic disease of deer	Deer	No	No
Epizootic diarrhea of mice	Mouse	No	No
Arbovirus group[b]			
East equine encephalitis	Human, horse, mule Birds	Yes	Yes
West equine encephalitis	Human, horse, mule Birds	Yes	Yes

RNA-Containing Viruses (*Continued*)

Common name of virus	Host(s)	Susceptible to ether	Budding
Venezuelan equine encephalitis	Rodents, human	Yes	Yes
Tick-borne encephalitis	Rodents, human	Yes	Yes
Californian encephalitis	Human, rabbit, Squirrel	Yes	Yes
Yellow fever	Human, monkey	Yes	Yes
Ross River	Human, horse, cat	Yes	Yes
Dengue, types 1 to 4	Human, monkey	Yes	Yes
Phlebotomous fever	Human	Yes	Yes
Kyasanur Forest	Human, monkey, rodents	Yes	Yes
Osmk hemorrhagic fever	Human, musk rat, and other rodents	Yes	Yes
Tensaw	Human, rabbit, squirrel	Yes	Yes
Tahyna	Human, squirrel	Yes	Yes
Louping ill	Sheep	Yes	Yes
Bukalasa	Bat	Yes	Yes
Daktar	Bat	Yes	Yes
Entebbe bat salivary gland	Bat	Yes	Yes
Semliki	Birds	Yes	Yes
Whataroa	Birds	Yes	Yes
Japanese encephalitis	Birds, pig	Yes	Yes
St. Louis encephalitis	Birds	Yes	Yes
West Nile fever	Birds	Yes	Yes
Quaranfil	Pigeon, cattle, egret	Yes	Yes
Genus arenavirus			
Junin	Human, rodent	Yes	Yes
Tacaribe	Bats, rodent	Yes	Yes
Machupo	Human, rodent	Yes	Yes
Genus rhabdovirus			
Vesicular stomatitis	Mammals	Yes	Yes
Rabies	Mammals	Yes	Yes
Chandipura	Human	Yes	Yes
Marburg	Shrew	Yes	Yes
IbAn 27377	Monkey	Yes	Yes
Bovine ephemeral fever	Cattle	Yes	Yes
M-1056	Rodents	Yes	Yes
Mt. Eglon	Bat	Yes	Yes
Lagos	Bat	Yes	Yes
Kern Canyon	Bat	Yes	Yes
Piry	Opossum	Yes	Yes
Flanders-Hart-Park	Birds	Yes	Yes

RNA-Containing Viruses (*Continued*)

Common name of virus	Host(s)	Susceptible to ether	Budding
Genus orthomyxovirus			
Influenza, Type A	Human, horse, pig, birds	Yes	Yes
Influenza, type B	Human	Yes	Yes
Influenza, type C	Human	Yes	Yes
Genus paramyxovirus			
Parainfluenza, types 1 to 4	Human, monkeys, cattle, mice	Yes	Yes
Mumps	Human	Yes	Yes
Measles	Human	Yes	Yes
SV5	Monkey, dog	Yes	Yes
Rinderpest	Cattle, sheep, goat, water buffalo	Yes	Yes
Canine distemper	Dog	Yes	Yes
Pneumonia virus of mice	Mouse	Yes	Yes
Newcastle disease	Bird	Yes	Yes
Turkey virus	Turkey	Yes	Yes
Genus coronavirus			
Human respiratory virus	Human	Yes	Yes
Transmissible gastroenteritis of pigs	Pig	Yes	Yes
Mouse hepatitis	Mouse	Yes	Yes
Avian infectious bronchitis	Bird	Yes	Yes
Genus leukovirus[c]			
Mason–Pfizer	Monkeys	Yes	Yes
Feline leukemia	Cats	Yes	Yes
Feline sarcoma	Cats	Yes	Yes
Bittner factor	Mice	Yes	Yes
Murine leukemia	Mice	Yes	Yes
Rous sarcoma	Chickens	Yes	Yes
Reptilia			
Arbovirus group			
Arbo encephalitis viruses	Wild gopher snake Garter snake Blue racer snake	Yes	Yes
Amphibia			
Arbovirus group			
West equine encaphalitis	Leopard frog	Yes	Yes
Pisces			
Genus rhabdovirus			
Viral hemorrhagic septicemia	Rainbow trout	Yes	Yes
Infectious hemopoietic necrosis	Salmon	Yes	Yes

RNA-CONTAINING VIRUSES (*Continued*)

Common name of virus	Host(s)	Susceptible to ether	Budding
Oregon sockeye	Salmon	Yes	Yes
Sacramento River chinook disease	Salmon	Yes	Yes
Genus reovirus			
Infectious pancreatic necrosis	Trout		
Unclassified			
Walleye sarcoma	Walleye	Not known	Yes
Walleye epidermal hyperplasia	Walleye	Not known	Yes
IPN	Trout	Yes	Yes

DNA-CONTAINING VIRUSES

Common name of virus	Host(s)	Susceptible to ether	Budding
Mammalia and aves			
Genus poxvirus			
Sparrow pox	Sparrow	No	No
Rabbit pox	Rabbit	No	No
Squirrel fibroma	Squirrel	No	No
Yaba monkey tumor	Monkey	No	No
Goat pox	Goat	No	No
Variola	Human	No	No
Genus iridovirus			
African swine fever	Pig	No	No
Genus adenovirus[c]			
Human, 32 serotypes	Human	No	No
Simian, 23 serotypes	Monkey, apes	No	No
Bovine, 7 serotypes	Cattle	No	No
Porcine, 4 serotypes	Pig	No	No
Canine, 2 serotypes	Dog	No	No
Murine, 2 serotypes	Mice	No	No
Sheep adenovirus	Sheep	No	No
Avian, 9 serotypes	Chicken, goose	No	No
Genus papillomavirus[c]			
Warts	Human	No	No
Rabbit oral papilloma	Rabbit	No	No
Deer fibroma	Deer	No	No
Genus polyomavirus[c]			
SV40	Monkey	No	No
Polyoma	Mouse	No	No
K	Mouse	No	No
Rabbit vacuolating	Rabbit	No	No

DNA-CONTAINING VIRUSES (*Continued*)

Common name of virus	Host(s)	Susceptible to ether	Budding
Genus parvovirus			
Porcine parvovirus	Pig	No	No
Feline panleucopenia	Cat	No	No
RV	Rat	No	No
Minute virus of mice	Mouse	No	No
Genus herpesvirus[c]			
Herpes simplex	Human	Yes	Yes
Varicella	Human	Yes	Yes
Epstein–Barr	Human	Yes	Yes
B virus	Monkey	Yes	Yes
Herpesvirus T	Marmoset	Yes	Yes
Infectious bovine rhinotracheitis	Cattle	Yes	Yes
Malignant catarrh	Cattle	Yes	Yes
Pseudorabies	Pig	Yes	Yes
Equine herpes	Horse	Yes	Yes
Herpesvirus cuniculi	Rabbit	Yes	Yes
Mouse cytomegalovirus	Mouse	Yes	Yes
Marek's disease	Fowl	Yes	Yes
Avian herpes	Pigeons, owls, parrots	Yes	Yes
Reptilia			
Genus iridovirus			
Gecko virus	Gecko	No	No
Genus herpesvirus			
Snake herpes	Snakes	Yes	Yes
Amphibia			
Genus iridovirus			
Amphibian cytoplasmic virus	Amphibians	No	No
Genus herpesvirus			
Lucké carcinoma	Frogs	Yes	Yes
Pisces			
Genus iridovirus			
Lymphocytic fish virus	*Stizostedion*	No	No

From Maramorosch and Kurstak (1971), Nowinski *et al.* (1971), Wildy (1971), and Wolf (1966).

[a] These have been grouped because many of the viruses listed infect both mammals and birds.

[b] Arthropod vectors are not listed.

[c] Oncogenic members are known for this genus.