GENETICS OF RESISTANCE OF ANIMALS TO VIRUSES: I. INTRODUCTION AND STUDIES IN MICE

Frederik B. Bang

Department of Pathobiology, The Johns Hopkins University, Baltimore, Maryland

I. INTRODUCTION

Inherited resistance to animal viruses may be conveniently classified into three types: monogenetic, following simple Mendelian ratios; polygenetic; and cytoplasmic.

A virus is a unique cellular parasite, dependent upon the host for reproduction and nourishment in a variety of different ways. Since, as with other types of parasites, the host and the parasite have necessarily evolved together, it is a distortion to consider the resistance of the host without considering the evolutionary steps in the development of this extreme form of parasitism. Therefore this discussion is initiated with a review of some of the ideas put forward about host–agent interactions in plants as well as animals.

The importance of genes in regulating resistance to disease (including parasites and parasitoids) is apparent if disease is considered an important evolutionary force. Haldane (1949), in his essay "Disease and Evolution" stated, "I want to suggest that the struggle against disease, and particularly infectious disease, has been a very important evolutionary agent, and that some of its results have been rather unlike those of the struggle against natural forces, hunger, and predation or with members of the same species." There have been several subsequent studies of evolution along these lines (Allison, 1964; Ford, 1964), one of which led to the discovery of a specific example of balanced polymorphism in human populations: the sickle cell gene and partial protection against falciparum malaria (Allison, 1964). Person and Ebba (1975) have suggested that polymorphism is a characteristic manifestation of a reaction to parasites, and indeed Ford's (1964) studies of polymorphism in butterflies point to parasitoids as the selective force in maintaining a particular wing pattern, presumably through a secondary effect of the gene for resistance on the formation of spots on the underwing. So it is established that parasites are selective forces in nature. The selective effects of viruses have not yet been adequately studied. Continued attempts to find a correlation between different blood groups and differing severity of smallpox infection clearly searched for selective forces (Chakravartti et al., 1966), but the results were inconclusive.

Most of our knowledge of genetic resistance to virus disease rests on the study of resistance to selected agents in various inbred strains of mice and chickens rather than on any knowledge of the effects of genetic resistance in a natural heterozygous population. The increasing frequency, however, with which genetic resistance is found is in itself evidence that these genes are important in natural outbred populations. In addition, there are increasing numbers of virus diseases in which the viral agent seems to be inherited in a Mendelian fashion.

A. Plant Pathology

In studying the genetic systems of flax and its parasitic rust, Flor (1956) showed that there were genes for virulence and avirulence in the parasite and that each of these genes was matched by a particular gene in the host. Thus pathogenicity and resistance cannot be dealt with separately. He summarizes: "The type of pustule developed on a host variety following inoculation with a race of rust is the criterion both of the reaction of that variety to the race and of the pathogenicity of that race to the variety." Clearly, then, a study of rust reaction involves the genetic systems of both host and parasite. Because of the close correspondence of the segregation ratios of the F2 generation of the rusts with the number of genetic loci for resistance in the host, Flor suggested that complementary gene systems in host and parasite control rust reactions. In certain rusts of apple trees, it is estimated that there may be as many as 19 such pairs. A gene-for-gene relationship has been demonstrated in host–parasite systems, which include rusts, smuts, mildews, and toxic insects, and has been suggested for bacteria and viruses (Day, 1974).

Viruses, as small parasites, have lost a great portion of their nucleic acid, and usually, but not always, have been reduced to the presence of either DNA or RNA, but not both. The amount of genetic information contained within them is limited. This means, in turn, if Flor's thesis of complementarity of host and parasite genes is valid, that there are limited numbers of genetic loci in the host which are available for resistance to viruses. Indeed, larger agents such as vaccinia would be complemented by larger series of genes which regulate resistance in the host, and smaller agents such as viroids would have fewer complementary genes. This does not of course bear on the question of the number of alleles at a given locus, but only on the number of different genetic loci on the chromosome complex.

Flor's study of the genetics of this interacting system led him to the further step of creating isogenic strains of flax which differed from each other only in the presence or absence of a gene for resistance or suscepti-

HOST

	rs/rs	+/+
p⁻	—	+
p⁺	+	+

VIRUS

FIG. 1. Compatibility between host and virus in the *Drosophila* sigma virus.

bility. This prepared the way for more specific analysis of the biochemical differences between resistant and susceptible races, or between incompatible and compatible interactions. Finally, this has been formalized in terms of a "quadratic check" in which it is assumed that most of the extraneous biochemical reactions not related to the particular interaction of complementary genes have been excluded (Flor, 1956, 1971). Ellingboe (1972) says "There are four possible parasite/host genotypes involving one locus in the parasite and one locus in the host (Fig. 1). Only one genotype P^1/R^1 gives an incompatible relationship between host and parasite (host resistant and pathogen avirulent) as originally identified by infection type. The other three parasite/host genotypes (P^1/r^1, p^1/R^1, and p^1/r^1) condition compatibility between host and parasite. The simplest hypothesis is that a specific host–parasite interaction yields an incompatible relationship. If specific interactions occur yielding compatible relationships, there may still be differences among the three genotype pairs which specify compatibility between host and parasite" (Fig. 1).

It is not known to what extent this system applies to animal virus–host systems, but the high degree of parasitism of viruses suggests that it is applicable. Indeed, a seemingly similar system has been described for mouse hepatitis virus and two strains of mice (Shif and Bang, 1970; Bang, 1972). In Part I of this review we explore the question of its application to mouse leukemia, and in Part II we will examine its relationship to Rous sarcoma infection of chickens. Although plant rust is diploid, and many viruses are dependent upon single strands of RNA or DNA, this does not affect the basic theory.

B. Drosophila

With the overwhelming amount of work which has been done on the genetics of *Drosophila* (fruit flies), one might suppose that the study of resistance to viruses in this species would be extensive. However, the information for this species is limited.

For instance, a study of the spectrum of susceptibility of *Drosophila* and several other dipterans and some lepidopterans showed a wide range of susceptibility from strain to strain (Jousset and Jousset, 1976). No genetic analyses were, however, carried out. Information on the differing susceptibility of strains of *Drosophila melanogaster* to the sigma (σ) virus is, however, of direct relevance to the idea of quadratic checks. σ is the name designated by the students of L'Héritier in France, who with Teissier (1937) discovered an agent which conferred on *Drosophila* the capacity to be killed by anesthization with carbon dioxide. Normal *Drosophila* are put to sleep by exposure to carbon dioxide but awaken afterward. Flies infected with σ do not. This property has been demonstrated to be infectious by inoculation into the appropriate strains of flies and may also be inherited by the progeny of infected females (more regularly) and males.

There are strains of virus and strains of *D. melanogaster* which apparently match each other in terms of compatibility. This may be presented in diagrammatic terms (Fig. 1).

The heterozygote re^+ seems to be intermediate in susceptibility (Gay and Ozolins, 1968; Gay, 1968). Though this work has not been taken up outside of France, it is of interest that it has in many ways foretold the present complicated situation involving combined hereditary and infectious transmission of three animal tumor viruses: mouse mammary tumor (Heston *et al.*, 1976), mouse leukemia (Rowe, 1972), and chicken leukemia (Crittenden, 1976).

For many years the variation in susceptibility of different geographic strains of culicines to infection with viruses such as dengue and western equine encephalomyelitis (WEE) has been noted, as summarized by Hardy *et al.* (1978). However, until the work of these investigators, there had been no attempt to select resistant and susceptible strains and test the resistance in hybrids, F2 generations, and backcrosses. The results showed that a strain of *Culex tarsalis* resistant to WEE crossed with a susceptible strain yielded a hybrid which was susceptible, and that segregation occurred in backcrosses and F2 generations. However, the data did not allow a determination of how many genes were involved. Finally, resistance was apparently due to a block in the penetration of the gut, since inoculation of virus into the thorax showed no difference between the resistant and susceptible strains.

C. Other Insects

In honeybees, it has been shown that lines resistant and susceptible to a presumed virus causing the hairless black syndrome may be developed (Kulincevec and Rothenbuhler, 1975). Resistance to the growth of a

nuclear polyhedrosis virus in an armyworm (caterpillar) has been studied on the basis of virus yield from experimentally infected animals. F1 and F2 backcross results followed those predicted on the basis of one gene (Reicheldoerfer; Benton, 1974). There is a scattering of other studies on silkworms and cabbage butterfly larvae, and so on, but as yet there is no coherent body of knowledge comparable to that for plant pathology.

D. Dominance

Since the time of Mendel the capacity of one gene to be phenotypically dominant has been well recognized, even though the variety of intermediate effects obtained in various heterozygotes is equally well known. Many of these intermediate effects are related to the fact that single doses of a gene may lead to production of about half the amount of a protein that a double dose produces. In several virus systems, there is intermediate susceptibility in the F1 generation.

It might be supposed that, if a gene for susceptibility is shown to be dominant, as is the case in mice reacting to mouse hepatitis, it would follow that the susceptible phenotype has manufactured some substance essential to the growth of the virus, whereas, if resistance is dominant, as in arbovirus infections of mice, the phenotype is able to make a substance which blocks the growth of the virus. However, the demonstration by Dickenson that the same gene for resistance to scrapie may be dominant in one genetic background and recessive in another (Dickinson and Miekle, 1971) puts a burden on such a simple explanation. A more important objection is inherent in the repressor–operon theory of gene action. Since a repressor gene suppresses the action of both alleles, this means that a genetic heterozygote may *fail* to produce the necessary substance even though only one allele carries the repressor (Hartman and Suskind, 1964). Thus the current classification of dominance and recessiveness of genes for susceptibility and resistance tells us nothing about the mechanisms of interaction of host and parasite genes.

The study of the genetics of resistance and susceptibility of vertebrates to viruses started with an examination of inherited differences among strains of animals to particular agents (Webster, 1933). It led to the brilliant demonstration by Sabin (1952a) of the Mendelian and unifactorial nature of a dominant gene in mice for resistance to arboviruses, particularly yellow fever. Following this, it was shown that resistance may be studied in specific cells in tissue culture in the case of mouse hepatitis (Bang and Warwick, 1960), Rous virus (Vogt, 1969), and West Nile encephalitis (Goodman and Koprowski, 1962). More recently, dominance–recessiveness relationships in terms of virus interaction have been analyzed by somatic cell hybridization techniques. (This is not discussed

here.) The greater ease of study of cell virus relationships in dispersed animal cell systems has meant that, at the cellular level, advances in animal virology have passed those of plant virology. Genetic analysis of the resistance of chicken cells to various strains of Rous virus has led to new concepts in tumor virology and opens up the possibility of using cell and virus variants to analyze in greater detail the steps in virus growth within cells (Vogt, 1977). Finally, population analyses of the prevalence of certain virus markers, such as Au antigen in hepatitis and intracellular tumor virus antigens, have in recent years brought the subject back to considerations of evolution, an area investigated originally by Haldane (1949). At the present stage of beginning knowledge, a critical review can proceed only by discussing each virus or group of viruses individually. In this process there are basic questions which can be asked concerning all the groups. They include:

1. *How has the genetic compatibility of host and virus interaction been measured?* Is the difference between resistant and susceptible strains consistently and statistically significant?

2. *Does the inherited resistance follow the predictions of Mendelian ratios?* The minimum amount of evidence for judgment on this includes demonstration of segregation ratios of the F2 generation and backcrosses. Occasionally even this is insufficient, as in Wright's classic morphological studies on guinea pigs (Wright, 1934).

3. *Are one, two, or three genes involved, and are they independent or separate?* Linkage usually indicates association on one chromosome, but there are claims of para-linkage due to other factors in mice (Michie, 1955–1956).

4. *Are there cytoplasmic factors in the uninfected host which modify the virus infection?* Cytoplasmic resistance to viruses has not yet been adequately described, but there is increasing knowledge of mitochondrial and chloroplast inheritance in general (Sager, 1972; Jinks, 1964). Possibly the phenomena of helper viruses and dependent viruses can be included under this heading. In one way, helper viruses may substitute for the defective matching of host and virus—supplying a component that might be furnished by the host. Mitochondrial genetic effects would certainly confuse the study of phenotypic changes in host cell susceptibility in tissue culture. Day (1974) has summarized data which indicate that cytoplasmic inheritance of resistance to pathogenic fungi in a variety of plants may be due to viruses.

5. *Is genetic resistance manifest in the animal at all ages (ontogeny of resistance)?* This is a particularly difficult problem, since the ontogeny of the immune lymphocyte system overlaps with the apparent cellular resistance.

6. *In what cellular type or tissue has resistance been demonstrated?* Differences between susceptibility of macrophages and fibroblasts from the same animal have now been shown in both mouse hepatitis virus and chicken oncornaviruses.

7. *Is there any way of modifying genetic resistance by drugs or environment?* These modifying factors may be the essential ones for survival of viruses in natural heterozygous animal populations.

8. *Are there viral variants which overcome cellular genetic resistance?* Such information is standard for bacteria–bacteriophage systems, but it is just beignning to be available for animal virus systems. Such variants are probably frequent but have not been searched for consistently. In other words, has a beginning inquiry into a quadratic check been made?

9. *Does a compatible pair of virus and host cells yield a one-hit curve of lesions and, when genetic resistance is overcome, does the curve change?* Information on this question may help determine whether separate components act together to produce infection. Recent studies of Rochovansky and Hirst (1976) on the reconstitution of infectious activity from influenza virus, in which a curve of infectivity consistent with seven infectious units was obtained, serve as an example. Considerable data have accumulated on the mouse leukemia system.

Some of the material presented in this article has already been covered in Fenner's excellent review on the genetic aspects of diseases of animals (1970), but his review is more oriented toward potential molecular mechanisms and includes considerations of wide genetic differences between species and different hosts. Other earlier reviews are those of Dickinson and McKay (1967) and of Allison (1965). We have sought in the present review to limit the discussion to differences between genetically manipulable members of a species. We have not included differences between viral interactions in cell lines from the same host, such as those of encephalitis and two rat cell lines (Bang *et al.*, 1957; Bang and Gey, 1952) or influenza virus and human cell lines (Wong and Kilbourne, 1960).

Finally we have not included consideration of the relationship of viral susceptibility to the *H-2 locus*. Doherty and Zinkernagel (1975) showed that a requirement for the killing of virus-infected cells by T cells was that both cells be of the same histocompatibility type. This discovery will aid greatly in analysis of viral immune mechanisms but is not directly relevant to the genetics of immune response, since differences in the histocompatibility of these two cells would occur only in chimeric animals. However, the association of certain virus diseases such as human hepatitis with a particular blood type or *H-2* antigen is partial evidence for genetic variation in susceptibility and is included (Hillis *et al.*, 1977).

A general review of this subject may be found in Klein's recent book, "Biology of the Mouse Histocompatibility-2 Complex" (Klein, 1975).

II. GENETICS OF MICE

Almost all our knowledge of genetic resistance to viruses in animals comes from studies on mice and chickens. Since this may have introduced a strong bias, it is of interest to examine how these two species came to play such a large role. In this section we consider the origin and development of inbred mice, and in Part II (Section X) we will discuss the development of inbred chickens.

Schwarz and Schwarz (1943) suggest that there were three major centers in the world where commensal mice developed from wild stocks. One was the borderland plateau of contemporary Turkistan and Iran, where wheat and barley were originally cultivated. The second was the wheat-growing area of southern Persia, and the third was the intensively cultivated portion of Japan. Most of the commensal mice developed from *Mus musculus wagneri* which came from Russian Turkistan. The laboratory mouse was apparently derived from a Japanese pet mouse, the "fancy mouse," which was kept more for display than for research.

"It is difficult to ascertain how long varieties of the house mouse have been recognized in China. The word for white mouse is ancient, and that for spotted mouse appears in the earliest Chinese lexicon, written in 1100 B.C. The waltzing variety has been known since 80 B.C." (Keeler, 1931). The Japanese waltzer was apparently derived from a strain or subspecies in Tibet. In their fancy strains the Japanese favored albinism, nonagouti, waltzing, dominant and recessive spotting, lethal yellow, and other characteristics. These different fancy strains, without waltzing, spotting, or lethal yellow characters, may be considered the precursors of modern strains, having doubtless been subject to a degree of inbreeding through the process of domestication. Several fancy varieties of the house mouse were taken from Japan to Europe and, by the turn of the century, "muriculture" had spread to America. Other present-day lines were derived from European mice—such as the "Swiss" mouse.

Despite this inevitable start on inbreeding, it is important to note that inbreeding of various random-bred strains of mice has not always succeeded. Indeed, Falconer (1964) recorded that, when 30 different lines of mice from a random-bred population were inbred by full sib mating for only three generations, two of the lines were lost. Continued inbreeding produced further depression and inevitably selection.

With the development of the Mendelian theory of heredity, Bateson (1909) and others turned to the mouse to test the heritability of different

traits. From their work came studies on coat color (Little, 1913). When Jensen (1908) showed that certain rat tumors could be transmitted by grafting from one rat to another, interest in the hereditary nature of cancer quickened, and a controversy as to the nature of this genetic factor arose. It was not possible to analyze these factors until it was clearly emphasized both by Little (1956) and Strong (1976) in the 1920s that only with relatively pure strains of mice, produced by continuous inbreeding, would it be possible to determine the relative roles of heredity and environment. This led to the establishment of a series of strains of mice at the Jackson Laboratory, Bar Harbor, Maine, and elsewhere, and it is through the use of such strains that knowledge of genetic resistance and susceptibility has been furthered. Actually relatively little fruitful work was carried out by selecting mice on the basis of their resistance or susceptibility to infectious agents from outbred stocks (see Section III). Thus the goal of inbreeding aimed at the study of cancer led to the definition of viral and host genome in the production of leukemia in some strains of mice, and to determination of the origin of mammary cancer, i.e., transmission of the agent by milk in some cases (Bittner, 1940; Andervont, 1940) and by genes in others (Heston *et al.*, 1976).

Again it was a chance observation by Tyzzer (1918) who, while working with transplantable tumors in Japanese waltzing mice, found that an epidemic bacterial liver disease caused by *Bacillus piliformis* affected the waltzing mice but had no apparent effect on the Swiss mice to which the tumors were being transplanted. In addition, various F1 generations of mice from crosses between regular laboratory mice and waltzing mice did develop the disease. Finally, since the F2 generation often had the disease but was not uniformly killed by it, he was led to emphasize the hereditary aspects of susceptibility. In a later, almost direct follow-up of this study, Gowen and Schott (1933) observed susceptibility to *B. piliformis* in the F1 generation and in various backcrosses of waltzing mice with various Chinese lines and black-eyed whites which were not susceptible. They concluded that susceptibility to the disease may be dependent upon a single major factor difference, with modifying factors playing a small part in expression of the disease. Dominance of the resistant allelomorph appeared to be complete in one cross, but incomplete in another. They further showed that the factor was not linked to the character for waltzing or coat color. This work is of interest to virologists, because it is concerned with a spontaneous disease caused by an intracellular bacterial parasite and supports the idea of the unifactorial nature of genetic resistance in some situations.

The degree to which viruses that are clearly associated with a particular host genome may nevertheless be transmitted to a totally different

species is unknown. It has been suggested that viruses which incorporate a small part of the host genome may act as evolutionary forces (Zhdanov and Tichenko, 1974), and it has been proposed that xenotropic type-C viruses may have been transferred from *Mus caroli* to primates through the carnivorous habits of the latter (Lieber *et al.*, 1975). Whether such a transfer also acts as a mechanism of transferring mouse genes to primates is an open question.

Since the mouse has been the model animal in studies of gene mapping and linkages, it is pertinent here to point out that linkages of characters in the F2 or backcross generations have not always been explicable by the association of the two characters on the same chromosome. This para-linkage is discussed by Robinson in his book, *Gene Mapping in Laboratory Mammals* (1972). Specific examples relate to species crosses in mice (Gates, 1926; Little, 1927; Green, 1931). This has occasionally been a problem in mapping the genes for viral resistance, as seen in Section VIII (Michie, 1955–1956).

III. Arboviruses (Flaviviruses)

The agents in this group are nearly always transmitted by mosquitoes or ticks (Russian spring encephalitis can be transmitted by milk) and have similar antigenic, cultural, and morphological characteristics. The first work on genetic resistance to an animal virus was done on group-B arboviruses (now known as flaviviruses) (Webster, 1933). Since then there have been more studies on the genetics of resistance, the mode of action of this resistance, and the different types of tissue affected, both *in vivo* and *in vitro,* than with any other nontumor viruses. Thus it is appropriate to commence with this group. The flavoviruses are taxonomically in the middle of the RNA virus group and are neither as complex as the oncornaviruses nor as small as scrapie virus, which is presumably on the border line of "true" viruses.

More than 50 years ago, Leslie Webster, at the Rockefeller Institute in New York, became interested in the multiple factors responsible for epidemics of different diseases and searched among different animal models for appropriate examples. He concentrated first on bacterial diseases such as paratyphoid of mice and, impressed by the increasing knowledge of the genetics of mice, sought to obtain paratyphoid-resistant and -susceptible mice by selective breeding. He succeeded in establishing several lines and found that some were either resistant or susceptible to louping ill virus (a disease of sheep whose viral etiology was early established). Resistance was not absolute in any line, but sharp differences in the percentage of mice that became ill following a standard intranasal

inoculation were maintained (Webster, 1924). The availability of St. Louis encephalitis virus (later known to be within the same group) following the epidemic of 1933 in St. Louis meant that another important series of studies could be undertaken (Webster and Clow, 1936; Webster and Johnson, 1941). Despite the absence of a *complete* difference in susceptibility (the susceptible strain had a 95% mortality and the resistant strain a 15% mortality) Webster and Clow (1936) were able to make the first analyses of the genetic nature of the difference. In mice strains that had been inbred by brother–sister mating for 12 generations, the amount of virus was about 100 to 100,000 times greater in the brains of the susceptible mice than in those of the resistant mice, even though it appeared to enter by way of the olfactory lobe of both strains following intranasal inoculation. The virus was successfully transferred by intracerebral inoculation to the resistant mice but did not seem to acquire any new host range characteristics after several successive passages.

Webster (1937) made several crosses between resistant (15%) and susceptible (95%) lines of mice and obtained intermediate susceptibility (31%); he also made backcrosses of the F1 mice with both the resistant and susceptible lines. In the offspring of these animals he obtained intermediate results. He interpreted this to mean that resistance was dominant and unifactorial.

Webster and Johnson (1941) then made the first tissue culture analysis of the phenomenon of genetic resistance. They showed that, when minced brain of mouse embryos was grown in Tyrode's solution with 10% serum (see Section III,A), the virus attained a titer of 1/10,000 in cultures made from susceptible mice but reached only 1/160 in cultures from genetically resistant mice. They found this difference even after eight tissue culture passages (made presumably in susceptible brain cultures) and always obtained 10 to more than 100 times as much virus from the cultures derived from susceptible mice. They showed that it was immaterial whether the serum in the culture came from the susceptible or resistant mice or from rabbits. The source of the brain tissue was crucial.

Two years later, Casals and Schneider (1943), at the same institution, showed that Russian spring-summer encephalitis (another group-B member) was also best able to grow in and kill the same strains of mice which were susceptible to louping ill and St. Louis encephalitis. Resistance was manifest following both intracerebral and subcutaneous inoculation.

With the adaptation of yellow fever virus to the mouse (Theiler, 1930), this virus also became available to the laboratories of the Rockefeller Foundation in New York, and very soon afterward Lynch and Hughes (1936) analyzed the genetic nature of the susceptibility to this virus.

(Fortunately for future events, this virus was also later found to belong to the flavovirus group.) They analyzed two other strains of mice that had been irregularly inbred, one of which (Swiss) had 100% mortality after intracerebral inoculation and the other 77% mortality. They found the F1 generation intermediate, 83.3%, and the backcrosses with the resistant and susceptible lines were also intermediate between the two constituents. They concluded that hereditary factors for resistance to yellow fever existed in mice and that the factors for susceptibility and resistance segregated in the F2 generation. They did not indicate how many factors were involved.

The stage was thus set for the chance discovery by Sabin (1952a) that a strain of partially inbred mice maintained at the Rockefeller Institute's Division of Animal and Plant Pathology at Princeton was completely resistant to yellow fever virus when adults were tested by intracerebral inoculation, and that the appropriate crosses and backcrosses showed that resistance was due to a unifactorial dominant gene. Through wartime duties, Sabin had been brought in contact with the Princeton branch of the Rockefeller Institute, which was at that time rather out of touch with the New York institute so that the sharp genetic difference between Webster's Swiss mice and the Princeton Swiss mice (afterward referred to as PRI) had been missed. Sabin quickly established three basic principles in the genetic analysis: (1) the unifactorial nature of resistance to group-B arboviruses, (2) that the young mice of the resistant strains were susceptible, and (3) that different members of the group-B viruses (French neurotropic yellow fever virus, and later mouse passages of Japanese B encephalitis) were less affected by this genetic resistance.

This semihistorical analysis of genetic resistance and susceptibility does not continue into the next section, but two conclusions may be drawn from this discussion. Chance and personal interest both had important roles in the selection of the models that were studied. It is fair to predict that the power of genetic tools in understanding pathogenesis and molecular sequences in virus growth in many other families of viruses will grow as new chance discoveries are made.

Table I summarizes the main points in the study of genetic resistance to the flavovirus group B. All the agents, louping ill, St. Louis encephalitis, Russian spring-summer encephalitis, yellow fever, and West Nile virus belong to the same group and thus may be subject to the same host mechanisms of resistance and susceptibility. However, none of the points listed in the table have been demonstrated for each of the different viruses, and future work should include both a search for other mechanisms of resistance and a study of ways in which genetic resistance may

TABLE I

CHARACTERISTICS OF GENETIC RESISTANCE TO FLAVIVIRUSES

		Reference
Nature of inheritance of resistance		
Mendelian, dominant, unifactorial	Clear-cut inherited differences in susceptibility, agreeing with expected values in backcrosses	Sabin (1952a,b)
	Continued 50% resistance in appropriate backcrosses	Groschel and Koprowski (1965)
	Coisogenic lines show sharp differences	Vanio (1963); Darnell and Koprowski (1974)
Macrophages of spleen and peritoneum	Somewhat higher yield and greater destruction produced by virus in cultures from susceptible animals	Goodman and Koprowski (1962)
Lung and brain	Higher yield of virus	Webster and Johnson (1941)
Lung, brain, and kidney	—	Jacoby and Bhatt (1976)
Cellular mechanisms of resistance		
Inadequate data on adsorption		Vainio (1963)
No difference in interferon production, but resistant cells are more susceptible to specific effect of interferon; not confirmed but increased interference on passage in resistant cells		Hanson et al. (1959)
		Darnell and Koprowski (1974)
Virus multiplies in brain of resistant animal, but titer remains low and host survives		Jacoby and Bhatt (1976); Goodman and Koprowski (1962); Sabin (1952a)
Gene has no effect on group-A arboviruses or other neurotropic viruses		Sabin (1952a)
Ontogeny of resistance		Sabin, (1954)
Eight gm mice not resistant, 20 plus gm mice were resistant		
Phenotypic alteration of genetic resistance		
Resistant macrophages and lymphocytes confer some immunity on susceptible strains; cortisone did not eliminate resistance		Goodman and Koprowski (1962)
Genetic resistance and virus mutants		
17D yellow fever kills no resistant mice; French neurotropic yellow fever virus multiplies and kills some resistant PRI		Sabin, (1954)
Mouse-adapted Japanese B encephalitis kills resistant PRI		Sabin, (1954)

be phenotypically modified. Sabin (1954) did a preliminary study on the mode of inheritance of partial resistance of PRI mice to French neurotropic yellow fever virus.

The evidence that a unifactorial dominant gene operates to produce resistant strains of mice is very good. Resistance was demonstrated both in the mice derived from Webster's original selected virus-resistant mice and in the PRI mice. This resistance was inherited in an astonishingly regular and predictable way in Sabin's test for susceptibility to the famous 17D yellow fever vaccine virus. In subsequent studies by Goodman and Koprowski (1962), the resistant gene from the PRI mice was introduced into susceptible C3H mice by making crosses between the two and then crossing these F1 mice with the susceptible C3H mice and testing for susceptibility to yellow fever virus. The survivors of this challenge (presumed to be genetically resistant), were kept and crossed again with the susceptible C3H. By continuing this for eight generations, a congenic strain was obtained (Groschel and Koprowski, 1965) which, according to arithmetical estimates should have been 99.6% similar to the susceptible C3H strain but which contained the gene for resistance. Repeated tests showed that 50% of the offspring of backcrosses resulting from the mating of the heterozygotes with the susceptible mice were resistant. The resistant backcrosses were inbred, susceptible offspring were discarded, and this procedure was repeated until a coisogenic group of mice was obtained. A comparison of the susceptibility of macrophage cultures from these mice showed that virus grew in cultures from the susceptible mice but not in those from the resistant line. This evidence of unifactorial resistance was confirmed by Vainio and Koprowski (1962). Genetic resistance to St. Louis virus was not conferred on susceptibles by foster nursing (Wright, 1940).

Although some strains of resistant mice (C3H and BRVR) have been shown to be better able to regulate their temperature (Lagerspetz et al., 1973) than susceptible (C3H/He) mice, direct experimental manipulation of body temperature failed to alter the course of the disease in the two strains (Darnell and Koprowski, 1974).

A. Cellular Representation of Genetic Resistance

In a study of the mechanism of action of the gene for resistance, a series of primary cultures was made (Goodman and Koprowski, 1962). These cultures of spleen cells, consisting predominantly of macrophages, were inoculated with West Nile virus, and the amount of virus was tested on days 2 through 7. Differences in virus yield became apparent on day 4. In other cultures of peritoneal macrophages, the susceptible cultures

yielded 650,000 and 250,00 plaques, while the resistant ones yielded 0, 30, and 0, respectively. Lung cultures studied in the same laboratory (Vainio, 1963) also showed a difference, but kidney cultures grown in human serum and yeastolate, showed no significant (Goodman and Koprowski, 1962) or small (Vainio, 1963) differences. These experiments therefore emphasized the role of macrophages in disseminating the infection.

Cultures were also tested for susceptibility by inoculating infectious or ultraviolet-inactivated virus and determining both cell destruction and the presence of specifically stained antigen, using fluorescent antibody. This was also done with West Nile virus (Vainio, 1963). There was diffuse staining in 65% of the susceptible cells, and 12% of the cells in the resistant line showed antigen. Again there was no difference between resistant and susceptible kidney cultures. The lower *in vitro* yield from resistant brain tissue found by Webster and Johnson (1941) was later confirmed by Goodman and Koprowski (1962). Finally, Darnell and Koprowski (1974) showed that fibroblasts (second passage) from mouse embryos yielded much lower titers of virus than similar cultures from susceptible embryos. This difference persisted even when the cells were transformed by SV40 virus before infection with West Nile virus.

B. Ontogeny of Resistance

Although no extensive work has been done on this subject, Sabin (1954) has repeatedly emphasized that very young mice are susceptible, even when they are from a uniformly resistant mouse line. Mice of the PRI (resistant) line were affected when they were 8 gm in weight (7/10 died) and at 12–13 gm (2/20 died), but not by the time they had reached 20–25 gm (0/20 died).

Jacoby and Bhatt (1976) found that resistance not apparent at 3 weeks of age became manifest at 4 weeks and increased up to 8–12 weeks.

C. Cellular Mechanism of Resistance

The data on adsorption of the virus to resistant and susceptible cells are probably not adequate, since thermal degradation of the virus was so rapid that no difference could be found. Two studies showed that the production of interferon was greater in susceptible cells, which yielded more virus (Hanson et al., 1959; Vaino et al., 1961), but Hanson, Koprowski, Baron, and Buckley (1959) have indicated that cells resistant to flavoviruses are more susceptible to the action of interferon against flaviruses than are susceptible cells. They have adduced five sets of data in support of this:

1. The differential growth of West Nile virus, which occurred in cultures from coisogenic lines, appeared late in the growth curve (20–50 hours).

2. Inoculation of cultures with high concentrations of virus in brain tissues (presumably more interferon) caused a decreased virus yield in the resistant cultures, but not in the susceptible ones, while inoculation with low doses of virus produced little difference in virus yield from the two kinds of cultures.

3. The differential effect was blocked by actinomycin D.

4. There was a greater sensitivity of the resistant cells to interferon when the growth of West Nile virus or Ilheus virus (group B) was tested, but this differential effect did not appear with group-A viruses or vesicular stomatitis virus.

5. Cultures from the three strains of mice (homozygous, heterozygous-resistant, and homozygous-susceptible) differed from each other on the basis of gene dosage. The heterozygotes showed an intermediate sensitivity to interferon.

In a later paper dealing primarily with fibroblasts, however, Darnell and Koprowski (1974) found that resistant cultures were *not* more sensitive to the action of endogenous interferon. Although exogenous interferon suppressed virus multiplication a little more in resistant cells, "It seems likely, however, that interferon-mediated suppression of WNV production in resistant cells was merely superimposed on an already existent, genetically controlled restriction of replication of group B arboviruses." However, passage of undiluted virus in resistant cultures interfered with hamster brain-propagated virus, and similar passage fluid in susceptible cultures did not produce such interferon. Thus the latter workers suggest that the interference apparent late in the growth curve of the virus in resistant cells is due to a greater production of defective interfering particles.

D. Host

Resistance and susceptibility to virus infection is now known to be a complex series of events involving route of inoculation, rapidity of antibody response, T-cell activity, interferon production, and a variety of other factors. Genetic factors, especially monofactorial Mendelian ones, theoretically may function at any step in this complex sequence. The study of the pathogenesis of any virus infection seeks to specify the particular action of the various factors in a given infection. Thus a comparison of the pathogenesis of infection in genetically resistant and in susceptible mice is of particular interest, especially when it is carried out

in congenic mice, i.e., mice that differ genetically only in the gene for susceptibility or resistance (Section IV).

In the earliest experiments on genetic resistance to flavoviruses, Webster and Clow (1936) showed that virus injected into resistant mice (not completely inbred) had a distribution similar to that in susceptible mice. The minimum infecting dose was 1000 times more for resistant mice (as tested by mortality) than for susceptible mice.

Jacoby and Bhatt (1976) recently made a careful pathological comparison of the progress of Banzi virus (another member of the flavovirus group) in congenic strains of resistant and susceptible mice obtained from the Koprowski group. They also subjected the resistant mice to various regimes of immunosuppression. The use of the same congenic strain makes a comparison with previous work easier, but since they used another virus it is possible that other factors were involved. In this connection it is important to point out that this strain of congenic mice was established after only eight backcrosses and therefore the two strains probably differed from each other at several loci, including the locus for genetic resistance to flavoviruses.

However, the two strains of mice differed markedly in susceptibility as measured by mortality when the virus was given intraperitoneally. This group of arboviruses is well known to contain members that are avirulent if given intraperitoneally but which kill if given intracerebrally. Thus the two strains of mice, C3H/He and C3H/RV, did not differ in susceptibility when inoculated intracerebrally. In other words, the resistant mice did not inhibit the early multiplication of virus and may have allowed sufficient multiplication so that immunization took place even after minimal amounts of the agent were used in the resistant animals. Similar amounts of virus developed in the spleen and thymus of the resistant and susceptible mice but, as in the original work of Webster and Johnson (1941), about 100 times more virus was found in the brain of the susceptible animals.

E. Phenotypic Alteration of Genetic Resistance

Immunosuppression by X ray, cyclophosphamide, and thymectomy were all effective in phenotypically changing resistant mice to susceptible mice. In contrast to mouse hepatitis, the ontogeny of resistance was somewhat delayed, in that resistance was not clearly manifest until 4 weeks of age. Although Jacoby and Bhatt (1976) did not test for in vitro tissue susceptibility, they agree that their results do not support the idea of differential tissue susceptibility as the cause of differences in susceptibility. This is endorsed by extensive histological studies on different tissues.

Goodman and Koprowski (1962) performed a series of experiments in

an effort to transfer cells between an inbred resistant mouse strain and its congenic susceptible. Neonatal mice of the PRI resistant strain were given spleen or bone marrow cell suspension from adult C3H susceptible mice. Runts were produced by the bone marrow injections. In these combined transplanted chimeras, 7/11 of the tolerant mice in which skin transplants were maintained became susceptible and died with good titers of virus in the brain. None of the untreated PRI mice was susceptible.

When resistant mice were treated with thioguanide and subsequently inoculated with cells from susceptible mice, 10/15 were shown to be susceptible, whereas 0/19 controls were susceptible, as reported by Goodman and Koprowski (1962). Since the number of successful experiments in this series was small, these workers were cautious in their conclusions.

Cortisone treatment of resistant mice did not make them susceptible, and irradiation increased mortality but did not increase virus titers. Resistance was suppressed by 6-thioguanide treatment, and virus concentration was increased in one strain of resistant mice but not in the other. Endotoxin given 24 hours before the virus, caused resistant mice to succumb to infection in 3/4 cases.

F. Interaction of Genetic Resistance and Virus Mutants

Webster and Clow (1936) were unable to adapt St. Louis virus to improved growth in mice with a high inborn resistance, but the virus was not passed through the resistant mouse brains very many times. Sabin (1952a) emphasized the susceptibility of the resistant strain of mice to mouse-adapted strains of virus. This is of particular interest in light of the idea that interference is responsible for resistance, since the French neurotropic strains titered in PRI (resistant) mice showed the same titer as in susceptible mice but the titration figures indicated considerable autointerference.

In a search for the resistance gene in 11 different inbred strains of mice, Darnell et al. (1975) found that no previously untested strains contained this gene. Each of three sources (a total of 15 mice) of wild house mice was resistant, and 80% of wild mice from a source in Maryland were resistant. In the last instance, the mice were laboratory descendants of wild-caught mice, so that acquired resistance could be ruled out. They concluded that wild mice and strains BRVR, PRI, and C3H/RV contained the resistance gene.

IV. Mouse Hepatitis Virus

Mouse hepatitis virus, a member of the coronavirus group (Andrews and Pereira, 1967; McIntosh, 1974) and the only one of this group which has been studied from the point of view of genetic resistance and suscep-

tibility, has several unique characteristics. It is often latent in colonies of mice (Gledhill, 1956) and has been discovered or evoked on several occasions which seem to have in common disturbances of lymphocyte activity. It has been evoked twice following the passage of leukemia cells (Nelson, 1972; Brounsteiner and Friend, 1954); once in runts, produced in neonatal thymectomy or in nude mice (East *et al.*, 1963; Hirano *et al.*, 1975), and by administration of antilymphocyte sera (van der Riet *et al.*, 1973). Certain strains are dependent upon a concomitant microbial associate (epierythrozoon) for full pathogenic effects in the host (Gledhill *et al.*, 1952; Piazza, 1969). There are three recognized strains: MHV-1, MHV-2, and MHV-3.

The study of mouse hepatitis virus offers unique opportunities to virologists interested in mechanisms of host resistance. First, the difference as measured by mortality between the susceptible and resistant strains is as great as 10^8 in mice and 10^6 in macrophage cultures. Second, the specific role of macrophages interacting with lymphocytes in determining the outcome of infection has been established in several different laboratories with both MHV-2 and MHV-3. Third, phenotypic alteration of genetic resistance has been demonstrated for both strains. Finally, the virus itself has been extensively characterized.

The genetic component of host resistance and the selective effect of the mouse-adapted strains of MHV-2 on macrophages were discovered by chance. The virus available for study grew well in PRI mice. Attempts to grow the virus in several varieties of liver culture had failed until, by chance, cultures of PRI liver grown on a collagen substrate produced an excess of macrophages and it was noted that these cells were destroyed *in vitro*. (Bang and Warwick, 1959). When similar cultures from another strain of mice (C3H) were tested for susceptibility to the virus, they appeared to be resistant (Bang and Warwick, 1960), and subsequently the series of genetic studies outlined in Table II was carried out.

It is important to emphasize that the genetic aspects of the compatibility of the virus and the host have been investigated over a very limited range of variation. There has been insufficient study of different strains of virus adapted to resistant hosts and of adult mice of different ages. It is therefore quite likely that other patterns of compatibility will be found which have very different molecular mechanisms. Equally susceptible (in terms of LD_{50}) strains of mice have been shown to differ markedly in the rate of multiplication in the tissues and the time of death (Taguchi *et al.*, 1976) (Fig. 2).

Since MVH-3 has been shown to mirror the capacity of MHV-2 to grow in and kill inbred strains of mice and in addition causes giant cells in macrophage cultures taken from susceptible mice, data from the

TABLE II

CHARACTERISTICS OF GENETIC RESISTANCE TO MOUSE HEPATITIS

|	Reference
Nature of genetic resistance .	
MHV-2 or MHV(PRI), Mendelian, unifactorial, resistance recessive	Bang and Warwick (1960); Kantoch et al. (1964)
Coisogenic line after 20 backcrosses still segregates at 50% susceptibility	Weiser et al., (1976)
Tissues affected in vitro	
Macrophages by unadapted virus	Bang and Warwick (1960)
Liver on continued passage	Kantoch et al. (1964)
Other cells with tissue culture-adapted strains; MHV-3 has degrees of effect on macrophages in culture directly comparable to pathogenicity for mice	Virelizier et al. (1976)
Ontogeny of resistance	
Newborn C3H resistant fully susceptible; gradual acquisition of resistance; macrophages reflect this	Gallily et al. (1967)
Combination of spleen macrophages and T cells from adult mice confers resistance	Levy-Leblond and Dupuy (1977)
Neonatal thymectomy prevents development of resistance	Piplani and Aikat (1970); Sheets (1975)
Cellular mechanism of resistance	
Unknown; adsorption similar in resistant and susceptible; interferon production apparently not responsible; possible block to virus development after penetration	Shif and Bang (1970)
Effect of resistance gene on pathogenesis	
Does not prevent infection, but death with wild virus never occurs; immunizes against virulent adapted virus	Willenborg et al. (1973) Sheets (1975)
Phenotypic alteration of genetic resistance	
Cortisone, X rays, cyclophosphamide, and epierythrozoon all break down genetic resistance; genetic resistance altered by lymphokines produced by mixed lymphocytic reaction in tissue culture	Willenborg et al. (1973); Lavelle and Bang (1973); Weiser and Bang (1976)
Genetic resistance and virus mutants	
Large inoculum of wild virus placed on resistant C3H cells overcomes resistance and regularly produces new virulent adapted strain	Shif and Bang (1970); Lavelle and Bang (1971); T. Cody (personal communication, 1978)
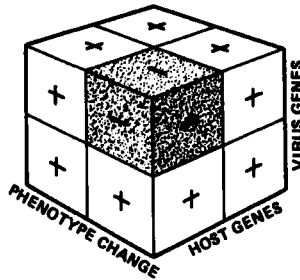
Fig. 2. Variations in compatability between host cell and virus system in Mouse Hepatitis. There are three variables, host genes, virus genes and phenotypic change. The right hand face shows that an incompatable system (−) may be converted to a compatable (+) by change in virus genes, or by a phenotypic change in the host cell.

MHV-3 strain of virus (Virelizier, 1975) are therefore included in each part of this section.

A. Nature of Genetic Resistance

Within the strictly defined area of transmission of the adapted mouse hepatitis virus (PRI) (i.e., MHV-2) to other strains of mice, susceptibility is due to a unifactorial dominant Mendelian gene (Bang and Warwick, 1960; Kantoch et al., 1964). This is demonstrated both in young adult mice and in cultures of macrophages from either livers or peritoneal washings. The F1 generation is fully susceptible, in that virus preparations titer just as far on these cells as on homozygous susceptible cells, and destruction is just as rapid. About 25% of the F2 generation is resistant and, when continued backcrosses of the susceptible F1 generation are made with the resistant (C3H) strain, 50% of the offspring show susceptibility (Kantoch et al., 1964). After 20 such backcrosses, a coisogenic strain of mice was bred (Weiser et al., 1976), and this strain presumably differed from the resistant strain by only one gene. The genetics of resistance of mice to MHV-3 (in contrast to MHV-2) is not as yet clearly defined. There is a striking genetic difference in the susceptibility of different strains, but there is also an intermediate degree of susceptibility, perhaps reflecting incomplete penetration (Virelizier, 1975).

B. Tissues Affected

The most marked pathological changes in the animal occur in the liver, spleen, and lymph nodes (Reubner and Bramhall, 1960; Piazza, 1969; McIntosh, 1974). Many of these changes are due to the direct effect of

the virus on macrophages, an effect which is highly visible in tissue culture (Bang and Warwick, 1959). However, the effect of the virus on lymphocytes *in vitro* has not been studied, and it is not known whether, as with macrophages, such an effect would correlate with the genetic susceptibility of the mouse. Macrophages from resistant mice are changed to susceptible by lymphokines, much as the mouse is changed, and susceptible cells and mice may be made resistant by concanavalin A or Freund's adjuvant.

A variety of other cell types, including tumor cells, is destroyed by mouse hepatitis virus *in vitro*, but in all cases the virus had been adapted to tissue culture before the studies were made. This may then represent a change in the tissue compatibility of the virus, a phenomenon which readily occurs with passage.

C. Ontogeny of Resistance

Both individual newborn mice of the resistant strain and macrophage cultures from these mice are susceptible (Bang and Warwick, 1960). In order to demonstrate the susceptibility of cultures from newborn resistant mice, it was necessary to grow liver cultures as explants on reconstituted collagen, so that macrophages would migrate from the explant. When precautions were taken to have a significant number of macrophages in the cultures, it was possible to show that the gradual acquisition of resistance of the C3H occurred both in the mice themselves and in the cultures. Thus macrophages grown from liver cultures of very young mice were susceptible, but macrophages grown from livers of weaned mice were resistant (Gallily *et al.*, 1967). However, this maturation of the resistance did not occur in the tissue cultures when they were maintained over a period of several weeks. The ontogeny of resistance, which has been described for MHV-2 and which occurs by 4 weeks of age, probably does not represent maturation of the *only* genetic effect. In the studies of Le Prevost *et al.* (1975), several of their partially resistant strains did not develop resistance until 12 weeks of age. They were still susceptible at 6 weeks of age. A somewhat similar development of increased resistance of the susceptible congenic strain of C3H mice has been observed at 2 months.

The fact that thymectomy of newborn mice prevents the development of resistance to both MHV-2 and MHV-3 (Piplani and Aikat, 1970; Sheets, 1975; Le Prevost *et al.*, 1975; Dupuy *et al.*, 1975; Levy-Leblond and Dupuy, 1977) suggests that resistance is not developed in macrophages alone as an isolated event, but that the interaction of macrophages

with lymphocytes may be of significance (Section III,E). This inference is further supported by Levy-Leblond and Dupuy (1977) who showed that susceptible newborn mice were protected by injecting a *combination* of T cells and macrophages. A remarkable finding was that cells obtained by peritoneal washing, although containing both T cells and macrophages, did not protect newborn mice. Cells from the spleen were necessary and, when adherent spleen cells (macrophages) were added to T cells from the peritoneum, protection was obtained.

D. Cellular Mechanism of Resistance

No difference in the adsorption of the original PRI-adapted virus onto susceptible and resistant cells has been demonstrated (Shif and Bang, 1970). Furthermore, resistant cells do not produce interferon (tested against vesicular stomatitis virus). In light of the findings of Hanson *et al.* (1959) on arboviruses, however, this subject needs to be further explored to see whether there may be autointerference of greater specificity. The fact that the cells from susceptible mice are destroyed *in vitro* even in the presence of resistant cells at a concentration of as high as 50% (Bang and Warwick, 1960) suggests, however, that interferon is not an effective method of blocking virus multiplication.

E. Effect of the Resistance Gene in Pathogenesis

When the PRI-adapted virus is inoculated into C3H mice, the mice all survive even when concentrations of inoculated virus are as high as 10^8 units. Cultures of C3H macrophages maintained in a standard medium of 90% horse serum are equally resistant. This has given rise to the idea that macrophages present a genetic barrier to the progress of infection. Infection must, however, take place in resistant animals, since mice have been shown to become immune to a virus strain that is adapted to these genetically resistant mice, if they have been given the PRI virus previously (Sheets, 1975). In addition, Virelizier *et al.* (1976) showed that genetically resistant mice had an altered immune response when inoculated with MHV-3, even though no apparent disease was caused.

Taguchi *et al.* (1976) followed the progression of MHV-2 in the liver of mouse strains differing only in terms of the time of death. The strain of mice which died quickly following virus inoculation had a more rapid growth of virus in liver, spleen, and blood than the strain which succumbed more slowly. In addition, Kupffer cells were more extensively infected early, and macrophages in culture yielded more extracellular virus when derived from the more rapidly killed strain.

As with flavoviruses, interferon has been considered the mechanism of resistance. J. L. Virelizier and I. Gresser (personal communication, 1977) showed that genetically resistant mice are made susceptible by treatment with antibody to interferon.

F. Phenotypic Alteration of Genetic Resistance

Gledhill and Niven (1957) showed that the MHV-1 strain of hepatitis interacted with an epierythrozoon to produce severe disease in mice. This was also found to be true of the MHV-2 strain (Lavelle and Bang, 1973). Thus the genetic resistance of mice to the virus may be phenotypically modified. Cortisone reduces resistance to several viruses, including mouse hepatitis (Lavelle and Bang, 1971), and Gallily et al. (1964) showed that cortisone was remarkably effective in eliminating the resistance of genetically resistant mice; although cortisone had a slight effect on rendering cells susceptible in vitro, it had only a fraction of the effect demonstrated in vivo. This failure to produce similar effects in the two systems suggested that a major component of the in vivo system was missing in tissue culture.

In the original experiments on the conversion of genetically resistant macrophages to susceptibility, extracts of PRI cells were placed in the resistant C3H cell cultures. This produced a definite, but not a very great, increase in susceptibility (Kantoch et al., 1964). Subsequently, Huang and Bang (1974) showed that there was a highly significant conversion when the cells (presumably lymphocytes) which survived in the supernatant cultures of the peritoneal exudate of the susceptible mice were transferred to the resistant cultures. This resulted in consistent conversion to susceptibility.

The possibility that in Huang's system an in vitro mixed lymphocyte reaction took place in the culture, and that a lymphokine so released caused the increased susceptibility, was tested (Weiser and Bang, 1976). First, no increase in susceptibility was conferred on the resistant macrophages by the addition of lymphocytes from the spleen of congenic susceptible mice, but there was a 100- to 1000-fold increase in susceptibility of the resistant cultures which contained lymphocytes when cells from an allogenic strain of mice were added. Second, the cell-free product of mixed lymphocytes produced an equal increase in susceptibility. Finally, spleen cells from cortisone-treated resistant mice caused an increase in susceptibility without altering the host adaptation of the virus (W. Weiser and F. B. Bang, unpublished). This explains the increase in susceptibility of mice given cortisone treatments.
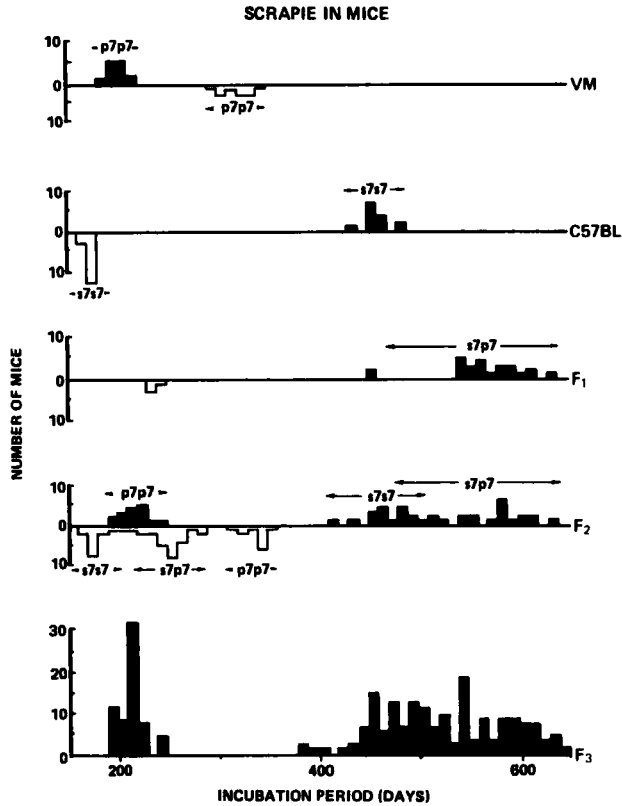
SCRAPIE IN MICE

FIG. 3. Relationship of incubation period of two strains of scrapie in two strains of mice, in their F1, F2, and F3 offspring. Notice that segregation of the character (incubation period) is exactly opposite in each case.

G. Genetic Resistance and Virus Mutants

Mouse hepatitis virus is unique in showing a very rapid adaptation to resistant C3H cells when large amounts of the virus are placed on these cells. The change in the host specificity which accompanies this destruction of cells is demonstrated by inoculating the virus in lower dilutions on the C3H resistant cells. The newly produced virus then has the capacity to destroy both the C3H and PRI cells at the same dilution.

The mechanism whereby the MHV(PRI) virus adapted to growth in PRI cells changes into MHV(C3H) adapted for growth in C3H cells is not known. It may of course be the selection of a mutant present in the original stock of virus, or some other adaptive process may occur. When C3H cells were grown in various sera, including that of fetal calf, they became as much as 1000-fold more susceptible (Lavelle and Bang,

1973), but it was not determined whether the virus that emerged was MHV(PRI) or MHV(C3H).

In conclusion, there are three aspects of the host–virus interaction in this system, i.e., the genetics of the host and of the virus, and phenotypic alteration of the host. The last-mentioned is apparent in the ontogeny of resistance, in alteration by cortisone, and in the effects of mixed lympocyte cultures on susceptibility *in vitro* (Fig. 2). Genetic analyses combined with studies on pathogenesis have emphasized the role of the macrophage system in determining the outcome of the infection. In addition, there may be other host genetic systems which control the rapidity of virus multiplication within the macrophage system but are not strong enough to alter the final outcome (Fig. 3).

V. INFLUENZA AND MISCELLANEOUS ACUTE VIRAL INFECTIONS

Of the three types of influenza, only influenza A has been studied for genetic resistance and susceptibility, and this susceptibility in turn has been studied only in mice. Since mice are a very unlikely host for the virus in nature, the evolutionary meaning of genetic resistance to the virus is unknown. Influenza A is, however, now recognized as having a wide host range including a large number of birds, swine, and horses, as well as humans. Thus it is not impossible that genetic resistance as detailed in Section V,D does have a meaning for closely related, as yet undescribed, agents. In this connection, it is interesting that wild mice have resistance genes (Haller, 1975).

The most complete studies of the effect of inheritance on susceptibility to this virus have been made with neurotropic and hepatotropic variants. A neurotropic (NWS) strain was developed by Stuart-Harris (1939) by making continuous intracerebral passages of the virus in mice, in which it produces hemorrphagic encephalitis and death. The hepatotropic virus was developed by Haller from a strain of fowl plague originally isolated from turkeys. However, resistance in mice is not limited to *these* strains but also occurs with some, but not all, pneumotropic strains (Lindenmann *et al.*, 1963).

A. Nature of Genetic Resistance

Lindenmann (1962–63) noticed that an inbred strain of mice, A2G, maintained at the Glaxo Laboratories for testing pertussis vaccines, was very resistant to influenza virus. Following intracerebral inoculation, these mice were resistant to 100,000 times as much virus as that which killed standard strains of mice, including C3H. This strain was also resistant to the neurotropic virus adapted to Ehrlich ascites tumor cells

(Lindenmann, 1963), even though the virus destroyed most of the implanted tumor. A careful Mendelian analysis of resistance to this virus strain was then made. Analysis of the F1 and F2 generations, and of the results of backcrosses with both the resistant and the susceptible strains, gave results which agreed closely with the hypothesis of one autosomal dominant resistance gene. This has been called Mx and is manifest against all three types of virus: pneumotropic, neurotropic, and hepatotropic. The gene for resistance was searched for in a large number of strains and not found. It is inferred that it was introduced by a stray British mouse (Lindenmann and Klein, 1966). Both resistant and susceptible mice showed equal susceptibility to a variety of other viruses, including yellow fever and West Nile virus (Lindenmann and Klein, 1966).

B. Tissues Affected

Since there is undoubtedly genetic selection of the virus for different capacities to grow in different tissues (tropisms), we first review the three different tropisms.

1. Pneumotropic

Less work has been done on these standard strains, but Lindenmann and Klein (1966) and Lindenmann et al. (1963) showed that there was a 100- to 1000-fold greater resistance on the part of A2G mice to standard intranasal inoculation of four strains of pneumotropic influenza A, but two Asian strains had low titers in both resistant and susceptible mice. Death presumably was primarily due to pneumonia.

2. Neurotropic

Following intracerebral inoculation multiplication of the neurotropic strains occurred, which produced 10^6 $TCID_{50}$/ml in brain tissue of susceptible mice. These mice died 6–8 days after inoculation. Resistant mice yielded almost 1/100 to 1/1000 as much virus and had no virus by days 6 to 8. They survived the infection.

3. Hepatropic Virus and Growth in the Liver

Virus multiplies rapidly in the liver of susceptible mice following both intraperitoneal inoculation and injection into a branch of the portal vein. It attained a high titer (10^9–10^{10}/ml) in the liver of susceptibles, but attained only 10^5 EID_{50} in the liver of resistant mice. This was accompanied by a marked difference in cell destruction (see Section V,C).

C. Cellular Mechanism of Resistance to All Three Strains

It may be inferred from the fact that immunosuppression by cyclophosphamide or X ray fails to change significantly the resistance of A2G mice to both the neurotropic and hepatotropic strains that resistance to the virus resides in the tissues (Fiske and Klein, 1975; Arnheiter *et al.*, 1976). However, the virus grows equally well in trypsinized kidney cells of both resistant and susceptible cells. Macrophages of resistant (A2G) mice are resistant, as are macrophages obtained from the F1 generation of a cross with susceptible mice. Macrophages from susceptible mice are susceptible (Lindenmann *et al.*, 1978). Evidence that interferon is the mechanism of resistance was nonconclusive.

D. Effect of Resistant Gene on Pathogenesis

A comparison of the extent of inflammatory lesions and of the spread of the neurotropic strain in the brain as measured by fluorescent antibody has been made by Fiske and Klein (1975). In general, the spread of infection is greatly limited in the brain of the resistant strain. Infection in A/J mice (susceptible) was characterized by a severe ventriculitis, necrotizing ependymitis, and a progression of the lesions in the paraventricular regions. In addition, there was extensive necrosis of the choroid plexus. In resistant mice, the ventricular inflammation was almost as intense, but there was less ependymal necrosis and little extension of the inflammation. Degenerative lesions of the neural and glial elements were absent in resistant mice. The spread of virus measured by fluorescent antibody was equally limited in resistant animals. Widespread unusual inclusions were found in the nuclei of the epithelial cells of the choroid plexus of resistant mice, whether immunosuppressed or not. Such inclusions were not present in the infection of susceptible mice or in sham-operated normal mice. Numerous intranuclear helical structures similar to the ribonucleoprotein of myxoviruses were apparent with electron microscopy. This clue suggests to the reviewer that infection in resistant mice fails to be liberated from the nuclei or the cells of the choroid plexus.

A similar comparison of the pathogenesis of the hepatotropic strain in resistant and susceptible mice has been reported by Haller *et al.*, (1976) and Arnheiter *et al.* (1976).

Apparently resistance is unassociated with the thymus, since in a cross with nude mice, which were presumed to be free of thymus tissue, there was no change in the ratio of 75% resistance/25% susceptible in the F2 genes (Haller and Lindenmann, 1974).

Although differences have been described (Briody *et al.*, 1953) in the

capacity of virus coming directly from humans to adapt to different strains of mice, all the work analyzed here has been done with adapted strains of influenza. Thus it is not surprising that the clear difference between the two strains of mice, which seems to rest on a single dominant gene, was much less apparent with other strains of virus. PR8 (influenza A), which is not neurotropic, produced pneumonia and death in resistant mice at the same dilutions as in susceptible mice, but a marked prozone effect was apparent in resistant mice, in that many inoculated with larger amounts of virus survived and did not have pneumonia. This prozone effect is reminiscent of the partial resistance of PRI mice to the neurotropic strain of yellow fever (Section III).

E. Virus Mutants

No mutant obtained directly from standard strains of NWS virus capable of growing in resistant mice were obtained, and no resistant mutants have been found in macrophage cultures inoculated with large amounts of virus (Lindenmann et al., 1978). It is interesting that two strains of mouse-adapted Asian influenza grew equally well in resistant and susceptible mice (Lindenmann et al., 1963) (Table III).

F. Newcastle Disease Virus

Neurotropic or velogenic strains of this avian virus produce encephalitis in mice, even when the virus is inoculated intranasally (Liu and Bang, 1952). It probably gains entrance to the central nervous system via the olfactory bulbs. When the effect of this was studied in five different inbred strains of mice at Bar Harbor, clear-cut differences were noted in three parameters (Liu and Bang, 1952). First, one strain of mice, DBA/1, was more prone to develop encephalitis than the rest, and this seemed to be a characteristic of DBA/1 but not DBA/2. Finally, C57 BL mice did not develop pneumonia, but did develop encephalitis which was much more frequently accompanied by the myoclonic twitching characteristic of Newcastle disease. Thus the inbred mice showed differences in response as manifested by encephalitis, myoclonic twitching, and pneumonia. No tests for the type of inheritance were made. Maturation of resistance to encephalitis with age was demonstrated in DBA/1 mice.

In an entirely unrelated study 17 years later, De Maeyer and De Maeyer-Guignard (1969) investigated the genetics of interferon production in serum following the intravenous injection of another strain of Newcastle virus into different strains of mice. They discovered that the C57 BL strain of mice was a good interferon producer and that the

TABLE III

	Reference
Nature of genetic resistance	
Strain A2G resistant to 10^5 LD_{50} of neurotropic influenza virus; Mx gene also responsible for resistance to pneumotropic and hepatotropic strains; all other strains susceptible to 1000- to 10,000-fold less virus	Lindenmann et al. (1963)
F1 generation resistant; F2 and backcrosses indicate an autosomal zone; gene not related to arbovirus resistance	Lindenmann (1962)
Tissues affected	
Resistant mice fail to develop pneumonia, encephalitis, or hepatitis	Lindenmann et al. (1963); Haller et al. (1976); Arnheiter et al. (1976)
Macrophages of susceptible mice, susceptible in vitro; macrophages of resistant mice, resistant in vitro	Lindenmann et al. (1978)
Ontogeny of resistance	
Virus multiplies longer in baby mice from resistent strain than in adults	Lindenmann et al. (1963)
Cellular mechanisms of resistance	
Unknown; no evidence that interferon is a result of the Mx gene; interferon production in response to Newcastle virus varies with dierent strains, and this variation is inherited as a Mendelian character(s); probably a separate gene	De Maeyer and De Maeyer-Guignard (1969)
Effect of resistance gene on pathogenesis	
Infection of resistant mice to neurotropic virus is initiated by the same low dose as in the susceptible strain, but virus increases in the latter to a 100-fold greater amount; cellular infiltrate is present in the brain and liver of resistant mice	Lindenmann et al. (1963)
Phenotypic alteration of genetic resistance	
Immunosuppression fails to increase susceptibility	Haller et al. (1976)
Splenectomy and ^{87}Sr treatment have no effect on resistance	O. Haller and J. Lindenmann (personal communication, 1977)
Genetic resistance and virus mutants	
PR8 strain (not neurotropic) produces infection in resistant strains, but with a marked prozone effect; two Asian strains of influenza grew equally poorly in resistant and susceptible mice	Lindenmann et al. (1963)

BALB/strain was a poor producer of interferon. The amount of inter-
feron produced was measured on L cells as activity against vesicular
stomatitis virus. Determination in individual mice showed that the two
strains could be separated into two classes with no overlap between them.
The amount of interferon produced in the F1 generations of the two
reciprocal crosses was determined, and the average value in both cases
found to be intermediate between that of the parents. Greater variation
over a wider range was found in the F2 generation. Backcrosses with the
high interferon producer (C57BL) tended toward the high producer,
while backcrosses with the low producer were more like the low producer.

These workers conclude that a "single, partly dominant, autosomal
factor is responsible for the difference in circulating interferon production
between both strains." It seems to us that the effect of several Mendelian
genes has been by no means ruled out, since the range of variation within
each class was so great and since the different presumed segregants did
not clearly separate into discrete classes.

Tucker and Stewart (1976) have taken advantage of the higher suscep-
tibility of inbred C57BI and C57BR, as compared to outbred Swiss mice,
when inoculated with Sendai (parainfluenza 1) virus to study patho-
genesis of the intrauterine spread of this virus. The virus produced a
persistent (21-day) infection and was isolated from 20–30% of the new-
borns from intravenously infected mothers. The genetic aspects of the
infection were not determined in hybrids or backcrosses.

G. Foot-and-Mouth Disease

In a long and rather complicated paper Subak-Sharpe (1962) has dis-
cussed the interrelationship of 12 different mouse strains (both young and
old mice) and susceptibility to unadapted foot-and-mouth disease virus.
Four of the rather closely related strains were 100 to 10,000 times more
susceptible to intraperitoneal and intramuscular inoculations than were
adult P mice, which resisted up to 100,000 ID_{50} of virus. Since there was
a much greater range of maturation of resistance with age in one group
of strains than in another, this investigator suggests that the suscepti-
bility of young and adult mice is under two separate genetic control
mechanisms. Continued adult mouse passage (intramuscular route) over-
came the resistance of adult P mice. In crosses between P mice (which
had a large difference between adult resistance and infant susceptibility)
intermediate results were obtained in the F1 generation. Subak-Sharpe
suggests that a single gene with incomplete dominance determines the
difference in susceptibility between AX (susceptible) and P (resistant)
mice.

H. Mousepox

Despite extensive studies on another poxvirus in rabbits (myxomatosis) and the general interest in human smallpox epidemics, relatively little has been done with poxviruses and genetic resistance. Schell (1960), taking advantage of previous observations of Trentin (1953) and Briody (1957) that the C57BL strains of mice are relatively resistant to mouse-pox, followed the pathogenesis of the infection in these inbred mice and compared it to the same infection in "stock" mice.

The C57BL mice were approximately a million times more resistant (LD_{50}) to ectromelia, inoculated into the foot pad, than the stock mice (not inbred). However, the infectious dose was the same for the two strains. A cross between resistant and susceptible mice yielded resistant mice, and a backcross with the stock susceptible mice yielded 30% mortality in 106 mice. Thus the number of genes involved in resistance may well be more than one. No further analyses of the genetic nature of resistance were made.

Ermolaeva *et al.* (1972) similarly showed that C57BL mice were resistant (to 1000 ID_{50}), and in their study a cross with a susceptible strain A/HeY also yielded resistant mice. They carried the genetic analysis to the F2 generation, the results of which agreed with a one-gene (independent of coat color) hypothesis.

The virus grows throughout the mouse in a large variety of tissues, including lymph node and liver, but it is not known whether macrophages are selectively affected. The virus grows equally well in monolayers of mouse fibroblasts from both strains (Schell, 1960).

I. Ontogeny of Resistance

Newborn mice of the resistant strain are apparently not resistant when 1–2 days old or at 8–12 months. High resistance was found at 4–8 weeks of age (Schell, 1960).

J. Mechanism of Resistance

It was found that neutralizing antibodies and hypersensitivity (measured in the foot pad after inoculation with a mild strain of virus) developed more rapidly in the resistant strain. Finally, the increase in size of the spleen (measured 5 days after infection) was shown to be greater in the resistant mouse. It is inferred that resistance may relate to an earlier immune response. The route of inoculation, as with other viruses, had a direct effect on the difference in susceptibility. Intranasal and intracerebral routes eliminated the greater resistance of the C57BL mice.

Since a variety of *in vitro systems* has substantiated Doherty and Zinkernagel's (1975) original finding that killer cells and virus-infected cells must be syngeneic at the *H-2* locus, it has been hypothesized that the *H-2* genes are involved in the production of new antigens on the cell surface of virally infected cells, and that these are necessary for cell killing by the host T cells. In the mouse pox system, Kees and Blandon (1976) showed that, with the cell transfer system developed by Blandon to study the T-cell activity against ectromelia-infected cells, neither the *K* or *D* region of the *H-2* complex played a necessary and sufficient role. A single mutation in the *K* region abolished the antiviral effect of the immune T-cell transfer.

In two papers (Duran Reynals, 1972, and Lilly and Duran Reynals, 1972) a marked variation among eight inbred strains of mice to vaccinia virus, and to two combined effects of vaccinia virus and cortisone in producing skin lesions, is recorded. The central purpose of the papers was to study the effect of a combination of virus and carcinogen (methylcholanthrene). Since the AKR mice (highly susceptible to leukemia) were highly resistant to the vaccinia infection the question as to whether the Gross virus was responsible for resistance was raised.

Crosses of the most resistant to vaccinia (AKR) with the susceptible (BALB/c) were done and backcrosses of these to the two parents. The F1 generation and the backcross to the susceptible were 100% susceptible to the combined action of the vaccinia and cortisone in producing skin ulcers. Since 78% (107/138) of the backcrosses to the resistant were susceptible, the authors suggest that two genes are involved. No further data supporting this suggestion are presented. These two genes for resistance to the virus were found to be independent of the H_2 locus, but the locus was found correlated with the subsequent development of papillomas. Again the presence of leukemia virus seemed to protect against skin tumorigenesis among mice F1xAKR.

VI. SCRAPIE

Scrapie is a natural disease of sheep and goats which occurs throughout the world but has especially affected the sheep of Britain and Europe over the past few centuries. It is an insidious chronic disease of the central nervous system which causes such itching that the afflicted animals scrape against fences or trees, hence the name.

The transmissable agent which initiates the disease in sheep, goats, mink, and mice has such peculiar characteristics that it is included in a discussion of viruses only when a reasonably broad definition is given to the term viruses. The agent is between 7 and 40 nm in size (filtration)

and is resistant to dry heat up to 160°C for 1 hour, to ultraviolet irradiation, and to 20% Formalin solution, yet it attains titers of only about 10^8 units/gm of brain tissue (Outram, 1976). Is is destroyed by Chlorox (J. Gibbs, personal comment, 1976).

The genetic aspects of scrapie were first studied by Parry (1962). On the basis of analysis of the genetics of different flocks of natrually infected sheep, he suggested that it was a "transmissible and hereditary disease of sheep." It was thought to be "due to a single autosomal recessive gene" in spite of the fact that the agent obtained from infected strains of sheep induced infection in other sheep, goats, and mice. Maternal transmission in sheep and the concomitant presence of several strains of the virus in one animal have made genetic studies difficult. However, the agent itself is so unusual in its small size and its resistance to heat and Formalin that it raises unique molecular problems about its growth and is a unique challenge in the analysis of effects of different host genotypes on virus behavior and growth.

The major contributions to understanding the interaction of virus and host genome have been those of Dickinson and his colleagues (1968). The unusual findings which have emerged match the peculiarity of the agent itself. Since scrapie is a classic example of a slow virus, and since the incubation period may exceed an animal's life span, determination of which animals are truly resistant is sometimes difficult. For example, there were striking differences in the incubation periods in individual animals within groups of sheep following both subcutaneous and intracerebral inoculation (Dickinson and Miekle, 1971). These seemed to fall into short (197 ± 7 days) and long (917 ± 90 days) classes. Evidence for a single dominant gene for susceptibility was obtained by studying the progeny of sheep with known short incubation periods which were mated with sheep lacking the expressed factor for short incubation. Stocks of sheep selected for susceptibility and resistance now have a difference of 90% incidence following subcutaneous inoculation. Among the animals apparently homozygous for the recessive resistant allele, there were some which developed scrapie after a very long interval; this has left the problem of the genetics of long-incubation scrapie in sheep unsettled.

By selective breeding and testing of offspring for susceptibilty to the SSBP-1 agent. (also used by Dickinson et al., 1968), Nussbaum et al., (1975) also established Herdwick sheep flocks of predictable susceptibility. Their results support the idea of a dominant allele which confers susceptibility. Their evidence was derived from comparisons of members of families and from the rapidity with which selection for susceptibility was effected. All 48 animals in the susceptible flocks, which were studied for 2–4 years, developed the disease after an incubation period of 178 ± 35

days, while in the resistant flock 3.9% of 52 sheep developed the disease 1145 ± 44 days after inoculation. No tests of crosses of the two strains have as yet been practicable.

When the agent was successively transferred to mice by Chandler and others (1961), a more satisfactory model for experimental virological studies became available. Dickinson and MacKay (1964) found that all of the nine strains of mice they tested were susceptible, and that virtually all succumbed, but that the incubation period differed from inbred strain to strain, varying from 20 to 40 weeks. The strain of mice that had the longest incubation period had been only partially inbred so that, when mice were further selected for susceptibility to long-incubation-period disease, they were at the same time inbred to apparent homozygosity. The formal Mendelian genetic tests of the relationship of long incubation periods (270 days) to short incubation periods (150 days) could then be carried out. All tests for viral susceptibility were by the intracerebral route. The F1 hybrids had an intermediate incubation period, and the F2 and the two backcrosses showed segregation ratios which fit the one gene–two allele hypothesis, with neither gene dominant.

The most striking finding in the continuing genetic analysis of incubation periods in mice came later. Results almost opposite those above were obtained when another strain of scrapie, 22A, which had been passed in the "long-incubation" mice before it was used, was employed in a genetic test of the effect of the short-incubation ("sinc") gene. In this new system the "short-incubation" mice (C57BL) had a longer incubation period, and the long-incubation mice (VM) had a short incubation period. Furthermore, the F1 hybrid showed the phenomenon of over-dominance; that is, the incubation period of these F1 mice was longer than in either of their parents. Segregation ratios in the F2 and F3 generations followed the expected pattern for one gene. These workers argue that the sinc gene is responsible for these reversed effects. Three reasons are given. First, detailed comparison of the expected and observed ratios of short to long incubation periods in different crosses following a test of the F2 generation with the first agent (ME7) and a test of the F3 generation with the second agent (22A) showed close agreement with the hypothesis that one gene or closely linked genes were responsible. In other words, there was no segregation of the two agents into two factors between the F2 and F3 generations. Second, the response of several other strains of mice to one agent can be predicted on the basis of the response of the other. Finally, as new agents were found, they fell into the same pattern of behavior.

Explanation of the pathogenic mechanisms is incomplete. There is a correlation between histological changes, disease, and virus growth. Rates

TABLE IV

CHARACTERISTICS OF GENETIC RESISTANCE TO SCRAPIE

	Reference
Nature of inheritance	
Short incubation in sheep controlled by recessive gene; mice uniformly susceptible, but two alleles at one locus control the incubation period. neither gene dominant.	Dickinson
Tissues affected	
Specific areas of central nervous system; virus also grows in many tissues, especially reticular-endotheliar system, but pathology not apparent in these	
Cellular mechanism of resistance	
Unknown; but heterozygous overdominance in one system suggests a heteromeric structure to which each allele contributes different subunits	Dickinson and Miekle (1971)
Effect of resistance on pathogenesis	
Growth of virus in spleen delayed 4 weeks in long-incubation-period disease; early growth of virus in central nervous system in short-incubation-disease but eventual titer is the same in diseases	
Phenotypic alteration of genetic resistance	
Unknown; immunodepression may delay	Outram (1976)
Ontogeny of resistance	
Increasing age associated with less variation in incubation period; infant mice may lack susceptible cells; thus there is also developmental maturation of susceptibility	Outram (1976); Dickinson and Fraser (1969)
Genetic resistance and virus mutants	
Gene for long incubation period functions to produce short incubation period when another strain of virus is used; F1 hybrid overdominant with this virus strain	Dickinson and Meikle (1971)

of growth of the first agent (ME7) were studied in short- and long-incubation mice. Initiation of growth in the spleen was 4 weeks later in long-incubation-period disease, even when the virus was given intracerebrally. Parenthetically, it is of course well established that intracerebral inocu-

lation is accompanied by a large spillover into the vascular system. Multiplication of the virus in the spleen in short-incubation-period disease occurred almost immediately after innoculation (Dickinson and Fraser, 1969). Following this initial difference, the amounts of virus in the two types of disease were almost the same and were maintained at these levels. The multiplication of virus in the brain was delayed in long-incubation-period disease, although the subsequent rate of increase and terminal titer were similar for both alleles (Dickinson et al., 1969).

The striking reverse in the susceptibility of the mice to long-incubation-period disease when a new strain of virus was used, and the phenomenon of overdominance, are apparently due to the same two alleles on the single sinc gene.

This raises interesting questions at the molecular level. Dickinson and Meikle (1971) suggest that the overdominance of the heterozygotes which was apparent in the infection of 22A heterozygotes may involve a heteromeric structure, an enzyme or replication site to which each allele contributes different subunits. With some agents such a heteromeric structure could be of intermediate efficiency, but with others it could be less efficient than the homomeric structure that would be present in the homozygotes. These workers point out that this hypothesis is similar to that put forth by Zimmermann and Gunderlach (1969) to explain the varying efficiencies of heteromeric enzymes in bacteria in which there is intra- or interallelic complementation.

Modifications of the pathogenesis and the incubation period are sometimes introduced by indirect genetic effects. For instance, when the agent is injected intraperitoneally, the incubation period is 20% longer in mice in which spleens are congenitally absent $(Dh/+)$ than in their anatomically normal littermates.

VII. Other Chronic Infections

One may suggest at least two mechanisms for the development of chronic disease. In one, the agent simply grows slowly and, because of the slow rate of growth, the disease develops slowly. In the other mechanism, there may be a balance between the host defenses and the agent, so that neither gains complete control. In such a system there must be a waxing and waning of both growth of the organism as well as the response of the host. This is analogous to a density-dependent control mechanism which controls the size of a population. In chronically infected tissue cultures, interferon or defective interfering particles may act in this manner. In animals we lack adequate knowledge of such balancing factors.

Genetic control of chronic nontumor virus infections is then an area

which warrants intensive study but, with the exception of the data on scrapie, the only published studies are the scattered ones reviewed in this section. They are discussed as completely unrelated situations.

A. Lactic Dehydrogenase Virus

This unique agent, with a distinctive morphology, grows particularly in macrophages, both in the animal and in tissue cultures (Darnell *et al.*, 1975; Fenner, 1970). Its presence is recognized by a failure of the animal to clear from the blood such enzymes as lactate dehydrogenase and malate dehydrogenase. However, no cell pathology has been evident. The virus grows well in freshly explanted mouse macrophage cultures but fails to grow in SV40-stimulated cultures. Since acute macrophage cultures usually contain lymphocytes, the possible role of lymphocytes in growth of the agents should be explored.

Genetic control of the response of SJL/J mice to this virus has been reported by Crispens (1972). These mice, instead of having a normal "elevated" response to infection of 3300–6250 units/ml, yielded an exaggerated level (9600–16,700 units). A cross between the elevated (BALB/c) and the exaggerated (SLJ/J) yielded an F1 generation which was at the lower range of the elevated level (2650–6300 units). The F2 generation yielded a 3:1 elevated/exaggerated segregation ratio, and a backcross with the exaggerated yielded a 1:1 ratio. It is suggested that the SJL/J mice have a recessive trait for extreme susceptibility. No studies of cell cultures have been reported, but in light of the known high genetic susceptibility of macrophages to mouse hepatitis, and now influenza, and the recent report on the differential genetic susceptibility of cells to cytomegalic virus (Diosi *et al.* 1974) (Plavosin and Diosi, 1974) along with the established capacity of LDV to grow in macrophages, such studies should be useful.

B. Lymphocytic Choriomeningitis

This agent was first described by Traub (1971) as a latent agent in stocks of PRI mice and is transmitted from mother to newborn offspring, persisting in them for life and causing usually little or no disease. It has since become a classic example of a disease in which the immune response is essential for the production of disease, and the latent situation is considered a true example of immune tolerance. Because of its importance as a standard model of basic problems in chronic infection, it has been studied extensively. The genetic aspects of the disease complex are of special interest. Oldstone and Dixon (1968) reported in a brief note that different strains of mice may differ as much as $10^{4.6}$ log in susceptibility. In a later paper entitled, "Histocompatibility-Linked Genetic Control of

Disease Susceptibility" (1973), these workers corrected the log difference to $10^{1.9}$ and then presented data which showed a correlation between (1) the presence of the locus q for immune response in the H-2 complex, and (2) susceptibility as determined by death following 10-fold graded doses of the virus. Since the difference between the resistant C3H mice and the susceptible SWR mice was 1.9 logs for male mice and 1.4 logs for female mice, and since the virus used was passed in SW mice, it is difficult to know the biological significance of such resistance and susceptibility when the differences obtained are just above statistical significance. The two F1 generations from these crosses were closer to the susceptible strain than to the resistant strain in the percentage of mortality when the k (immune response) gene was present in a double dose. Finally, there are illustrations which show extensive inflammatory response in the leptomeninges of the susceptible strain with the q gene and no inflammatory response in the strain with the k gene. This difference was noted in each of 10 mice in each group innoculated intracerebrally with a lethal dose of the E-350 strain of lymphocytic choriomeningitis virus. There are objections to this work.

First, there is a lack of a sharp difference between susceptible and resistant mice. It is not clear whether the resistant mice, when they died of infection, also had severe necrotizing disease. Second, as these workers themselves state in the discussion: "There is as yet no direct evidence showing that the immune response to LCM virus . . . separates genetically with H-2 types and with disease incidence in individual members of a segregating population."

Lehmann-Grube (1971), who has worked with lymphocytic choriomeningitis virus for many years, undertook to confirm and extend the above findings. His laboratory has been unable to find differences in susceptibility in different strains of mice, including the particular ones used by Oldstone and Dixon, when they are tested with the same strain of virus, and has been unable to find differences in the growth of the virus in tissues derived from different strains of mice. For these reasons then, the question of the genetic aspects of this classic virus infection is still open.

C. Cytomegalic Virus

In two brief but interesting papers, Diosi et al. (1974) and Plavosin and Diosi (1974), from Romania, have reported on cellular and in vivo resistance of Swiss (S) mice to a strain of cytomegalic virus isolated from wild (W) mice. This contrasts with reports on previously isolated strains of this virus, which grow readily in Swiss mice. They found that cells

from newborn mice, particularly kidney cells, maintained resistance in tissue culture if derived from resistant Swiss mice, and were susceptible (developed typical intranuclear inclusions) if derived from the wild mice strain presumably established in the laboratory. A cross of the two (W $\times$ S) yielded 5/5 F1 susceptible and 3/4 positive cultures. The F2 generation showed 3/4 and 2/3, respectively, while two backcrosses with resistant mice showed about 1/3 of the mice susceptible. Presumably, one gene dominant for susceptibility is involved. Long-term maintenance of infected resistant cultures suggested that an abortive infection took place.

D. Polyoma

There are two studies on the genetic aspects of susceptibility to polyoma virus in mice. Unfortunately, they are complicated by (1) the observation that susceptibility and resistance differed completely at different doses, (2) the fact that susceptibility was presented both on the basis of development of a runting syndrome and as a percentage of mice developing tumors, and (3) subsequent knowledge that the development of tumors following polyoma virus is greatly affected by the cellular immune system. The data are therefore understandably difficult to analyze and cannot at present be used to support any particular thesis of Mendelian inheritance.

In the first study it was shown that, when AKR mice were given virus as newborns, 100% either developed tumors or were runted, or both, while only 30% of the more resistant C57BL/6 mice developed the same syndrome. Increasing the dose of virus by 100-fold did not change the effect in AKR mice but increased the percentage of C57BL/6 to 79%. In general, the F1 generation had intermediate percentages, and backcrosses were also intermediate. It does not seem possible to us to argue that tumorgenesis appeared to be determined by a single autosomal gene with incomplete dominance.

In an analysis of the mechanism of the difference, Chang et al. (1968) found that cultured embryonic fibroblasts, macrophages, and kidney cells from the two mouse strains showed no significant difference in susceptibility to polyoma virus. This leaves unexplained the finding that there was a consistently higher titer of virus in all the tissues of the susceptible mice.

Jahkola (1965) approached the same problem using different strains of mice: DBA/2 in which the susceptibility was 93% and C57/BL with 7% susceptibility. After infecting appropriate crosses, backcrosses, and F2 generations, he found that percentages of susceptibles were intermediate between those of the original parents. This investigator concentrated

on the development of parotoid tumors and excluded from consideration the mortality before the development of tumors. Thus runting produced by the virus is not included in the above figures. Jahkola concluded that the factor(s) for resistance were incompletely dominant, and that resistance might be explained on the basis of several genes.

E. Slow Disease of the Central Nervous System

Because of the interest in the genetic aspects of amyotrophic lateral sclerosis, special attention has been recently paid to the chronic paralytic disease of mice first described by Gardner and seven coauthors (1973). This disease is apparently caused by a type-C virus given the awkward name WM 1504 E.

A recent article by Oldstone *et al.* (1977) describes variation in the susceptibility of 10 different inbred strains of mice. Of the 6 susceptible strains, 4 belonged to the *kk* subtype of the *H-2* haplotype. Of the four resistant, three were of the *dd* subtype. There were no susceptible *dd* and no resistant *kk* subtypes. Genetic analysis by reciprocal crosses between the two resistant and susceptible strains showed both F1 crosses to be resistant.

VIII. MOUSE LEUKEMIA

This entire review is concerned with the genetic aspects of animal host resistance to viruses. In considering the inseparability of the genetics of host and agent, Flor (1956) has pointed out the necessity of considering the genetics of the combination of host and agent. This is beginning to be possible in the case of mouse leukemia. It is, however, complicated by the rapid recent advances which have led to a great mixing of new and old data, and new and old concepts. The description of this historical development of knowledge about the genetics of susceptibility to this virus does not repeat the excellent review by Lilly and Pincus (1973). It is, however, necessary to review some of the earlier data on the subject so that the specialized material may be related to the general subject of resistance.

We have attempted to rearrange the data so that they may be considered in an orderly fashion. The most obvious components are presented in Fig. 4 as a pyramid, and we discuss them in the sequence of the numbers on Fig. 4, always remembering that there is constant interaction among all six components and that most present-day research on the subject is driven by the Eldorado of the virus theory of cancer, which introduces a bias of its own.
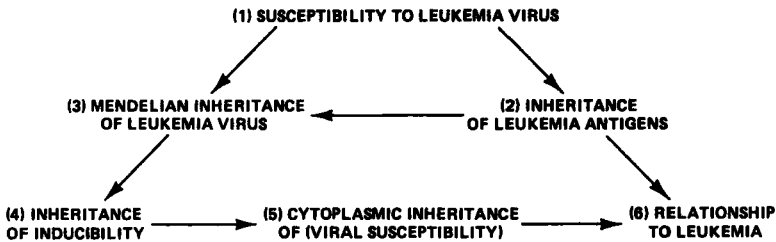
Fig. 4. Outline of relationship of genetic factors considered in mouse leukemia.

A. Genetics of Susceptibility to Mouse Leukemia Virus

The history of the genetics of host resistance to the leukemia–sarcoma virus complex in mice differs strikingly from that for chickens, in that it was only after the relatively recent demonstration of its viral etiology by Gross (1961) that it was possible to test the idea of Mendelian inheritance of compatibility. On the other hand, the great number of strains of mice created for the specific purpose of studying the genetics of cancer made progress in this field much more rapid.

Although Gross (1961) indicated from the start that the filterable nature of leukemia was demonstrable only in certain strains of mice, the first clear proof of the Mendelian nature of this susceptibility was furnished by Odaka and Yammamota (1962). A few years later, Axelrad and van der Gaag (1968), in Canada, showed that Friend virus, which causes leukemia in adult mice, also infected only certain strains of mice. Resistance to the agent was thought to be inherited as a single autosomal factor, but the F1 cross obtained from inbred resistant and susceptible mice was intermediate in susceptibility. Further analysis was carried out by establishing congenic lines for both the resistance factor and the susceptibility factor. The resistance factor, along with the chromosome segment containing it, was introduced into the susceptible strain by eight repeated backcrosses and, contrariwise, the susceptible factor was introduced into the resistant strain by the opposite backcrosses. This work showed that at least two genetic factors were involved in resistance to Friend leukemia. Rowe and Hartley (1972) then showed that several different viruses were involved with different tropisms, and that the tropism was characteristic of the virus. Subsequent work by Lilly et al. (1975) has continued at such a pace that further attention to the historical sequence has no particular usefulness.

The leukemia viruses of rodents may be divided into several groups or subgroups. The discovery of N-tropic and B-tropic viruses, which are limited to certain strains of mice, preceded the discovery of the xenotropic and now the amphatropic strains (Rasheed et al., 1976; Hartley

	HOST	
	RATS	MICE
ECOTROPIC	−	+
VIRUS		
AMPHOTROPIC	+	+

FIG. 5. Assumed derivation of compatability between host and C-type virus systems.

and Rowe, 1976). Since the newly discovered amphotropic (grows in mice, rats, and other mammalian cells) strains are found in wild mice with their broad genetic variation, it is possible that the true wild-type virus is amphotropic. Using quadratic analysis we have Fig. 5.

Xentropic viruses are agents which fail to grow on mouse cells but do grow on rat cells. This is true in the sense that they fail to destroy mouse cells or produce new virus, but since they originate in and are induced in mice, they are presumably transmitted vertically in these animals. They probably originated by mutation from the amphotropic strain, simply losing the capacity to be also transmitted horizontally in mouse cells (Fig. 6).

We now return to the viruses which have been studied in inbred mice and which are transferable horizontally from cell to cell only among mice. Hartley et al. (1970) showed that the different mouse leukemia viruses could be separated into two main classes dependent upon the type of mouse in whose tissues they grow. They designated the phenotypes of these mice N (NIH Swiss) and B (BALB/c) mice, which carry the basic types of genes (presumably dependent upon cell compatibility) at which locus variation may take place. Certain laboratory-passaged mouse leukemia strains infect both types of cells equally well, and these were designated NB tropic. Using the quadratic analysis method outlined for mouse hepatitis, we present the compatibility of the host gene with the virus in Fig. 7.

Two assumptions are made. First, that the gene for resistance and

	HOST		
	RATS	MICE	
AMPHOTROPIC	+	+	
VIRUS			
XENOTROPIC	+	(−)	
		HORIZONTAL	MUTATION

FIG. 6. Assumed derivation of Xenotropic type-C viruses for Amphotropic.

N HOST GENE	B (BALB/C)

	N HOST GENE	B (BALB/C)
B TROPIC VIRUS	−	+
NB TROPIC	+	+

	B HOST GENE	N (NIH)
N TROPIC VIRUS	−	+
NB TROPIC	+	+

FIG. 7. N and B tropic viruses in mouse leukemia.

susceptibility (*FV-1*) mutates from one state to another; this thesis has not yet been tested, but testing should be possible in the near future, as strains of mice are being continuously monitored for mutants. Second, the quadrates show that NB virus might arise from either N or B. The latter change has been effected *in vitro* (J. W. Hartley, T. Pincus, and W. P. Rowe, unpublished data, 1971, in Lilly and Pincus, 1973), and N has changed to NB (Lilly, 1967). The mutation thesis might also be tested by determining the capacity of the virus, growing on resistant cells following large inocula, to grow on resistant cells following small inocula. This was tested by O'Donnell *et al.* (1976), and no change in B or N viruses growing on resistant cells was found. However, the test was made after only a 1000-fold increase in virus, which would be unlikely to detect mutants at a low prevalence. In this connection, it has been shown by Ware and Axelrad (1972) that tissue culture cells from mouse embryos maintain their state of resistance or susceptibility for at least 25 transfers. The recently described cultivation of cell lines susceptible to both N and B viruses from a feral mouse embryo may suggest the presence of a third allele for susceptibility to both viruses at the same locus or the absence of the gene (Hartley and Rowe, 1975).

The presentation of these quadratic analyses emphasizes that the eventual goal of understanding is to determine the nature of the fit (compatability) or lack of fit (incompatibility) between the variety of virus mutants (Vogt, 1977) and the biochemical restrictions imposed by the genetics of the cells. In the case of these two strains, N + B tropic and NB tropic, this is currently under study by Faller and Hopkins (1977) who showed a basic similarity in that they shared 30 T1 RNase oligonucleotides and yet the N-tropic virus of BALB/c mice had 8 unique oligonucleotides and the B-tropic virus 6 unique ones. The same group of workers pinpointed other differences between the B and NB agents (Faller and Hopkins, 1977).

We now return to the development of our knowledge of the gene for cellular resistance. Resistance to leukemia viruses was first recognized by Odaka and Yamamato (1962) as a Mendelian resistance factor for the Friend virus operative in adult mice and was first studied by standard

Mendelian techniques by both the Canadian group (Axelrad and van der Gaag, 1968) and the Japanese group (Odaka, 1969). The virus was titered in adult mice by focus formation in the spleen. Subsequently, congenic strains of mice were developed by transferring the genes for susceptibility and resistance. The subsequent determination that there were two genes, one for tissue susceptibility (*FV-1*) and the other for reaction to the developing tumor (*FV-2*) has been carefully documented by Lilly and Pincus (1973).

The congenic strains of mice differing in susceptibility to mouse leukemia, which were developed by Axelrad and van der Gaag (1968), have been used by the Canadian group in several further ways. First, they showed that the gene for resistance in these mice was indeed the same one studied by Rowe et al. (1972) and that $FV-1^{nn}$ and $FV-1^{bb}$ were thereby represented. Second, they showed that resistant and susceptible cells may be derived from the appropriate mice and that such cell lines maintained their resistance and susceptibility for 25 tissue culture passages (Ware and Axelrad, 1972). Then they showed that the reciprocally related resistant and susceptible cells from these congenic mice both showed curves of the one-hit type; i.e.; they differed from each other by about 2 log dilutions but the curves were parallel (Schuh et al., 1976). Finally, they used the cell lines to analyze the nature of the resistance factor by infecting the two cell lines with defective virus (murine sarcoma virus) which can be helped by either B-tropic or N-tropic mouse leukemia virus. Under these conditions, the *FV-1*-mediated restriction acted solely by restricting the mouse leukemia virus, thereby inhibiting focus formation by the defective murine sarcoma virus in nonpermissive cells (Kochman et al., 1977).

1. Association of FV-1 Locus with Linkage Group VIII

The assignment of the *FV-1* gene to this linkage group (representing chromosome 4) was first made by Rowe et al. (1973), who showed that the character for brown coat color of the C57BR tended to be associated during segregation with the *FV-1* locus in the F2 and backcrosses with BALB/c or AKR mice. There was, however, a recombination frequency between the two characters *FV-1* and *Br*, so that they were estimated to be 39 map units apart. At the same time a preliminary study showed that *FV-1* was closely linked to the glucose-6-phosphate dehydrogenase electrophoretic variant in the two congenic mice used in the Canadian studies. This mapping has been extended further by Rowe and Sato (1973).

Finally the closeness of this last linkage was substantiated at the

Jackson Laboratory (Taylor *et al.*, 1977) where a series of multiple recombinant inbred lines derived from mice with *FV-1*^{b} or *FV-1*^{n} and four other markers were studied. The distance between the *FV-1* and the *Cpd-1* genes was shown to be 0.6 centimorgans. Only one recombinant was found among 45 mouse lines tested. These workers suggested that, since *Gpd-1* may have arisen by tandem duplication, they should search for the duplication of other genes, such as *FV-1*, in the same area.

2. Relationship of Resistance to Virus to H2 Histocompatibility (RFV-1) (FV-3)

The extensive work, including that of Lilly, associating the *H-2* locus for histocompatibility with resistance to mouse leukemia has been well summarized in Lilly and Pincus (1973). They review the evidence that within this complex the *H-2*^{k} is important in susceptibility and that *H-2*^{b} is related to resistance to leukemogenesis whether induced by means of chemical or radiation stimuli or by virus. They suggest that two genes exist within the complex, these being responsible for resistance to leukemogenesis. The complexity of the *H-2* locus, along with the inclusion of the *Ir* gene responsible for specific immune responses, makes this area of study a most active one. Chesebro *et al.* (1974) have studied the relationship of this locus to resistance in a new way. They followed the development of enlarged spleens in living mice by simple palpation following virus inoculation. They showed that recovery occurred in certain strains of mice receiving lower doses of the virus, dependent upon their *H-2* gene composition. F1 mice which were *H-2*^{bb} had a high incidence of recovery from spenomegaly compared to *H-2*$^{b/d}$ and *H-2*$^{b/a}$ mice. They suggested that recovery was influenced by a new gene (designated *FRV-1*) within the *H-2* complex. This gene itself may be made up of several genes, since backcrosses of susceptible hybrids with resistant mice showed only 3/27 to regress (Chesebro and Wehrly, 1976a). The relationship of this work to Doherty and Zinkernagels' studies (1975) on the effect of the histocompatibility complex on the recognition of foreignness of virus-infected cells by T cells is unknown.

In two subsequent studies, Chesebro and Wehrly (1976a,b) showed that specific antiviral antibodies, anti-cell surface antibodies, and killer cells functioned during the recovery process. However, there does not seem to be a crucial difference among mouse strains in these parameters.

3. Cell Type Affected

The great majority of the studies on tissue culture are done with trypsinized cultures of whole embryos. In these fibroblasts predominate

and, since the tumors are mostly sarcomatous, presumably the susceptible cells are fibroblasts. However, other cells are present. In studies on hybrid cells produced by combining SV40-transformed human cells with mouse macrophages, Huebner and Croce (1976) showed that one type of mouse (BALB/c) which harbors B-tropic virus produced macrophages which released B-tropic viruses, whereas the macrophages from C57BL mice did not. These studies were made with cells hybridized with human SV40-transformed cells.

4. Phenotype Change in Genetic Resistance

Relatively little work has been done on this subject with known incompatible pairs of leukemia viruses and cells. Blackstein and Kochman (1976) showed that both the resistance of cells from $FV-1^{bb}$ and $FV-1^{nn}$ mice may be reduced in tissue culture by the administration of dexamethasone at 10^{-4}–10^{-8} M concentrations. Most of the effect was manifest as an increase in the number of infective foci produced. The same drug also increased the yield of virus from susceptible cells. Restrictive cells have also been made susceptible or permissive by double infection (Niwa et al., 1976), but since this study depended largely upon the idea of one-hit and multiple-hit susceptibility curves, the work is discussed later.

Tennant et al. (1976) studied the effect of extracts of cultured cells and of young adult tissues from the two types of resistant pairs on the susceptibility of the compatible cells. Limiting the strength of the extract to less than 500 mg/ml to avoid nonspecific effects, they showed that a 50% reduction in susceptibility of the cells by administering only the opposite cell type (N or B) could be regularly produced. This meant of course that the tissue extract failed to equal the natural genetic restriction, which is from 99.0 to 99.9%, but introduced a new parameter in the methods of working with this particular type of cell resistance. Considerable care was taken to differentiate the reduction in susceptibility, which is *specific* for the susceptible cells, by demonstrating a correlation between the source of the extract and the genotype of a particular genetic cross.

5. One Hit versus Multiple Hits in Producing Infection

Axelrad and Steeves (1964) showed that inoculation of varying dilutions of the highly efficient virus–cell combinations produced a one-hit curve; i.e., there was a direct relationship between the number of lesions produced and the concentration of the virus. A sharp decrease in activity (much greater than that produced by dilution alone) occurs when virus is inoculated into the resistant cells; i.e., a multiple-hit curve is obtained.

Several explanations for the multiple-hit curve have been proposed, including the hypothesis that the leukemia agent is dependent upon a helper agent which is limiting. A detailed analysis of how this resistance factor ($FV-1$) operates based on the susceptibility of resistant cells to multiple hits has been offered by Pincus et al. (1975). They showed that resistant cells may fall into two classes, those succumbing to two hits and those succumbing to three. They suggested that refractoriness may be similar to a gene dose factor. Decleve et al. (1975; Decleve and Niwa, 1976) showed a similar curve change from one-hit to multiple-hit. It is important to know whether the progeny virus produced from such multiple-hit infectious curves has the same characteristics as that of the original inoculum. No change in the virus harvested from resistant cells, as compared to susceptible cells, was detected by O'Donnell et al. (1976), but the yield from only one cycle of virus growth was tested.

Many of the B-tropic strains of virus available produce a two-hit curve when inoculated on hybrid cells obtained from resistant and susceptible cells, i.e., $FV-1^{nb}$, as reported by Decleve et al (1975). This is because resistance is dominant. However, in a murine leukemia virus isolated from irradiated mice, they showed that a one-hit curve was produced on one type of hybrid, but a two-hit curve on another type of hybrid, both of which were $FV-1^{nb}$ but were otherwise different. The mouse cross which yielded the one-hit titration curve with this virus and one other B-tropic virus was NIH Swiss $\times$ C57B, whereas the cross yielding a two-hit curve was NIH Swiss $\times$ BALB/c. Both curves were intermediate in level of sensitivity between those of the resistant and susceptible parents. This has led these workers to propose that another gene, SVR, must be present in BALB/c mice together with nb heterozygosity at the $FV-1$ locus in order for the conversion from two-hit to one-hit kinetics to occur. To test this a variety of crosses was made, and the individual mice so derived were tested to see what kind of titration curves would be forthcoming. A ratio of mice yielding a one-hit curve to those yielding a two-hit curve was then determined and compared with the predicted results. There was good agreement between the two, and these investigators therefore feel that they have established another modifying gene in these mice. The gene, however, has no apparent effect on N-tropic viruses.

By use of a sophisticated statistical analysis of these curves for one-hit and two-hit infections, Niwa et al. (1976) studied the interaction of the two viruses which are apparently necessary to establish productive infection in nonpermissive cells. They showed that under proper conditions virus infection produced by one nonproductive particle converted the cell to one that was subsequently susceptible to a second one-hit infec-

tion. The effectiveness of the first nonproductive infection is lost after 24 hours, destroyed by ultraviolet radiation, and is specific for ecotropic viruses. (Exposure to xenotropic agents did not change the curve to a one-hit curve.)

One of the greatest values of genetic strains of mice, especially when they are coisogenic, is that the specific location where virus growth is stopped in the resistant strain may be examined in detail. It is now generally recognized that after the entry of leukemia viruses into susceptible cells the RNA genome is copied into linear and closed circular double-stranded DNA. Some part of this is the precursor of the DNA integrated later in infection, and this in turn is transcribed into the viral RNA, which may be measured by the amount of virus-specific reverse transcriptase.

First, by using the two types of resistant and susceptible cells and N- and B-tropic viruses, it was shown by Huang et al. (1973) that the cells could absorb and take in pseudotypes of vesicular stomatitis virus (i.e., with N and B coats on their surface), as demonstrated by the subsequent growth of these compatible viruses in cells which were resistant and susceptible to N- and B-tropic leukemia viruses. They concluded that resistance was manifest after attachment and penetration. Krontiris et al. (1973) simultaneously and independently reported the same results, and in addition demonstrated that virus was absorbed with equal efficiency to the resistant and to the susceptible cells. These data, together with the work of Yoshikura (1973), who used murine sarcoma virus as a pseudotype of leukemia strains, indicate that resistance to infection by the FV-1 gene takes place after primary absorption. However, Bassin et al. (1975) have questioned the value of Yoshikura's model.

At the other end of the sequence RNA-DNA-RNA, the output of infectious virus was measured by following the amount of reverse transcriptase produced in tissue culture. Jolicoeur and Baltimore (1976b) showed that virus production in the fluid was reduced 70 to 100-fold in resistant cells, and that virus-specific RNA was equally reduced in the cytoplasm and nuclei. The stage was then set for two groups to determine more exactly where the incompatibility occurs (Sveda and Soeiro, 1976; Jolicoeur and Baltimore, 1976a). In these experiments, the use of a virus-specific DNA probe and a new procedure for the extraction of unintegrated double-stranded forms of proviral DNA were important. Compatible and incompatible pairs of viruses and cells both yielded the same amounts of unintegrated proviral DNA after infection, but only in the compatible cell systems was this new DNA integrated into the host cell.

Jolicoeur and Baltimore (1976a) conclude their discussion as to where the FV-1 gene acts with the following:

The possible models of a Fv-1 action have been sharply restricted by the experiments of Rein *et al.* (1976) and Bassin *et al.* (1975). Their experiments indicate that the viral structure susceptible to the Fv-1 product is one provided by MuLV to murine sarcoma virus. Furthermore, the Fv-1 sensitive structure can be phenotypically mixed in the yield of MuLV from cells con-infected with N-tropic and B-tropic virus. Such a structure would probably be a protein. The only known virus-coded protein that functions early in the MuLV growth cycles is the reverse transcriptase and, because so much of the coding capacity of the genome has been assigned to known functions, there may not be new viral proteins to discover. To reconcile these facts with our observation that the bulk synthesis of reverse-transcribed DNA occurs normally in Fv-1 resistant cells, we must postulate a function for reverse transcriptase beyond the manufacture of DNA. This function would be the one specifically affected by products of Fv-1 alleles.

6. The FV-2 Gene

Is is one of the basic tenets of this review that, just as with bacteriophages and their host bacteria, there is a continuum of genetic adjustments between host and agent. Each pair represents a degree of adjustment that may be changed by a mutation (or by phenotypic modification of the host) of either member of the pair. New compatible host–virus pairs will be recognized only as the old ones become standardized.

However, the first gene to be identified as a resistance gene in any strain of mouse leukemia was the one now known as *FV-2*. It is separate from the histocompatibility locus (Chesebro *et al.*, 1974). It was originally recognized in a general study of the resistance of mice to Friend leukemia by Odaka and Yamamota (1962). Axelrad and van der Gaag (1968) later studied the same groups of resistant and susceptible mice, but also developed congenic mice in which the selection procedures (on the basis of performance of progeny) were somewhat different. In their work it soon became clear that two genes were operative. The gene subsequently demonstrated to be effective in tissue culture by Rowe *et al.* (1973) is *FV-1*, whereas the gene which represents the originally described resistance of the mice themselves to Friend virus has been called *FV-2*. There is much less knowledge of how the latter gene functions. In contrast to *FV-1*, resistance of the mice is almost absolute, but the situation is similar to that in the mouse hepatitis system in which adult mice from the resistant strain are also completely resistant (in terms of death), but tissue cultures of these mice allow for a change in the virus, and thus the tissues appear to be only relatively resistant.

The mechanism of the resistance produced by *FV-2* in preventing the development of mouse leukemia has been intensively studied by a group at Boston University (Kumar *et al.*, 1974; Kumar and Bennet, 1976). They investigated resistance and susceptibility to Friend virus in several different strains of mice and in resistant mice of differing ages. Because

there was an apparent association of the capacity of mice to reject bone marrow grafts and to be resistant to the Friend strain of mouse leukemia virus, they were led to see if ^{89}Sr, a bone-seeking element, would abrogate the resistance of the genetically resistant mice to the virus as well as to bone marrow transplants. This was found to be so, and since ^{89}Sr apparently does not affect T or B cells, the putative cell responsible for resistance was called the M cell. The marrow cells of the resistant strain of mice are not in themselves completely resistant, since bone marrow transplantation from resistant mice into susceptible, irradiated mice which have been given virus do develop leukemia.

Friend virus itself suppresses or represses the general immune response of susceptible mice, but not of resistant mice. Using this action of the virus as a marker to differentiate how the gene for resistance to virus may function, they conclude (Kumar *et al.*, 1976) that there is a repressor cell intermediate between the M cell and the target cell—and that this is one of the T-cell series, since it requires thymic influence for maturation, has Thy-1 antigen, adheres to nylon wool, and is lysed by cortisol. These intermediate cells are present in susceptible mice and absent in resistant mice. Although they play a part in resistant mice in the immunodepression caused by virus, their relationship to susceptibility to the disease, leukemia, is not clear.

7. The FV-4 Gene.

An apparently totally different gene for resistance to murine leukemia viruses has now been reported by Suzuki and co-workers (Suzuki, 1975; Suzuki and Natsubara, 1975; Kai *et al.*, 1976). This gene, present in a strain of mice called G, shows resistance both in the mouse and in embryo cell cultures to N-tropic, B-tropic, and NB-tropic viruses. The degree of relative resistance in culture varies from 100-fold to as much as 765-fold, but in the exceptional virus strain (Maloney NB) it was reduced to 44. The gene has been named *FV-4*.

B. Inheritance of Leukemia-Associated Antigens

Historically, essentially two major aspects are involved. The first is concerned with the antigen found on the surface of Gross virus-induced tumors, now called Gross cell surface antigen (GCSA), and the less studied Friend, Maloney, and Rauscher (FMR) antigen induced by this group of agents. The second aspect arose with the study of differentiating antigens on the surface of mouse thymus cells developed by Old *et al.* (1965) at Sloan-Kettering Cancer Center. The most relevant of these is the GIX antigen which appears on thymus cells of some mice and not

on those of others but is also present on the surface of tumor cells induced by virus, such as those of AKR mice.

These two sets of data are rapidly being brought together by the recent demonstration that the glycoprotein of MW 69,000–71,000 (gp 69/71) of purified mouse leukemia virus is closely related to the GIX antigen, and that the other antigen (GCSA) is closely related to the smaller proteins of the virus (Obata et al., 1975; Snyder et al., 1977). How these different antigens function will eventually be of great interest. A great deal of the fascination, however, is covered over by an excess of confusing initials and nomenclatures, for example, gs (group-specific antigen), G (Gross antigen), GIX (originally a thymus differentiation antigen), and gp (glycoprotein of murine leukemia virus), as well as the intials of the different mouse strains, each with its own backcrosses and its own tumors, and the need to describe specific antisera that have been developed for a particular cytotoxic test. In addition, there are the newly created names for markers: "gag" for group-specific antigen, "pol" for polymerase, and "onc" for oncogenicity. Thus, in a general review of this sort, it becomes very difficult to evaluate independently the relationships of these different antigens.

A whole new approach to the study of the genetics of virus tumors was opened up in 1968, when Payne and Chubb found that a soluble antigen present in normal chick embryos, which could be detected by sera from rats which had been inoculated with the Schmidt-Ruppin strain of Rous virus, was apparently inherited as a Mendelian trait. Rapidly thereafter a group working with Huebner at NIH, (Taylor et al., 1971), Meier et al. (1973) at the Jackson Laboratory at Bar Harbor, and a group with Old and Boyse at the Sloan-Kettering Institute (Hilgers et al., 1972) took up the problem in mice. An antigen apparently similar to that of chicken leukemia was detected in mice by using sera either prepared against the gs tumor antigen itself and highly absorbed, or by using sera from (1) rats which had been inoculated with mouse leukemia or (2) rats inoculated with tumors induced by any of the mouse leukemias or sarcomas or (3) rats that had developed rat leukemia. These sera have a sufficient degree of specificity so that, if they are used carefully at critical dilutions, they differentiate clearly between the presence or absence of the antigen, mostly by means of complement fixation tests. Major emphasis has since been placed by the Sloan-Kettering group on the G antigen which is one of the gs antigens. This antigen, now called GCSA, is carefully defined by the injection of cells of a specific Gross virus-induced tumor into C57BL/6 mice.

Mice from a standard high-leukemia strain (AKR/J) were mated, the presence and absence of gs antigen was determined in the spleens of

individual F1 mice, and the F2 generation and various backcrosses were tested in the same way. The antigen was fully expressed in the F1 generation, regardless of whether the mother or father was of the high-tumor strain. However, when the F2 generation and backcrosses with the tumor strain were studied, there was a 15:1 and a 3:1 ratio, respectively, indicating that more than one, probably two, genes were involved. When the F2 generations were carried further by inbreeding from different pairs of F2 mice, and the presence of gs antigen in the different lines thereby derived was determined, the 16 lines fell sharply into several classes— those that had continuous 100% positive results for gs antigens, those that had 50–75% positive results and, finally, those that carried no positive antigen. The last-mentioned result suggested that one dominant gene was responsible for the occurrence or appearance of gs antigen. In general, when gs antigen was present in a line or a mouse, infectious virus could be demonstrated, and, when antigen was absent, virus was not found; of 110 mice, 4 were antigen-negative and nevertheless virus-positive.

Taylor *et al.* (1973) studied a situation in which the presence of gs antigen was regulated by recessive genes at one locus, but was not accompanied by the production of infectious virus. This chance finding arose during a study of the congenic lines B10 and 58N which were thought to differ also in the presence or absence of gs antigen. Genetic analysis of the difference in gs antigen titers between B10 and 58N led them to conclude that 58N contained a single contaminant gene from one of the parent strains which controlled gs-antigen expression. This difference between the two lines (B10 and 58N) was then analyzed by the appropriate F1, F2 and backcrosses, and from these it was determined that the gene was completely recessive. Furthermore, the presence of the antigen (again tested with appropriate rat antisera) was unaccompanied by liberation of the infectious virus. These workers argued from this set of data that the locus was regulatory, or repressor (the presence of the gene suppresses the appearance of the antigen), and gave it the label *MLV-1*.

Hilgers *et al.* (1972) made similar analyses of the inheritance of gs antigen in various stocks of mice having a high and low incidence of leukemia. They found that the mice could be classified into three groups: one with high titer (1/4 to 1/8), one with intermediate titer (1/1 to 1/2), and one negative. Using these titers as phenotypic characters they analyzed a series of crosses and F1 and F2 and suggested that the results could be explained by a single gene locus, which may well be regulatory, but they did not indicate whether expression of the antigen was dominant or recessive.

With the availability of purified virus preparations and highly specific

antisera against them, it is now possible to compare the complex G antigen with different parts of the purified virus. Both by absorbing specific G antisera with parts of the core virus proteins and by testing the leukemia cells for virus antigens, three different studies have shown a close relationship (Tung et al., 1977; Snyder et al., 1977; Ledbetter and Nawinski, 1977). Thus much if not all of the inheritance of this antigen may be explained as inheritance of the virus.

GIX Thymocyte Antigen

There are several specific gene-regulated antigens [thymus leukemia (TL), LY1, and GIX] on mouse cells. One of these, the GIX, has been identified as being related in some way to mouse leukemia virus. It was originally thought to be part of linkage group IX which contains the *H-2* locus. Interest in these antigens and their relationship to leukemia arose in an indirect way, through the interest of Boyse, Old, and their associates in the expression of various surface antigens on thymocytes. For a detailed review of this work, their reviews and articles should be consulted, but since there is clearly a relationship to leukemia viruses, we summarize them here. The TL antigen, which is peculiar to the process of differentiation of the thymus, was identified after thymocytes of one strain of mice were injected into another strain. Mice can apparently form TL antibody to any TL antigen that they do not themselves express on their own thymocytes. The same antigen has been found in several leukemia cells, including some that were clearly virus-induced, but the relationship of this "normal thymus" antigen to the leukemia agents of mice is an open question.

The GIX antigen on thymocytes is identified by a rat anti-mouse leukemia (Gross) antiserum. The antibody, which also occurs in direct response to infection with mouse leukemia agents, is used in a cytotoxic test for both rat and mouse leukemia cells; it is also active against thymocytes of certain nonleukemic mice. The relevance of this overlap is now much clearer, since it has been demonstrated that GIX is one of the antigens of the mouse leukemia virus gp 69/71 (Obata et al., 1975; Tung et al., 1975). The genetics of the inheritance of thymocyte antigen (GIX) has been studied in different strains of mice. The usual Mendelian analysis by Boyse et al. (1972) led to the proposal that two unlinked genes are required for the expression of GIX on normal thmocytes: one dominant, the other semidominant. They now call these genes *GV-1* and *GV-2*. The expression of the GIX antigen can be equated with the presence of a specific murine leukemia antigen. Indeed, infection of mouse fibroblasts with a large variety of murine leukemia viruses, both ecotropic

and xenotropic, induces antigen which absorbs antibody directed against GIX (O'Donnell and Stockert, 1976).

In an extension of this work, Stockert et al. (1972) showed that the second gene (GV-2) necessary for the expression of the Gross antigen (GIX) is in an entirely different linkage group, i.e., group I. This latter linkage group also contains the locus for inducibility of mouse leukemia (see below under Rowe et al.), but it is suggested by Stockert et al. (1972) that the genes for Gross antigen and inducibility are different, because AKV-1 (inducibility) has been located on the other side of Gpi-1 and Hbb1 from GV-2.

The determination that a particular gene is linked with another is of course measured by the degree to which the characters fail to segregate independently of each other in the F2 generation and other crosses. The linkage of separate characters is the basis for association in a particular linkgroup, and therefore it is assumed that the two genes are on one chromosome. However, several linkage-like segregations in European and Asiatic races of mice have been obtained, which suggested to Michie (1955–1956) that a mechanism other than association on a particular chromosome is necessary for an adequate explanation. This mechanism, in Michie's opinion, might be due to an attraction of centromeres of like origin to the same pole of the mitotic spindle, thereby bringing about an association of different chromosomes during the meiotic process. Whatever the mechanism, it seems to be an established phenomenon and becomes of direct interest in studying the genetics of the association of different leukemia antigens.

Stockert at al. (1976) point out that their previous data had shown an association of the phenotype GIX and H-2 on the one hand, and of GIX and Gpd-1 (glucose-6-phosphate dehydrogenase) on the other. However, since the histocompatibility antigen, H-2 and Gpd-1 are on different chromosomes, the apparent association cannot be totally through location on the same chromosome. For this reason the original association of the three genes was reinvestigated in heterozygous mice with a similar genetic background. It was shown that GV-1 and GV-2 consistently deviated from the expected random recombination ratio of 0.50 (i.e., the ratio was 0.35), and GV-1 and Gpd-1 also differed (ratio equaled 0.32). It was proposed that this deviation was due to a quasi-linkage, a mechanism other than true linkage, and for this they invoked the theoretical considerations of Michie. Among these are a postmeiotic mechanism, a differential fertility of spermatozoa for eggs, and a differential fertility imposed by the antigenic pattern dictated by the murine leukemia gene (GV-1). The possible mechanisms of quasi-linkage as it applies to this virus antigen system have been reviewed with clear diagrams by Boyse (1977).

C. Mendelian Inheritance of the Agent of Mouse Leukemia

The naturally occurring leukemia virus of AKR mice is readily detectable as infectious virus by means of tissue culture techniques using a combination of cocultivation with susceptible cells and the formation of plaques which occurs when these cells are further cocultivated with certain rat tumor cells (XC) (Rowe, 1972). The virus appears early in the life of AKR mice, can be repeatedly cultured from tail tissue snips, and thus may be followed in individual mice. In contrast, the virus is much less common in low-leukemia strains having a low incidence of disease and is absent in early life. Thus this phenotypic character can be analyzed by formal genetic procedures, i.e., the presence of virus in early life. The characteristics of the mouse may be defined by the presence or absence of virus at 2 weeks and at 6–10 weeks. Rowe (1972) first studied crosses between AKR mice and a series of low-leukemia-strain mice, all of which were, however, limited to those which carried the gene for susceptibility to N-tropic viruses ($FV-1^n$). This provision was necessary, since (as was shown by Rowe and Hartley, 1972) most of the spontaneously occurring virus in AKR mice is N tropic, and therefore the presence of genetic resistance to *propagation* of the virus would in itself affect the spontaneous presence of the virus and should be eliminated. When the appropriate crosses were made (Fig. 8), Mendelian inheritance was clearly demonstrated by the dominance of the phenotypic character of the presence of the agent in the F1 generation in a series of crosses, and by segregation in the F2 generation and in backcrosses of the specific character (absence of virus at either 2 or 6 weeks of age). The ratio of the number of mice with virus to those lacking virus in the F2 generation was 15:1, and in the backcrosses it was 13:1, making it extremely unlikely that the genes for occurrence of virus are at one locus and strongly suggesting that two autosomal loci are involved. One of the loci, *V1*, was shown to be associated with linkage group I by the observation that in many of the backcrosses there was an association of coat color (white) with the presence of the virus at 2 weeks of age. This linkage was further tested by searching for an association with the gene which determines hemoglobin type. Virus occurred approximately one and one-half times more frequently in mice which had acquired their gene for hemoglobin or coat color from AKR mice. Rowe (1972) points out that, in cases where two independently segregating genes give rise to the same phenotype, the closest possible linkage with a given marker would give only a 2:1 ratio. Further evidence for linkage of the one gene was obtained by studying the offspring of different families in which only one gene for the presence of the virus remained after backcrosses and segregation. This locus, referred to as *VI* (now referred to as *AKC-1*), was

25 to 30 map units from c (albino coat color), and the order appeared to be $V1$-c-Hbb.

Other families derived from the different backcrosses and segregations of the two dominant genes could also be shown to have lost one of the genes for the presence of the virus. These latter families (called $V2$) showed no linkage between the phenotypic presence of virus and either coat color or hemoglobin type, demonstrating that the second gene for presence of the virus was independent of the first.

Rowe (1972) suggests that the type of inheritance which has been studied here should not be considered similar to that of classic Mendelian inheritance, even though Mendelian methods have been used to study it. First, some of the strains of mice which are scored negative for the virus early in life actually do show virus later in life and, second, the presence of the virus depends upon its release from a few cells and then its spread by infectious processes to other cells.

An excellent demonstration of the role of resistance to spread of the virus is presented in the paper by Rowe and Hartley (1972). It is remembered from Section VIII,A that mouse strains tested in embryo tissue culture may be shown to carry a gene for resistance to propagation of the leukemia virus, and dependent upon whether the virus is of the N- or B-tropic type, have either the allele FV-1^n or FV-1^b. Since most of the AKR mice carry the N- tropic virus, it was possible to analyze the effect of FV-1^b (which produces resistance to propagation of the N- tropic viruses) on the spontaneous appearance of leukemia virus by making crosses of AKR mice with low leukemia strains which carry the gene FV-1^b instead of FV-1^n, as was done in the previous analysis. Some of the results of these crosses, as reproduced from their paper, are presented in Fig. 8. The marked reduction in the number of mice of the F1 generation that had virus present at two weeks of age and the ocurrence of mice that did not show any virus at six to ten weeks was apparently a reflection of this resistance to propagation (Fig. 8). Furthermore, in the backcrosses there were very few mice that had significant amounts of virus at two or six weeks, in direct contrast with the backcrosses between AKR and low leukemia strain mice with the permissive gene FV-1^n,

That the FV-1 gene is acting by suppression of the ability of the virus to propagate in susceptible tissues, and not acting on the actual spontaneous occurrence of the virus, was nicely demonstrated by induction of virus by means of 5-iododeoxyuridine in tissue cultures of the appropriate generations and crosses. The presence of virus in the induced cells was tested by co-cultivation with susceptible cells immediately after induction and then determining the presence of virus. Thus it was shown that the presence of FV-1^b had no influence on the evolution of the virus, but, as shown above, prevented the spread within the animal.
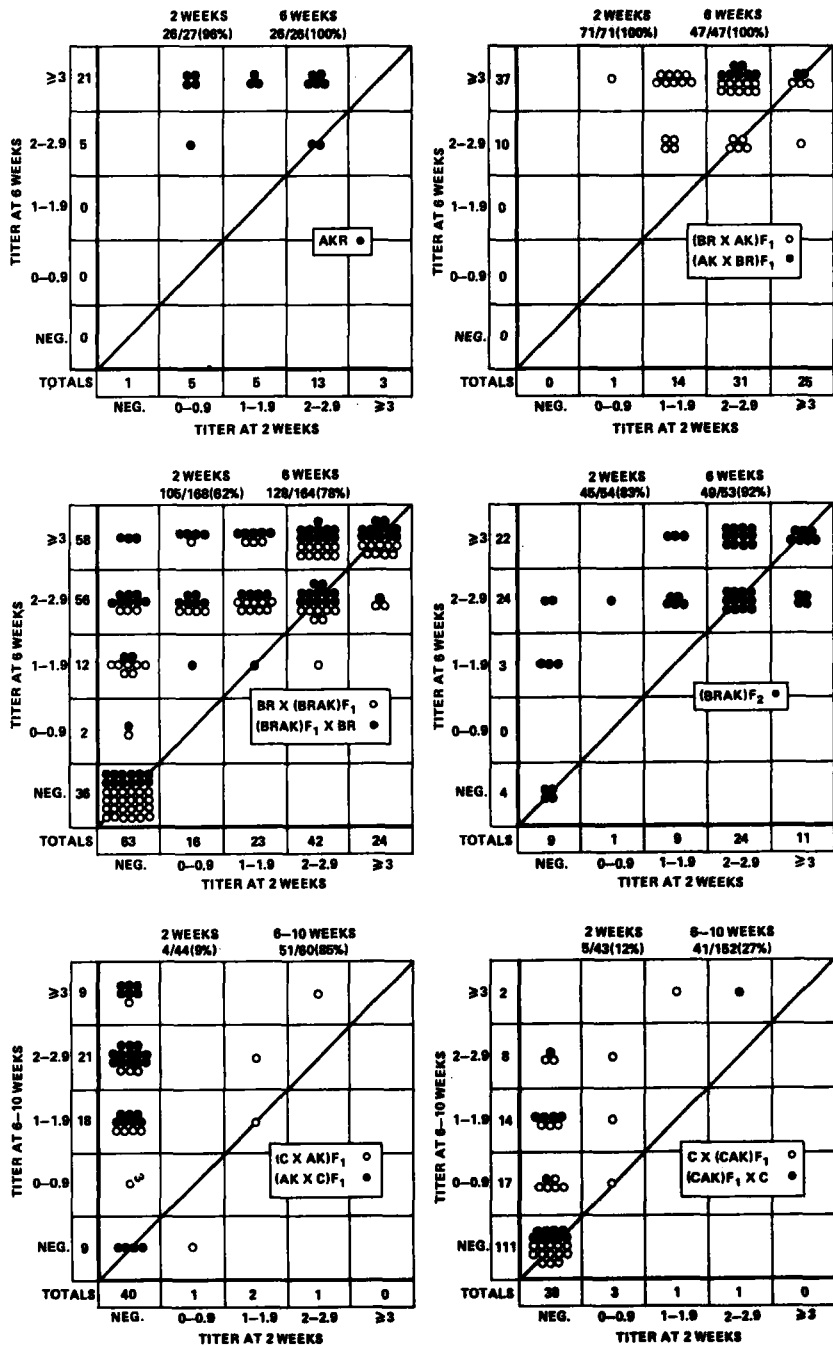
FIG. 8. Inheritance of the character (presence of mouse leukemia virus in the tail blood) in the original high tumor mice (AKR) with crosses with low tumor mice (BR) and the F2 generations. Frequency distributions of the MLV titers in tail extracts of hybrid mice. The circles show the relationship of the titers at 2 and 6–12 weeks in individual mice. Mice tested at only one time point are included in the totals along the edges.

The apparent Mendelian inheritance of the gs antigen for chicken leu-
kemia, in some cases of the mammary tumor agent, and of several differ-
ent manifestations of mouse leukemia has now been demonstrated. The
nature of the integration of the virus with the host genetic material has,
however, not been clarified. The main purpose of Rowe and Hartley's
investigation was to determine whether the virus released from the hy-
brids had the characteristics of the various hybrid hosts from which it
was actually released, or whether it was like that of the dominant parent,
the AKR mouse. Since viruses that cause leukemia in mice may be
divided into two main classes, N tropic and B tropic, and since a cross
between AKR mice (which carry N-tropic viruses) and various FV-1^b
low-leukemia mice (which carry B-tropic agents) had been made, it was
then important to determine the host range of the virus which appeared
in this set of hybrids. The host range of this virus was almost always
that of the AKR mice, which was interpreted by Rowe and Hartley as
strong evidence that the virus-inducing genetic loci of the AKR mice
contained the mouse leukemia virus genetic loci and not that the agent
was derived directly from the host.

This idea was carried further by Jaenisch (1976), who apparently in-
duced integration of the virus for leukemia in the genome of the develop-
ing mouse. In order to follow the reasoning involved in this procedure,
which for the first time combines the contradictory evidence of Men-
delian inheritance of the agent with the knowledge that it is an infectious
agent, a few preliminary statements are necessary. As Gross originally
showed, infection of an AKR mouse at birth leads to the development
of leukemia in the growing animal. However, the virus does not show the
integrated into the genome, since Mendelian analysis does not show the
agent to be appropriately distributed in the F2 and backcross generations.
On the other hand, the spontaneously infected AKR line, as indicated
above, yields Mendelian ratios. Jaenisch exposed mouse embryos at the
four- to eight-cell stage, after digestion of the zona pellucida with pro-
nase, to Maloney leukemia virus. These early embryos were transplanted
to foster mothers, and the offspring were studied for leukemia virus, for
disease, and for further transmission.

Of 140 transplants, 45 mice were successfully reared. Of these, one male
and two females developed the virus and later the disease. The male, no.
339, then transmitted the disease to 4/25 of his offspring early in his
mating career, but this capacity was gradually lost so that, at 12 months
of age, 0/28 of his immediate offspring contained the agent. This insta-
bility of inheritance of the virus is reminiscent of the paternal inheritance
of the carbon dioxide sensitivity factor in *Drosophila* (L'Héritier, 1958).
However, following mating with unaffected females, a regular inheritance

of 50% disease was obtained from offspring which were positive, i.e., from mice that were originally infected by male no. 339, thus establishing the apparent Mendelian nature of the inheritance. Molecular hybridization experiments showed that these males carried one copy of the murine leukemia virus gene per diploid mouse genome, thus supporting the idea that viremic backcross animals are heterozygous for a single Mendelian locus carrying this gene. Finally (Jaenisch, 1977), these heterozygotes were mated and the expected three types of offspring obtained. These were $++$, $+-$, and $--$, and as far as the presence of Maloney murine leukemia virus-specific DNA sequences in their liver nuclei was concerned, all three developed normally.

D. Inheritance of Inducibility

This section deals with (1) mouse leukemia (2) xenotropic agents, and (3) electron microscope studies on C-type particles in the pancreas.

1. Mouse Leukemia

When it was shown that the leukemia agent could be recovered from tissue cultures of AKR (high-leukemia) mice which had been maintained as noninfectious mouse embryo linse, that most if not all of the cells could be activated to produce infectious virus (Rowe and Hartley, 1972), and that the spontaneous release of virus by mouse embryo cells in several cell lines occurred after long-term culture (Hall et al., 1967), the similarity to a lysogenic or virogenic system became much greater. This rapidly led to the discovery that X rays and DNA inhibitors, such as 5-iododeoxyuridine, could greatly increase this spontaneous release.

The genetic background for such release was then studied by Stephenson and Aaronson (1972a,b). In the first study they used the amount of reverse transcriptase inhibited by specific sera and obtained from the supernatants of embryo tissue cultures of the different mouse strains as a measure of the amount of virus released. In the second study virus activation itself was also determined by XC plaque assay. Initial release of virus from the B (BALB/c) embryo cells was apparent after treatment of secondary cultures with iododeoxyuridine and cocultivation with 3T3 cells. The same treatment of the N (NIH Swiss) embryo cells did not evoke the virus. The factor for inducibility was dominant (F1 yielded the same amount of virus); and when backcrosses and F2 generations were tested, it semed as if one gene was responsible. This gene was given the label *IND*.

The C58 strain of mice has a high susceptibility to spontaneous leukemia, and replicating virus can be detected in primary embryo cultures

of this strain. Therefore the *IND* gene was introduced into the various crosses and the initial burst of virus release on induction with iododeoxyuridine was followed by an increase in and persistence of the virus. Finally, by comparing the incidence of spontaneous appearance of the virus in tissue cultures of embryos of the different strains and backcrosses, and of virus in cultures of spleens of adult mice, a close correlation between spontaneous induction and inducibility by 5-iododeoxyuridine was demonstrated, suggesting that the same gene was involved. These workers reason that inducibility represents the presence of a structural viral gene in the mouse. This is given further credence by the finding that the induced viruses from different parents had different biological properties.

In an extension of the mapping of the locus for the induction of mouse leukemia in the case of a high-virus phenotype, Rowe *et al.* (1972) indicate that AKR mice possess two independently segregating chromosome loci, either of which leads to appearance of the virus early in life. They showed that one locus was on linkage group I—about 3 and 12 map units from the loci for albino (*c*) and for the B chain of hemoglobin (*Hbb*) genes, respectively. The other locus (*V2*) was not mapped. This differentiation into two loci was made possible by the use of backcross mice in which it was shown that, when the character for high inducibility was present in 50% of a particular cross, some of the mice had the character associated with the genes on linkage group I and others did not. Since the presence of the character in 50% of the offspring of the backcross suggested that it was due to one gene, and yet was sometimes associated but other times not associated, the results meant that two dominant genes both functioning in the same manner had been separated. The *V1* locus was further mapped by three-point crosses, and the order determined to be *C-Gpi-AKV-1*. The specificity of the mapping is then direct evidence of a chromosomal locus; second, the linkage to *Cpi-1* makes testing for allelism with other phenotypes much easier; third, since *Gpi* (glucose phosphate isomerase) can be tested in tissue culture, work on somatic hybridization in tissue culture will be facilitated. Finally, localization of the gene in association with *Cpi-1* "may provide a unique means of examining the most crucial, and the most difficult to test, portion of the oncogene hypothesis, that is, that subinfectious expression of the inherited MuLV genome is a major determinant of malignancy, not only of leukemia, but of solid tumors as well."

Two subsequent papers (Chattopadhyay *et al.*, 1974, 1975) from this group offer very strong evidence that the *AKV-1* locus responsible for appearance of the leukemia virus, which previous work had shown to be part of the Mendelian genetic mechanism of the host mouse, is viral

genetic material. A more sensitive single-stranded DNA probe made *in vitro* by using mouse leukemia virus showed that at least 87% of the sequences present in the 70S viral RNA were matched by the DNA of AKR mice (both embryos and tissue culture), but that NIH mouse cells which lacked inducibility also lacked many of these sequences. Second, the "gene" for the virus from the AKR mice was introduced by appropriate backcrosses into the NIH mice, and association of the sequence in the host gene and inducibility of the virus were determined. This association was maintained through a three-point cross segregating for *AKV-1* on the MH background.

It is appropriate here to quote from Lwoff's original review of lysogeny (1953) in which he discusses the relationship of lysogeny to the neoplastic process:

> For the time being, no evidence may be advanced against the hypothesis that the potential power of a cell to become neoplastic may be perpetuated in the form of a gene-like structure and that the carcinogenic agents induce the expressions of the potentiality of this genetic materal. . . . Thus the neoplastic potentiality of a cell could be visualized as perpetuated in the form of the genetic material of the neoplastic particle. . . .

2. Xenotropic Agents

There is increasing recognition that virus induction can be elicited so frequently among mouse strains that most mouse cells must be assumed to carry type-C viruses in some latent state. Only with the most rigorous proof can it be stated that a strain or cell line does *not* contain a virus. For instance, NIH mice (Stephenson *et al.*, 1974; Aaronson, 1974) can rarely be induced to yield virus, but a type-C mouse virus was evoked by passing a human tumor cell line into immunosuppressed mice of this strain. Various immunological tests show that NIH cell embryo cultures contain antigens identical to viral antigens. In addition, there is now recognized a special class of these viruses, which are called xenotropic because their presence is detected by inoculation into rat, mink, or human cells, even though no mouse cells have yet been shown to be susceptible. They may differ from other indigenous mouse viruses, and therefore their genetic control mechanisms are of special interest.

With this situation particularly in mind, Aaronson and Stephenson (1973) looked for genetic loci for controlling the activation of these xenotropic agents. It had been found earlier that NZB mice (which also spontaneously develop a disease not unlike lupus erythematosus) readily released a type-C virus which was immunologically identical to an inducible agent of BALB/c mice and which was xenotropic. Thus it was assumed that NZB mice carried a gene(s) allowing release, wheras NIH

Swiss mice failed to release the presumed xenotropic agent that they carried. Both the NZB and the BALB/c mice had antibody to the agent and yielded embryo cells which were induced by iododeoxyuridine to yield an agent which was measured by its specific polymerase activity. Thus is was assumed that the inducibility in the mice and in the cultures was a dominant character. These two strains were subjected to further genetic tests by crossing and backcrossing (Stephenson and Aaronson, 1973), and the amount of virus produced was followed by determining the specific polymerase activity of 1000-fold concentrates of tissue culture fluid. The RNA-dependent DNA polymerase activity was shown to be specific for type-C viral activity as measured by inhibition by antisera prepared against purified enzyme. It was emphasized that these results were for type 2 of the endogenous viruses of this strain of mice, i.e., for a xenotropic virus. NIH embryo cells released no detectable enzyme, and NZB released high levels, whereas the F1 hybrid embryo cells released intermediate amounts. Backcrosses yielded amounts of activity similar to that of the original line with which it was backcrossed, but the intermediate group remained at about 50% prevalence. The F2 generation showed a ratio of 8 high levels, 23 intermediate, and 9 with no specific enzyme. As Aaronson and Stephenson (1973) state: ". . . these results are in agreement with those expected for the Mendelian segregation of a partially dominant locus influencing spontaneous expression of a xenotropic endgenous type-C virus."

Recent studies from the same laboratory (Greenberger and Aaronson, 1975) suggest that these xenotropic viruses, which are activated by both cyclohexamide and iododeoxyuridine, do so by different means. As yet no genetic differences in these types of activation have been reported.

Closely related strains of C3H mice differ in their mitogenic response to lipopolysaccharides (Watson and Riblet, 1974). It then is not surprising that lipopolysaccharide given to different mice differs in its capacity to induce type-C RNA viruses. Indeed, the same sharp difference found among C3H/HEJ and other C3H strains for lipopolysaccharide seems to hold for inducibility (Phillips et al., 1977), which presumably is a response to mitogenic activity.

3. Electron Microscope Demonstration of Pancreatic Agents

An entirely different method of studying genetic control of endogenous C-type virus production is presented by Beiocchi et al. (1975) who found that two strains of mice, C37L/He and C4/BL/6J, regularly had pancreatic acinar cells which contained moderate numbers of C-type particles. These particles increase greatly in number when mice are treated with dexamethazone and also become extracellular. Two other strains of mice,

C3H$_f$ and BALB/c, lack the particles and are not induced to produce them by dexamethazone. Crosses between the two C57BL strains (particles present) and the C3H$_f$ strain (particles absent) yielded F1 mice that had virus present. Thus the genetic expression from this cross is dominant. However, a cross with the BALB/c strain (also negative for particles) yielded an F1 generation which was negative, but which when backcrossed with the positive mice yielded about 50% positive mice. Thus the factor for expression is dominant with one cross and recessive with the other. These workers note that no particles are found in positive mice in tissues other than the pancreas. Thus the factor may also be expressed only in certain tissues. The relationship of this agent (known only by electron microscopy) to the other inducible agents remains to be determined.

In addition to the variety of mice under study, there has also been such a variety of methods used that even for a specific phenomenon, such as inducibility, it is difficult to compare the resuts of one laboratory with those of another so that a determination of the relationship of one gene to another may be made. The first systematic studies were those of Law (1972) on the induction of the disease leukemia. In these studies, in addition to the effect of presumed Mendelian inheritance, a maternal influence was apparent in the F1 offspring. Then came the detailed studies on the inducibility of the virus—without data on development of the disease. Now, with knowledge that a specific leukemia virus may be induced by X rays (Kaplan, 1967), the group at Stanford has determined the inheritance of susceptibility to the virus strain which had been originally X-ray-induced (Rad LV-1). Susceptibility to this virus turns out to be influenced greatly by the *H-2* locus; i.e., the *H-2D^d* allele confers resistance, and the *H-2D^q* and *H-2D^s* alleles are associated with susceptibility. Yet in a strain of mice other than that used in the above tests, a different gene, not linked to *H-2*, confers dominant susceptibility to the same virus, and this gene overrides the *H-2D^d* protection (Meruelo *et al.*, 1977a,b).

E. Cytoplasmic Inheritance

The separation of genetic factors into nuclear (Mendelian) and cytoplasmic has played a significant role throughout the history of the genetics of tumors. Lilly and Pincus (1973) discuss several instances of maternal influence on the resistance of offspring, but in the absence of concrete data it is not yet possible to differentiate between the acquisition of protective antibodies, the presence or absence of helper agents, and true cytoplasmic inheritance.

Decleve *et al.* (1977), in their study on the *in vivo* interaction between

RNA viruses induced by irradiation, suggest that certain nonthymotropic
viruses may act as agents which can facilitate similar defective agents. If
the nonthymotropic agent were itself transferred from mouse to mouse
by non-Mendelian inheritance, this would be an example of cytoplasmic
inheritance.

As pointed out by Sager (1972), cytoplasmic or organelle inheritance
became acceptable with the development of techniques allowing one to
study linkage groups of characters within the organelle and with the
finding of DNA peculiar to mitochondria and chloroplasts. Thus it seems
possible that true heritable cytoplasmic factors play a role in the com-
patibility of agent and host cells. However, until the mechanism for de-
termining linkages for such presumed cytoplasmic characters as drug
resistance in somatic cells is established, this very important area will
remain relatively unexplored.

There are already at hand established phenomena of ontogenetic
changes both in susceptibility and natural inducibility. In addition, the
apparent incubation period preceding the development of a tumor after
the virus is manifest may be considered an epigenetic phenomenon. This
has been subjected to very little experimental analysis.

F. Viruses and Leukemia

The pyramid diagram at the start of this section indicates how a
variety of genetic studies pointed toward the basic study of the relation-
ship of the agent to the production of the tumor. This was both the
starting point of genetic tumor research and is now also the place where
final synthesis is needed. The admirable and thorough review of Lilly and
Pincus is addressed to this very point, and in some ways has been
extended in the book "The Molecular Biology of Tumor Viruses" by
Tooze.

The demonstration that the virus is inherited by a formal Mendelian
genetic mechanism suggests that other nontumor viruses may also be
inherited by the same mechanism but may play no role in the production
of tumors. Indeed, the fact that NIH mice carry part of the viral genome,
but do not develop tumors, suggests this. The fact that cell populations
may carry more than one endogenous virus, and that some of them may
be xenogenic, i.e., able to produce their effect only when grown on cells
of other species, suggests that in the ultimate integration of virus and
host there may be such a complete and common matching of the two that,
as in the lysogenic bacterial system, the host becomes resistant to other
members of the same virus group.

It is important to emphasize again in this section why such a rapid

and complete analysis of this particular system was possible. Much of it is dependent upon the unique use of the mouse as an inbred standard animal whose genetic makeup is now much better understood through the use of congenic strains, pioneered by Snell in his transplantation studies (Snell, 1975), and now extended to several host–virus systems. There are no other such extensively known vertebrate systems. The work on chicken tumors is limited by the lack of such systems.

Doherty and Zinkernagel (1975) found that killer T cells must have the same histocompatibility type (*H-2k* compared to *H-2d*) as the virus-infected cells in order that the killer cells be effective *in vitro*. This has been confirmed in several but not all *in vitro* systems. Since it had previously been shown that the *H-2* type was influential in determining the prevalence of leukemia in mice (Lilly, 1969), it has become of great interest to determine how the histocompatibility genotype interacts with the spontaneous induction of the virus, in turn leading to manifestation of disease. Lilly *et al.* (1975) made various crosses and backcrosses between AKR (high-leukemia strain) and BALB/c (low-leukemia strain) and thereby established strains that were similar in having the *AKV-1* and *AKV-2* genes for spontaneous expression of the virus but which differed in both the genotype for *FV-1* (n versus b) and for the two variants of the *H-2* histocompatibility, *H-2d* and *H-2k*. First, as before, there was a close correlation between the presence of the virus in the backcrosses and the development of leukemia. The *FV-1* gene suppressed both viruses and thereby the subsequent leukemia. Finally, there was a highly significant nonrandom distribution of virus-negative mice among the backcross litters, suggesting a maternal effect on virus expression.

In this connection, three recent reports (Hartley *et al.*, 1977; Troxler *et al.*, 1977; Kozak and Rowe, 1978) show (1) that a new type of recombinant murine leukemia virus is found in the thymuses of leukemic and late preleukemic AKF mice and even in lymphomatous NIH mice with the *AKV-1* loci, and (2) that the Friend strain of spleen focus virus is also a recombinant between an ecotropic and a xenotropic C virus.

From the theoretical standpoint, one may return to Flor's hypothesis on the matching of host gene with agent gene and suggest that the ultimate form of parasitism, i.e., lysogeny, cannot be matched by a host gene, for the parasite has become the host. It is therefore to be expected that future investigations will include much more work on the genetics of the agent as related to host adaptation.

In order to analyze the different genetic stories that have been followed in the search for a viral etiology of cancer, and which have focused so directly on mouse leukemia, it was important to separate it into different parts, emphasizing primarily (1) susceptibility to exogenous

virus, (2) inheritance of leukemia antigens, (3) Mendelian inheritance of virus, and (4) inheritance of inducibility. These different parts are now beginning to be brought together. Genetic susceptibility to exogenous virus is a necessary prerequisite for virus multiplication, since early multiplication of the virus seems to be necessary for the later induction of tumor. However, genetic susceptibility to multiplication and Mendelian inheritance of the virus are now linked by the demonstration that exogenous virus (introduced into newborns) may be converted into endogenous virus by infecting embryos *in vitro* at the four- to eight-cell stage and reimplanting them in the mother. Inheritance of inducibility, as compared with inheritance of the spontaneously appearing virus, must follow very similar lines, perhaps modified by some as yet undescribed epistatic factor. The relationship of the specific normal thymus antigens which are directly related to an antigen of the virus itself now seem to have some connection with the question of susceptibility to the virus itself, since the *FV* gene (viral susceptibility) apparently controls the association of the *GIX* gene with a nonviral gene (that for glucose phosphate dehydrogenase). Although no data clearly indicative of cytoplasmic inheritance of any factor controlling the development of leukemia have been forthcoming, as with the story of genetics in general, exceptions to Mendelian inheritance can only be satisfactorily explored when the basic Mendelian inheritance has been established.

Rowe (1973), who has been responsible for so many of the genetic analyses of susceptibility, inheritance of the genome, and inducibility, has discussed all this in relation to the development of cancer;

> The growing understanding of endogenous viruses does provide an unexpected answer to the question, 'Do C-type virus genomes cause cancer?' In my view, the answer to this question is now, 'It doesn't matter.' That is, the C-type virus is so integral a part of the cell (and this almost certainly will be found for human cells as well) that its expression in malignant cells is in itself sufficiently important and potentially exploitable that whether the viral genomes are the cause, in a formal or semantic sense, is of little additional practical importance. What is important is that the viral genomes are there, often expressed, and thus may provide a specific point of attack against the cancer cell by either biochemical or immunological approaches.

IX. Mouse Mammary Tumor Virus

From the virologist's point of view, the mouse mammary tumor virus is among the most difficult and confusing chronicles in the literature. It has been reviewed thoroughly by several outstanding tumor workers, but we again review the material because this virus is rapidly becoming a classic example of the very different modes of transmission and types of

interaction with host genes that are possible within one virus species. It is also an excellent example of the unconscious bias that is built into all medical research, dependent as it is upon the chance selection of model systems. The major difficulties of the mouse mammary tumor virus (MTV)* model have been the lack of an adequate and rapid titration method and the consequent dependence on tumor incidence as the critical measure of virus activity. These have been partly overcome in the last decade by the use of electron microscopy, fluorescence microscopy, immunological tests for virus-excreted milk, and the measurement of increases in virus DNA and RNA.

There are really three independent stories about the virus: (1) the Bittner milk-transmitted agent (Bittner, 1942), (2) the agent associated with residual late-appearing mammary tumors (Andervont and Dunn, 1948), and (3) the Muhlbock agent, i.e., a virulent virus isolated in Europe from a new inbred mouse strain (GR) (Muhlbock, 1965) and transmitted apparently by both male and female mice.

In order to concentrate on the genetic aspects of resistance to these agents, each story will be told separately, and the evidence concerning genetic resistance assessed separately for each of these major strains. There are other biological strains of mouse mammary tumor virus but, since they have not been studied in terms of genetic resistance, they are not discussed here. Unlike mouse leukemia agents, the strains have not been separated on the basis of host range or, as with the chicken leukemia complex, on the basis of antigenic differences. These apparent dissimilarities between this other RNA tumor virus may be an accident of the history of investigation rather than a true difference.

As recounted in Section II, one of the driving forces for the production of mouse strains was the thought that cancer is an hereditary affliction. The early investigators of the origin of mouse tumors, such as Lathrop and Loeb (1918), showed that the incidence of mammary tumors in laboratory mice ran in families and that there tended to be a greater maternal influence in this inheritance. For instance, they found that after 24 matings of high-tumor and low-tumor mice, the mothers' influence was dominant in the progeny of 17. Also, in the progeny of 10 strains in which low-tumor rates dominated, the mothers' influence was apparent

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* Following Bentvelzen et al. (1970) we designate strains of the virus by MTV plus an additional letter. MTV-S is the standard virulent agent transmitted through milk and occasionally by males of the mouse strain C3H. MTV-L is the strain identified primarily by electron microscopy, which presumably produces late mammary tumors. It is not transmitted in the milk of mouse strain C3H$_f$. MTV-P is the newly identified strain which is transmitted both by milk and presumably with the genes of mouse strain GR.

in 8. After inbred mouse strains were specifically developed for study of
the inheritance of tumors, the Bar Harbor Laboratory under the leader-
ship of C. C. Little (1956) showed that a cross between an established
high-tumor strain (C3H) and a low-tumor strain produced hybrids in
which the incidence of tumors depended upon the mother. That is, if
tumors developed rapidly in the mother (within 1 year), the offspring
had the same high incidence, while if the mother was of a low-tumor
strain, then the offspring were also. The finding by Bittner (1940) and
others that this maternal factor was a virus particle transferred in milk
then led to a series of studies by Heston *et al.* (1940) in which genetic
resistance to this transmissible factor was studied. Individual strains of
mice differed not only in the incidence of tumors following a known
standard dose of virus but also in their capacity to transmit the agent
to their offspring (Heston *et al.*, 1956). This difference was apparent even
when the mice were raised under identical conditions with the same foster
mothers (Heston *et al.*, 1956). Heston and co-workers then undertook to
study the genetic control of the propagation and transmission of MTV-S,
the standard milk agent. For this, they made a series of backcrosses of
resistant C57B with C3H females. After two such backcrosses, the
amount of agent was greatly reduced, and in the third it was no longer
present in the milk, as judged by failure to induce tumors in the C3H*f*
(susceptible but milk agent-free) strain. A reduction in transmission of
the agent had already occurred in the F1 generation. Reintroduction of
susceptible C3H germ plasm by backcrossing the seventh backcross C57-
C3H series with C3H*f* males did not reactivate the agent. These workers
state that, because the agent was eliminated so early, it was possible that
the strains differed by only a few genes controlling propagation and
transmission, possibly by a single pair. However, later tests on the ability
of the second backcross with the resistant mice continued to show a
proportion of transmission in great excess over the expected 50% (Heston
et al., 1960). This made it necessary to abandon the theory that one gene
might be responsible for regulating transmission.

The milk agent was recognized and identified on the basis of the early
development of mammary tumors in mice infected with the agent. Yet,
histologically, identical tumors appeared in mice free of the milk agent
(Andervont and Dunn, 1948; Heston *et al.*, 1950), only about a year later
than the milk factor-induced tumors. When these late tumors were
examined in the electron microscope in mice free of the milk factor,
they contained identical particles (Bang *et al.*, 1956; Bernhard, 1960;
Dmochowski, 1957). This meant either that the particles were not the
cause of the mammary tumor, or that there was some other method of
transmission of this second presumed agent, now called MTV-L by

Bentvelzen. Moore (1962), in reviewing his own and other work, has argued that the virus particles are the cause of the tumor and therefore that some other method of transfer of this agent must be sought. Vellisto and Bang (1968) sought to free the milk agent-free ($C3H_t$) mice of the additional agent by establishing lines of mice derived from either the first litter of a female or from her last litter, and maintaining this procedure through five generations. The primiparous and multiparous lines were then subjected to identical stimuli, and it was shown that mice derived from the multiparous line developed fewer tumors from the eighteenth month on. However, the first daughters of the multiparous line reverted to the higher incidence, and there were equal numbers of tumors in the families of both lines. Virus (B) particles were found in tumors of both the primiparous and the multiparous lines. These workers suggested that the agents were transmitted as hereditary symbionts, as in the case of the carbon dioxide sensitivity factor of *Drosophila* or the kappa particle of *Paramecium* (Preer, 1971). It is impossible to determine whether the association of the agent with the host in such a situation is cytoplasmic or nuclear (Mendelian) unless a test for the segregation of the character is performed either on the F2 generation or on various backcrosses of the FI generation. These tests in turn are difficult to evaluate unless the characters can be sharply separated and the incidence of tumors is high. Bentvelzen and Daams (1969) argue that "the hereditary infection with MTV-L cannot be explained by a susceptibility gene," since hybrids of $C3H_t$ and BALB/c develop more tumors than $C3H_t$, and, since this is a maternal effect, they suggest that by exclusion is transmitted as a genetic factor in the host.

A. MTV-P

The most striking recent advance in the understanding of the genetics of transmission of mammary tumor viruses came with· the development of a new strain of mice which was selected for a high incidence of mammary tumors. This is the GR strain selected by Muhlbock (1965), and in this case a strain was produced in which the reciprocal crosses of high- and low-tumor strains showed a 100% incidence of tumors. In addition, when mice were delivered by Caesarean section and foster-nursed, they still gave a high incidence of the tumors and also continued to transmit the agent in their milk. Thus the agent was transmitted by milk and apparently also derived from the male parent. Zeilmaker (1969) showed that MTV-P in females was transmitted by ova, since the transfer of fertilized eggs to females of a tumor-free strain produced a high incidence of tumors. These experiments were done with F1 hybrid eggs of a GR

and either C57B2 (resistant) or BALB/c males. Yet, all 10 such female offspring developed tumors at 4–11 months of age.

Bentvelzen (1968a) suggested that this virus strain was itself transmitted as a genetic factor. Bentvelzen and Daams (1969) showed that backcrosses between a hybrid of low- and high-tumor lines, which had 100% tumors, with a low-tumor line yielded intermediate results, indicating some segregation of the characters. Reciprocal backcrosses, however, showed a maternal influence (92% versus 21%), and three other backcrosses yielded about the expected 50% incidence of tumors. From this it was theorized that the infection was due to transmission of the virus as a genetic factor of the host. When this virus is transmitted to other strains of mice, it loses this characteristic (Bentvelzen, 1968a).

The idea that MTV-P is inherited as a single dominant gene was based on the finding that the first-generation hybrids of reciprocal matings of the new GR mice and low-tumor mice yielded 100% mice which developed mammary tumors, and that approximately 50% of the backcrosses of these hybrids with the low-tumor mice yielded mice with tumors. These data have been amply confirmed (Heston et al., 1976). However, the idea that the agent is inherited or that its expression is regulated by a single gene may be further tested in two ways. First, the female mice may also be studied for the expression of virus in their milk (immunological tests) and, second, further backcrosses may be made with low-tumor mice. The picture then loses its sharpness (Nandi and Helmick, 1974). Although there is a high correlation between the presence of the agent and the development of tumors, a much smaller proportion of mice actually develops the tumors than carries the agent. Furthermore, in the second backcross, the percentage of mice developing either virus or tumors decreases sharply as compared to the first backcross. The immediate question arises as to whether the results are contaminated by the presence of virus in the milk, but this should increase rather than decrease transmission and tumors. It is of course possible to argue that the decrease from the first backcross to the second is due to the presence of increasing amounts of the basic resistant genotype in the second backcross, but the difference between this explanation and that preferred by us, i.e., that the inheritance of genetic susceptibility is affected by more than one factor, is not great.

One arrives then at the conclusion that the major transmission of MTV-P virus is through a genetic mechanism—possibly through a straight Mendelian mechanism—but that concomitantly milk transmission and several other genetic factors may influence the capacity of the agent to be carried with the host genome. Indeed, in the studies on the original milk agent, several genetic factors also seemed to influence transmission.

It is still not possible to inoculate cultures of uninfected cells with a strain of MTV and to prove infection by readily apparent changes in the cells. Thus titrations in tissue culture are not practicable. Therefore genetic analysis of the interaction of the virus and the host does not have the same exact status as that of the two other RNA viruses (mouse leukemia virus, Rous sarcoma virus) which are clearly capable of genetic transmission.

Part I of our review has concentrated on the genetics of resistance to viruses in mice. Part II will emphasize studies in chickens and will analyze the limited data available on humans. Discussion of the entire material will be presented in Part II.

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