THE CELLULAR NATURE OF GENETIC SUSCEPTIBILITY TO A VIRUS*

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(Received for publication, January 9, 1963)

It is now well established that a number of animal virus diseases (1) as well as plant diseases (2) are directly affected by the genetic makeup of the host. In some cases resistance is dominant; in at least one (mouse hepatitis) susceptibility seems dominant (3). The demonstration that the macrophage is the cell which is paramount in susceptibility to mouse hepatitis virus, MHV, (4) was followed by a demonstration that cultures of newborn mouse liver from susceptible mice yielded susceptible macrophages and from resistant mice yielded resistant cells (3). The susceptibility factor was manifested in the hybrids, F_2, and back-cross generations of cultures from young mice as well as in whole mice. It was, therefore, inferred that genetic susceptibility resides in the cells—in particular in the macrophages. In order to test this definitively, four kinds of tests have been carried out directly in the mice and in their cells, and the four methods compared: (*a*) individual mouse susceptibility (phenotype), (*b*) production of susceptible offspring (genotype), (*c*) susceptibility of macrophages from liver cultures, and (*d*) susceptibility of macrophages from peritoneal washings of adult mice. The first part of this paper reports the results of these correlations, describes the susceptibility of several back-crosses, and confirms the cellular nature of genetic susceptibility to this virus. The second part deals with the susceptibility of peritoneal macrophages in culture and the conversion of resistant to susceptible cells by the addition of extracts of the susceptible cells to cultures of resistant cells (5). If there is a correlation between the susceptibility of macrophages and the susceptibility of the individual animal to MHV infection, then different kinds of cross and back-cross generations of susceptible and resistant animals can be studied by examining their macrophages without sacrificing the mouse, and the progeny can be used for selective breeding.

* This investigation was conducted under the auspices of the Commission on Viral Infections of the Armed Forces Epidemiological Board, and was supported (in part) by the Office of The Surgeon General, Department of the Army.

‡ Rockefeller Foundation Fellow.

Methods and Materials

Virus.—Mouse hepatitis virus, carried in the PRI strain of mice, was originally obtained from Dr. John B. Nelson of The Rockefeller Institute. The virus was harvested from PRI mice, 3 to 4 weeks old. The infected mice were killed in the agonal stage, and 10 per cent liver suspension was prepared in Hanks' BSS. Ampules of virus were stored at −30°C. The titer of virus was determined by the LD_{50} in 3 to 4 week old PRI mice, and sometimes in PRI macrophage cultures 3 to 4 days old.

Macrophage Cultures.—Each mouse was injected intraperitoneally with 2 to 3 ml of sterile thioglycollate medium; the following day about 5 ml of phosphate-buffered saline containing penicillin, streptomycin and heparin (200 units, 50 μg and 5 U.S.P. units/ml respectively) was injected intraperitoneally and after a gentle massage of the area the exudate was withdrawn. The number of cells in the exudate was determined by staining a small sample with 0.01 per cent crystal violet in 0.1 M citric acid and counting in a Spencer chamber. The cells were then separated by centrifugation (1000 RPM for 10 minutes), washed with Hanks' BSS, and resuspended in Chang's (6) medium containing 90 per cent inactivated horse serum, 2 per cent beef embryo extract, and 8 per cent Hanks' BSS, to give a final concentration of 1×10^6 cells per ml. One ml of macrophage suspension was inoculated into standard size Wassermann tubes (10 × 100 mm). The tubes were placed in a stationary position for 8 to 10 hours and then rotated in a roller drum. The macrophages were usually attached to the glass after a few hours and began to spread over the surfaces after 1 to 2 days. The medium was renewed about every 4 days.

Mice.—Major effort was placed on two inbred strains of mice which have been maintained in our laboratory for the past 8 years, by continual brother-sister mating. The C_3H strain was originally obtained from Dr. H. B. Andervont as a strain free of the milk agent. It has been used for various tumor experiments since then. The PRI strain was obtained from Dr. John Nelson in 1954 and has now been maintained through some 25 generations of similar inbreeding.

1. C_3H strain
2. PRI strain
3. F_1 hybrid of C_3H and PRI strains
4. Back-cross generations of F_1 cross:
   (*a*) $BC_1 = F_1 \times C_3H$
   (*b*) $BC_2 = BC_1 \times C_3H$
   (*c*) $BC_3 = BC_2 \times C_3H$
5. Cross generations of back-crosses:
   $BC_3 \times BC_3$
6. DBA strain
7. C_{57} black strain

Details of method pertinent to given experiments will be described in context.

RESULTS

Genetic Aspects of Macrophage Susceptibility.—It was first necessary to see whether peritoneal macrophages from susceptible mice were susceptible. As has been briefly reported (5) we found the macrophages of the PRI strain of mice fully susceptible and those of the C_3H resistant. (Figs. 1 to 4.) A variety of tests and studies on possible variables in the *in vitro* systems were then undertaken and are reported in the latter part of the paper.

Having once established a correlation between susceptibility of macrophage

cultures and susceptibility of different breeds of mice, the susceptibility of cross and back-cross generations was studied.

PRI and C_3H mice were crossed and macrophage cultures from this F_1 population were found to be susceptible to MHV virus infection (Table I). F_1 mice were then crossed with C_3H mice to yield the first back-cross generation (BC_1); crossing the susceptible BC_1 progeny with C_3H gave a second back-cross generation (BC_2) and finally the susceptible BC_2 were crossed again with C_3H to give the third back-cross population (BC_3). In this way the factor for susceptibility was introduced into the resistant C_3H mouse strain. Segregation of the characters for susceptibility and resistance occurred in the first back-cross generation (BC_1). Hybridization of PRI and C_3H mice in all tests resulted in animals (F_1) which yielded susceptible macrophages. Back-cross generation BC_1 produced both susceptible and resistant animals as determined by macrophage susceptibility as did the BC_2 and BC_3 progeny of the susceptible back-crossed parents (Figs. 6 to 15).

TABLE I

Susceptibility of Mouse Macrophage Cultures

Animals	No. of animals	Macrophage cultures	
		Susceptible	Resistant
PRI	About 100	100%	0
C_3H	About 100	0	100%
F_1	21	100%	0
BC_1	27	22%	78%
BC_2	5	40%	60%
BC_3	3	33%	66%

The data on the susceptibility of the back-cross generations are summarized in Table I.

In another group of experiments resistant animals from the BC_3 generation were crossed with those of both resistant and susceptible mice from the same BC_3 generation, and macrophages were cultured from the progeny. The results are summarized in Table II.

It was apparent that crossing resistant back-cross mice resulted in production of resistant cells, but crossing one susceptible and one resistant mouse yielded both types.

While the above tests were carried out on the macrophages, other tests for susceptibility of the intact animal and of cultures were done. Including the peritoneal macrophage test, there were four in all: (*a*) direct test of mice which were infected with MHV virus as determined by mortality; (*b*) genetic performance of mice, *i.e.* a test of the susceptibility of offspring obtained by crossing with resistant C_3H mice; (*c*) susceptibility of macrophages from cultures of newborn mouse liver; and (*d*) susceptibility of peritoneal macrophage cultures from adult mice. These results are presented in Table III.

As can be seen, there is a consistent agreement among these four methods of measuring susceptibility, with the exception of macrophages from progeny of one mouse of the BC_2 generation, which produced six out of six resistant offspring. This result may have been due to chance, since over-all results would lead one to expect 2 of the 6 mice susceptible. The genetic performance (capacity to produce susceptible offspring) showed a higher per cent of mice with

TABLE II

Results of Crossing Back-Cross Mice Known to Be Resistant or Susceptible

Mating type	Mice	Resistant
BC_3 female x BC_3 male Resistant Resistant	7	7
BC_3 female x BC_3 male Resistant Resistant	9	9
BC_3 female x BC_3 male Resistant Susceptible	4	2

TABLE III

The Susceptibility of Back-Cross Generations

(No. susceptible/Total No. tested)

Mice	Phenotype	Genotype	Phenotype	Phenotype
	Direct test of mouse	Genetic performance of mouse	Macrophages from newborn liver cultures	Peritoneal macrophage cultures
BC_1	8/24	9/15	3/10	6/27
BC_2	45/199	3/7	3/7	2/5
BC_3	30/77		0/6	1/3
Total	83/300	12/22	6/23	9/35
Per cent	27	55	26	26

the gene for susceptibility than did the three direct tests for susceptibility. The genotype may then differ slightly from the phenotype. This problem will be discussed later.

It is also possible that more than one gene is responsible for susceptibility or resistance and therefore less than 50 per cent of the animals of any particular back-cross generation would be susceptible. This aspect of the problem needs further exploration. It seems clear that the gene(s) responsible for susceptibility is dominant.

Another way of testing the validity of methods for assessing susceptibility is to compare the phenotype, as tested by peritoneal macrophage susceptibility, with the genetic performance of the animal. This has been done for eight mice shown in Table IV. In one case, there is an exception ($BC_2♂$ #13)—the same as included in Table III.

TABLE IV

Susceptibility of Individual Mice to MHV by Genotypic and Phenotypic Tests

Mouse	Parents		Phenotype	Genotype	Genotype	Genotype
	Female	Male	Peritoneal macrophage cultures	Direct test of offspring	Macrophages from newborn liver cultured from offspring	Peritoneal macrophages cultured from offspring
BC_1 female #1	F_1	x C_3H	—	4/13	—	2/5
BC_1 male #2	F_1	x C_3H	Susceptible	5/13	—	—
BC_1 male #3	F_1	x C_3H	Susceptible	16/26	—	—
BC_1 male #7	F_1	x C_3H	Susceptible	7/12	—	—
BC_2 male #9	BC_1#1	x C_3H	Resistant	0/13	0/4	0/10
BC_2 male #10	BC_1#1	x C_3H	Resistant	0/4	—	—
BC_2 male #13	BC_1#1	x C_3H	Susceptible	11/26	0/6*	—
BC_2 female #9	BC_1#1	x C_3H	Susceptible	7/13	1/2	—
BC_2 female #10	BC_1#1	x C_3H	Resistant	0/16	0/8	—

* See text, p. 784.

TABLE V

Titer of MHV from Cultures of Macrophages from Susceptible and Resistant Mice and from Various Crosses*

Control (No cells)	PRI Macrophages	C_3H Macrophages	F_1 Macrophages	BC_2 Susceptible macrophages
$<10^4$	$10^{6.5}$	$<10^4$	$10^{6.3}$	10^6

(Original titer of virus $10^{7.4}$ — LD_{50} of PRI mice.)

* Expressed as LD_{50} of PRI mice when inoculated with appropriate tissue culture fluid.

The susceptible macrophage cultures from the PRI strain, the F_1 generation, and all back-cross generations of mice were also tested for the presence of virus (Table V).

The cultures were infected with a 10^{-2} dilution of virus, and after degeneration was completed, fluids were separated, diluted, and injected into PRI mice. An increase of virus occurred only in susceptible cells. Incubation with C_3H macrophages or without cells resulted in a decrease in the number of infective virus particles. This is not surprising since this virus is inactivated at 37°C.

In all our experiments, both those with liver macrophages previously reported (3) and those with peritoneal macrophages, it was noted that degeneration of F_1 and back-cross macrophage cultures occurred more slowly than in the case of PRI macrophages. PRI cultures were routinely destroyed in 2 or 3 days, whereas destruction of F_1 and back-cross cultures occurred after 3 to 4 days. In a specific comparison, when PRI and F_1 macrophage cultures were infected with the same virus preparation simultaneously, F_1 cultures degenerated about 1 to 1½ days later than PRI cultures.

Factors Affecting in Vitro Susceptibility.—We found that the susceptibility of macrophage cultures did not depend on the age of the mice; this was tested in macrophage cultures from PRI mice ranging in age from 3 weeks to about 1 year. It was also found that susceptibility of macrophage cultures did not depend on the age of the culture. 2- to 3-day-old and 2- to 3-week-old cultures were fully susceptible to the virus. Old uninfected macrophage cultures, however, contained increasing numbers of dead cells which were detected by a neutral red test.

TABLE VI

Titer of MHV on PRI (Susceptible) and C3H (Resistant) Mice and Macrophage Cultures

	Type of mice		Type of macrophage cultures	
	PRI	C3H	PRI	C3H
LD_{50}	$10^{-7.4}$	0	$10^{-5} - 10^{-6}$	10°*

* Varying degrees of effect of undiluted virus on macrophages. No effect of 10^{-1} dilution.

The titer of the virus suspension was always 1 or 2 log dilutions higher for PRI mice than for PRI macrophage cultures (Table VI).

On the other hand, C3H macrophage cultures were resistant to the virus infection. They survived infection without any visible effect if the virus suspension was diluted. In a number of cases undiluted virus preparations caused intense granulation of the macrophages which was usually followed by partial or complete destruction of these cultures. This was much slower than in the PRI macrophages. When, however, the undiluted virus was removed from the C3H cultures after a few hours' exposure (1, 4 and 24 hours), there was little or no destruction and only a little granulation (Table VII). There is, therefore, a strong correlation between the susceptibility and resistance of PRI and C3H mice on the one hand and macrophage cultures from the same mice on the other.

The destructive effect of undiluted virus on C3H cultures might be explained by a toxin. To test for this, the virus particles were inactivated with UV light[1]

[1] General Electric sterile lamp, 15 watts, 1760 ergs/minute mm².

by radiation at a distance of 42 cm for 5, 15, and 30 minutes. During exposure the virus suspensions were stirred and samples of virus removed. C_3H and PRI cultures were then inoculated with irradiated and non-irradiated virus suspensions. Virus which had been irradiated for 30 minutes had no effect on either type of cell (Table VIII).

Shorter irradiation times partially inactivated the virus for PRI cells and similarly reduced the effect of the virus on C_3H cells. This suggests that the degeneration of C_3H macrophage cultures resulted from an overwhelming concentration of virus, since the multiplicity of the ratio of virus to infected cells is about 10. Granulation was observed both in cultures inoculated with

TABLE VII

Effect of Undiluted MHV on C_3H Macrophages

Different Times of Exposure to the Virus.

Culture	Time of exposure to MHV	Effect of virus after		
		4 days	6 days	8 days
	hrs.			
C_3H macrophage (no virus)	—	N*	N	N
C_3H macrophage + virus	72	Cells granular	50–60% of cells degenerated	80–90% of cells degenerated
C_3H macrophage + virus	24	Little granulation of of cells	Foci of degenerated cells	20% of cells degenerated
C_3H macrophage + virus	4	N	N	N
C_3H macrophage + virus	1	N	N	N

* N indicates normal culture.

non-irradiated and irradiated viruses, but it decreased in the latter, was temporary, and was not accompanied by degeneration of cells. We suggest that this granulation in both situations was primarily due to phagocytosis of liver material in the undiluted virus suspension.

Absorption.—The question of the factors which might account for the resistance of C_3H and the susceptibility of PRI macrophages was explored first by absorption studies.

10^6 and 5×10^6 macrophages were cultivated on glass or suspended in medium in siliconed vessels. Virus was added and the cultures were incubated 2 to 3 hours at 37°C, much longer than the time necessary to infect PRI macrophages. Fluid was then separated and the titer of virus determined.

No absorption was demonstrated, but the loss of virus in control tubes without cells was sufficient to obscure a differential absorption.

In another group of experiments attempts were made to isolate infective nucleic acid from the virus using high concentrations of virus (LD_{50} 10^7 to 10^8) and the cold phenol extraction technique. Preparations were checked by UV absorption for the presence of nucleic acid; high concentrations were found. Separate groups of PRI and C_3H mice were inoculated with these preparations,

TABLE VIII

The Effect of UV-Inactivated Virus Preparations on PRI and C_3H Macrophage Cultures

Macrophage cultures	Virus preparations		Effect of virus after		
	Dilution	Time of UV irradiation	3 days	6 days	10 days
		min.			
C_3H	Undiluted	0	Granulation	Partial degeneration	Partial to complete degeneration
	Undiluted	5	Granulation	Little or no granulation	N
	Undiluted	15	Granulation	Little or no granulation	N
	Undiluted	30	Granulation	Little or no granulation	N
	No virus	—	N*	N	N
PRI	Undiluted	5	Degeneration	Degeneration	Degeneration
	Undiluted	15	Degeneration	Degeneration	Degeneration
	Undiluted	30	N	N	N
	10^2	5	Degeneration	Degeneration	Degeneration
	10^2	15	N	N	N
	10^2	30	N	N	N
	10^4	5	Degeneration	Degeneration	Degeneration
	10^4	15	N	N	N
	10^4	30	N	N	N
	No virus	—	N	N	N

* N indicates normal appearance of cultures.

but no pathogenic effect was found. Macrophage cultures were exposed to the extract in standard medium without effect. Other cultures were placed in a saline medium and then exposed to the extract at 37°C for 3 hours, at which time the standard medium was replaced. Again, no effect. Blind passages from culture to culture and to mouse, and from mouse to mouse were also negative.

Except for chicken macrophages, it is generally recognized that this cell type rarely multiplies in culture. Studies were therefore initiated with the mouse cultures to determine whether multiplication does take place.

This may be demonstrated most easily by counting the number of cells at different times after inoculation. Two groups of C_3H macrophage cultures and two groups of PRI cultures were cultivated for 10 and 20 days. After these periods of incubation 90 to 95 per cent of the cells were removed by trypsinization (0.85 per cent trypsin solution) and intense shaking and the numbers of macrophages determined (Table IX).

We found that the number of cells during the first 10 days decreased, indicating that some of the cells had disintegrated. The number decreased slightly during the second 10 days. The macrophage cultures were examined for mitotic figures. In preparations stained by hematoxylin and eosin, a very few mitotic figures were found. These cultures, however, remained alive for a long time, showing excellent uptake of neutral red dye after 8 to 10 weeks of incubation.

TABLE IX

Effect of Prolonged Incubation on Number of PRI and C_3H Macrophages

Macrophage cultures	No. of macrophages		
	Original inoculum*	After 10 days	After 20 days
PRI	10^6	5.8×10^5	5.0×10^5
C_3H	10^6	4.6×10^5	3.2×10^5

* Original suspension contained all the peritoneal exudate cells, of which about 50 per cent were monocytes. Thus the original cell population actually is very similar to that at 10 or 20 days. No count was made of the few spindle cells which appear in the background after 7 days of cultivation.

There was no difference between the rate of neutral red uptake of PRI and C_3H macrophages.

The conversion of resistant to susceptible cells by exposure to an extract of the susceptible cells has been briefly reported (3). This was originally attempted since both C_3H and PRI macrophages phagocytize large amounts of material *in vitro*. Extracts from C_3H (resistant) macrophages did not change the susceptibility of PRI (susceptible) macrophages, but C_3H macrophages exposed to extracts from PRI macrophages became susceptible. (Figs. 3 to 5.) Degeneration proceeded more slowly in these altered C_3H cultures than in the infected PRI cultures. Altogether, 19 experiments have now been done. In some, the alteration to susceptibility was complete, in others partial, and in a few no effect was demonstrated (Table X).

In control experiments the influence of extracts from other animals (hamsters, chimpanzee, C_{57} black mice, DBA mice, other C_3H mice), was studied, and none of these extracts was found to affect the susceptibility of C_3H macrophage cultures to MHV infection.

In order to test the permanency of the alteration produced by PRI extracts,

C_3H macrophage cultures were exposed for 4 days to extracts from 5×10^6 PRI cells per culture. The fluid was then removed, and some cultures were infected immediately, while others simply had the fluid medium replaced. A

TABLE X

Alteration to Susceptibility of C_3H Macrophages

No. of experiments	Extract from	Per cent of degenerated cells			
		0–20	20–60	60–80	80–100
11	2×10^6 PRI macrophages	3*	3	2	3
8	5×10^6 PRI macrophages	2‡	—	2	4

* Two negative.

‡ One negative.

TABLE XI

Susceptibility of Exposed C_3H Macrophages to the Delayed MHV Infection

Preparations	Infection of culture	Effect of virus infection after		
		3 days	6 days	10 days
C_3H macrophage + extract + virus	Immediately after 4 day exposure to the extract	<80%D*	100%D	100%D
C_3H macrophage + extract + virus	2 days after extract removed	N‡	30%D	40%D
C_3H macrophage + extract + virus	5 days after extract removed	N	N	N
C_3H macrophage + extract + virus	8 days after extract removed	N	N	N
C_3H macrophage (no extract and no virus)		N	N	N
C_3H macrophage + virus		N	N	N

Extract from 5×10^6 PRI macrophages. C_3H macrophage cultures exposed 4 days/37°C to the extract.

* %D indicates per cent of degenerated cells.

‡ N indicates normal culture.

second group of cultures was infected 2 days later, a third group 5 days later, and the fourth group 8 days later. Alteration of cells, as measured by destruction by the virus, was greatest with cultures which were infected immediately after the 4 days of exposure to extract. When the infection was introduced 2 days after the extract was removed, the percentage of susceptible cells was less. When the period of time between the exposure to the extract and the sub-

sequent infection was extended (5 to 8 days), no conversion was demonstrated (Table XI).

The extract was thermostable. A series of temperatures (56°C for 30 minutes, 70°C for 10 minutes, and 100°C for 10 minutes) only partially inactivated the extract (Table XII).

In six experiments, in which the effect of desoxyribonuclease (1 mg/ml) was tested by incubation with the extract for 1 hour in the presence of magnesium ion, five experiments showed moderate to slight decrease in the conversion activity. The average reduction in activity was estimated at 30 per cent. In no case was all activity removed. Ribonuclease had no effect on the extract.

TABLE XII

The Influence of Heating, Dialysis, and Centrifugation on the Activity of the Extract

Extract	Effect of virus infection after		
	2 days	4 days	6 days
Untreated extract	N*	60%D‡	90%D
Heated 56°C/30 min.	N	25%D	80%D
Heated 70°C/10 min.	N	15%D	80%D
Heated 100°C/10 min.	N	15%D	70%D
Extract before dialysis and centrifugation	N	50%D	85%D
After dialysis 24 hrs./4°C	N	30%D	75%D
Supernatant after 5000 RPM for 1 hr.	N	N	N
Supernatant after 40,000 RPM for 3 hrs.	N	N	N
Control C_3H macrophage cultures	N	N	N

* N indicates normal cultures.

‡ %D indicates per cent of degenerated cells.

In control experiments these enzymes had no effect on either C_3H or PRI macrophages themselves; C_3H macrophages remained resistant and PRI susceptible to the virus infection. Dialysis of the extract against buffered salt solution at 4°C for 24 hours had no influence on the activity of the extract (Table XII). Centrifugation at 5,000 RPM for 1 hour or 40,000 RPM for 3 hours removed the activity of the extract completely. Supernatant fluids had no effect on C_3H macrophage cultures which remained resistant to MHV.

DISCUSSION

Resistance to virus diseases is now clearly established in some instances as being genetically determined in animal as well as plant disease. Sabin was the first to work out the Mendelian ratios involved in resistance to an arbovirus, yellow fever in mice (7). He demonstrated that resistance was dominant, and that the character for susceptibility segregated in the F_2 generation and in

appropriate back-crosses. He also emphasized the relative nature of this resistance—the variation with age of the host and the route of inoculation. Finally he suggested that resistance was primarily the property of the individual cells which harbor the virus, and not a humoral factor, since tumors explanted in resistant and susceptible mice supported the growth of Russian spring-summer encephalitis equally well (8).

Several years ago, we discovered that our difficulty in growing mouse hepatitis virus in tissue cultures of PRI mouse liver was rapidly overcome when particular attention was paid to the macrophages present in cultures of newborn mouse liver grown on collagen. These macrophages were rapidly destroyed by the virus, leaving the fibroblast and liver cells unaffected (4). After continued passage of the virus in these cultures, the parenchymal liver cells were partially destroyed. We have not studied the process of adaption to new cell types described for this virus by Mosley (9).

Interest focused on the genetic aspect of the phenomenon when it was found that a strain of mice, C_3H, resistant to the virus, produced macrophages which were resistant in tissue culture. By crossing the PRI and C_3H strains it was found that the character for susceptibility was dominant and that segregation of the characters for resistance and susceptibility occurred in the F_2 generation and in back-crosses from the susceptible F_1 hybrids to resistant C_3H mice. The ratios at which this segregation occurred in the hybrids and back-crosses were the same for the adult mice and the liver cultures, and it was therefore concluded that the macrophage was the cell which was responsible for the genetic susceptibility of PRI mice (4).

Subsequently Goodman and Koprowski (10), who had been studying the genetic resistance of PRI mice to arboviruses, by crossing resistant PRI mice to susceptible C_3H mice and back-crossing the resistant F_1 hybrids to the PRI (continually selecting for resistance), showed that the progeny of the eighth back-cross yielded cells in tissue culture which were 58 per cent resistant and 42 per cent susceptible to the virus. They then suggested that the macrophages were the cellular expressors of inherited natural resistance (10, 11). It is to be noted that the same strains of mice have been used in both studies, but that the C_3H strain of mouse which is resistant to mouse hepatitis is susceptible to arboviruses, and contrariwise the PRI strain which is susceptible to mouse hepatitis is resistant to arboviruses. Preliminary comparisons based on exchanges of survivors from various back-crosses between the Philadelphia and the Baltimore laboratories indicate that these characters are independent (12).

In order to test the correlation between the mouse and its cells even further, two things were done in the present study. A comparison of the ratios of susceptible to resistant mice was carried out by four different methods in the BC_1, BC_2, and BC_3 generations. It was found that about 27 per cent of the mice in all of these back-crosses yielded susceptible macrophages either from peritoneal

washings of adult mice, or from newborn mouse liver cultures, and the same ratio of susceptibility was obtained in adult mice *in vivo*. This incidence of susceptibility, which is well below the 50 per cent expected on the assumption that one gene is functioning as a dominant, may indicate that more than one gene is responsible for susceptibility. On the other hand, in a limited number of tests for genetic performance in producing susceptible offspring, slightly more than half (12/22) were capable of transmitting susceptibility. Thus the character for susceptibility, though dominant, may in the geneticist's terms be incompletely penetrant. Only continued work along these lines will allow us to choose from the two hypotheses.

An additional method of testing the proposal that the macrophages reflect the genetic nature of resistance is to compare the four different tests for susceptibility in individual mice. Table IV shows that in nine mice where it was possible to compare by two or more methods the question of susceptibility or resistance complete agreement was obtained in eight mice. One mouse, No. 13, was found to produce susceptible offspring, and susceptible macrophages from its own peritoneum, but one litter of six from which liver cultures were obtained yielded no susceptible cells. This may have been a chance variation, for on an expected ratio of about 27 per cent, less than two susceptible mice which might yield susceptible liver macrophage cultures would be expected. Thus the evidence for a correlation between macrophage susceptibility and mouse susceptibility seems strong.

An additional facet in the genetic manifestation of resistance is beginning to emerge from our study. Hybrid mice carrying the gene for susceptibility furnish macrophages which are destroyed more slowly than the macrophages from homozygous susceptible mouse. This presumably means that the double genetic dose is more effective than the single dominant gene, or possibly that the entire C_3H genotype suppresses the effectiveness of the gene for susceptibility. It is hoped that appropriate crossing of susceptible back-crosses will give us the final answer to this question.

As in Sabin's studies, age susceptibility may play an important role. We have only tentative data as to the rapidity with which the PRI mice as compared to the F_1 generation, become more resistant with age. We have also limited our studies to the question of the immediate susceptibility of cells inoculated with one strain of virus obtained from one source—PRI mouse liver (13). We have not compared the susceptibility of these cells to tissue culture adapted virus, nor studied the effect of the Eperythrozoon which increases the virulence of mild strains of virus (14).

Explanation of the resistance of C_3H cells to mouse hepatitis may obviously fall into two possible categories. The cells lack a substance necessary for the growth of the virus (growth meaning all phases of penetration and multiplication) or the resistant cells may promptly form some inhibiting substance. With

this in mind the crude experiments of exposing resistant and susceptible cells to extracts of the opposite type, and then testing for susceptibility were done. When it was found that a frozen-thawed extract induced the resistant cells to manifest susceptibility, immediate questions concerning the nature of this conversion became important. Susceptible cells were not made resistant by extracts of resistant cells, and the conversion of resistant cells to susceptible had a high degree of specificity, that is, it was induced by extracts of susceptible cells, and not by the cells of a number of other resistant animals and strains of mice. The question of the similarity of this phenomenon to transformation as described in bacterial genetics became immediately apparent. This phenomenon as it is presently interpreted depends upon the uptake of DNA by the bacterium, which then is incorporated into the clone of multiplying bacteria. Tests of the phenotypic expression of the ingested DNA are usually not possible before the bacterium has multiplied. The role of an increase of the DNA of the host cells, which has received the material, for the incorporation of the donor DNA and continued function of the latter is unknown. Thus although transformation is considered as a relatively permanent genetic change persisting through a number of generations, and our conversion of cells is temporary (lasting only a few days), it is not clear whether the two conditions are actually different. The lack of multiplication of cells in our system may inhibit incorporation of the DNA, and certainly does not allow us to test the more permanent conversion of a small percentage of cells. A number of tests have been made to determine the nature of the substance which converts the cells to susceptibility. Within the limits of the crude quantitation so far available, it is a relatively large molecule, since it sedimented at 5000 RPM and did not pass through a dialysis bag. It is heat-stable, which is not unlike certain DNA extracts. However, continued tests with DNAse have shown only a partial destruction. We have as yet not tested the effect of DNAse after heat treatment or after digestion of the fresh extract with proteases. Thus the nature of the extract, which in turn bears on the nature of the conversion, is poorly understood. Niu *et al* have reported on ribonucleic acid-induced changes in mammalian cells (15), in which ascites tumor cells are partially reconverted to normal cells by treatment with a nucleic acid extract, which is destroyed by RNAse and unaffected by DNAse. Our material was not affected by RNAse.

The demonstration by Mountain and Alexander (16) and Holland *et al.* (17) that resistant cells may be made susceptible to poliomyelitis virus by inoculation with nucleic acid extracts of the virus, and the subsequent studies of Holland and McLaren (18) on the receptor substance important in the attachment of the virus to the cell, suggest that some similar mechanism may play a role here. Is there a substance in the susceptible cell which is important in the initial stage of infection, which can be transferred to the resistant cell? The fact that several days elapsed before the cells became susceptible after treat-

ment with the appropriate extract speaks against this. Do the susceptible and resistant cells differ in that a greater proportion of susceptible cells absorb virus? In our experiments we were unable to measure sufficient absorption by the cells to give an answer to the question. Furthermore, our nucleic acid extracts remained inactive.

Two other phenomena need discussion. Wahl and Fouace (19) have described an unstable phenotypic resistance to erythromycin in staphylococcus cultures, which can be transferred to susceptible cells. Until more is known about the factor from the bacteria which is responsible for this transfer, little gain is had from comparisons.

Parry (20) has shown that scrapie, a disease of sheep, has a strong hereditary history; the gene which causes its manifestation being a single autosomal recessive. However, it is possible to transmit the disease from one sheep to another in series by an extract of the brain which is highly heat-stable, withstanding 99.5°C for 30 minutes. The "infectious particle" passes through gradocol membrane of 210 mμ A.P.D. but not 27 mμ A.P.D. Thus the particle size and the heat stability are not unlike our substance, which, however, transfers susceptibility. Much more needs to be known about the two substances before they can be adequately compared.

Finally it is possible that we have been particularly fortunate in choosing the macrophage for our work, for its well known characteristic of large scale ingestion of foreign material makes it particularly favorable for the study of change induced in a cell following the ingestion of particulate matter. In all of our experiments it was apparent that large amounts of the extract were being taken into the cell. Perhaps this capacity is responsible for the occasional destruction of the resistant cells by undiluted extracts of virus contained in liver suspensions prepared from susceptible mice. This virus suspension contains extracts of the susceptible cells. However, current studies on the time of exposure of the cells to the extract before they become susceptible are slightly against this hypothesis.

SUMMARY

Using peritoneal macrophage cultures it was found that both PRI mice and their macrophages in culture were susceptible to mouse hepatitis virus and that C_3H mice and macrophages were resistant. All F_1 macrophages and some back-cross cell cultures were susceptible. The degeneration of F_1 and back-cross macrophages obtained either from adult mouse peritoneal exudate or newborn mouse liver, occurred more slowly than PRI macrophages. Segregation of susceptibility occurred in the first back-cross generation. Tests of three back-cross generations from susceptible mice yielded about one-quarter of the mice shown to be susceptible either by direct test or test of their macrophages. A clear correlation between susceptibility *in vivo* and *in vitro* was established both

in the test of the percentage segregation and in tests of individual back-cross mice. A small series of tests, however, indicated that 50 per cent of the back-cross mice had the genetic capacity to transmit susceptibility. Thus a hypothesis of two genes for susceptibility, although not excluded, may yield to a hypothesis of a single dominant gene, incompletely expressed.

Resistant cells were converted into susceptible cells by ingestion of a relatively large particle containing a heat-stable substance. This susceptibility, although complete, was temporary. The nature of the factor causing the change has been discussed.

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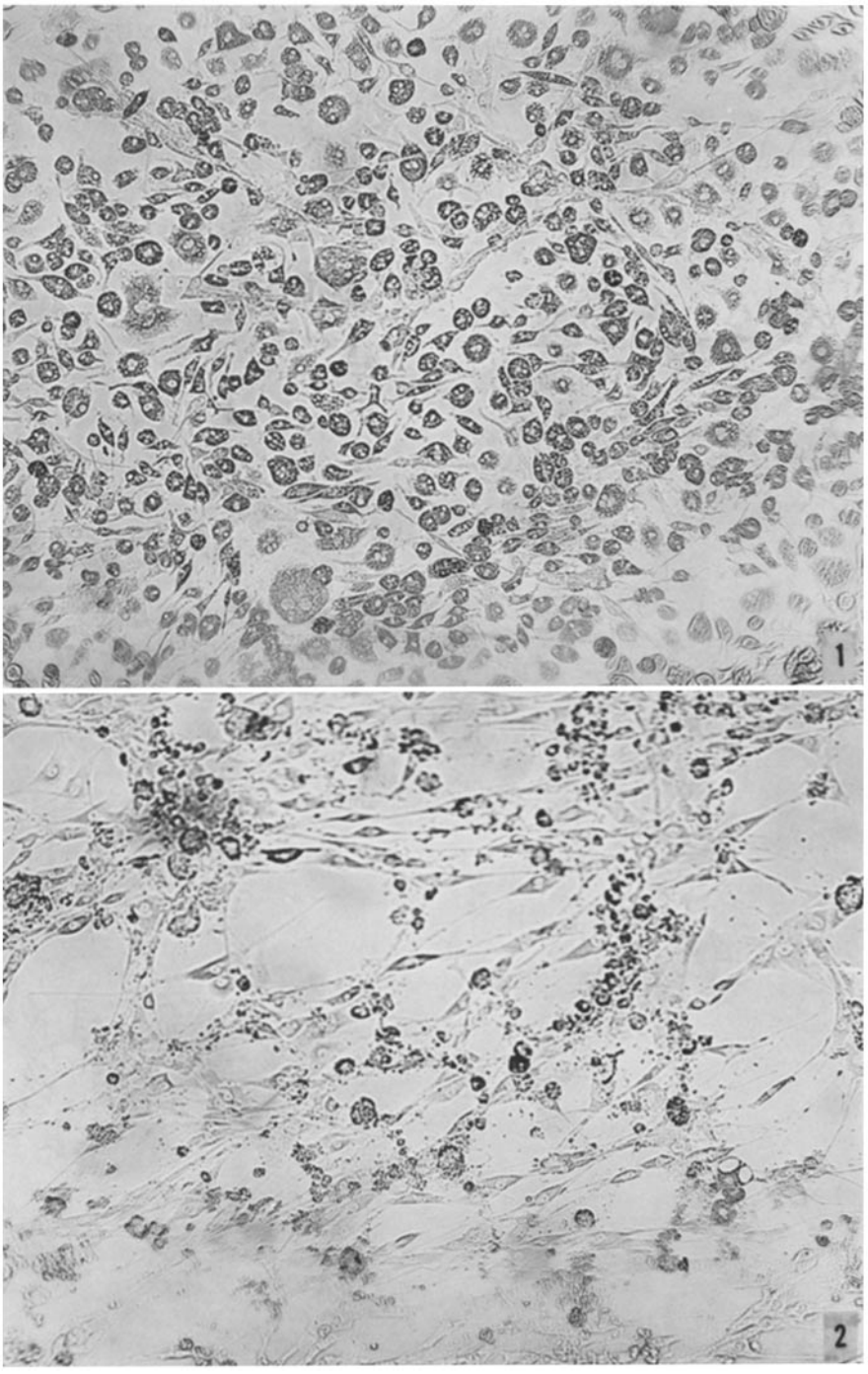
EXPLANATION OF PLATES

PLATE 45

FIG. 1. PRI culture, 10 days old, non-infected. × 100.

FIG. 2. PRI culture, 10 days old, 6 days after infection. × 100.



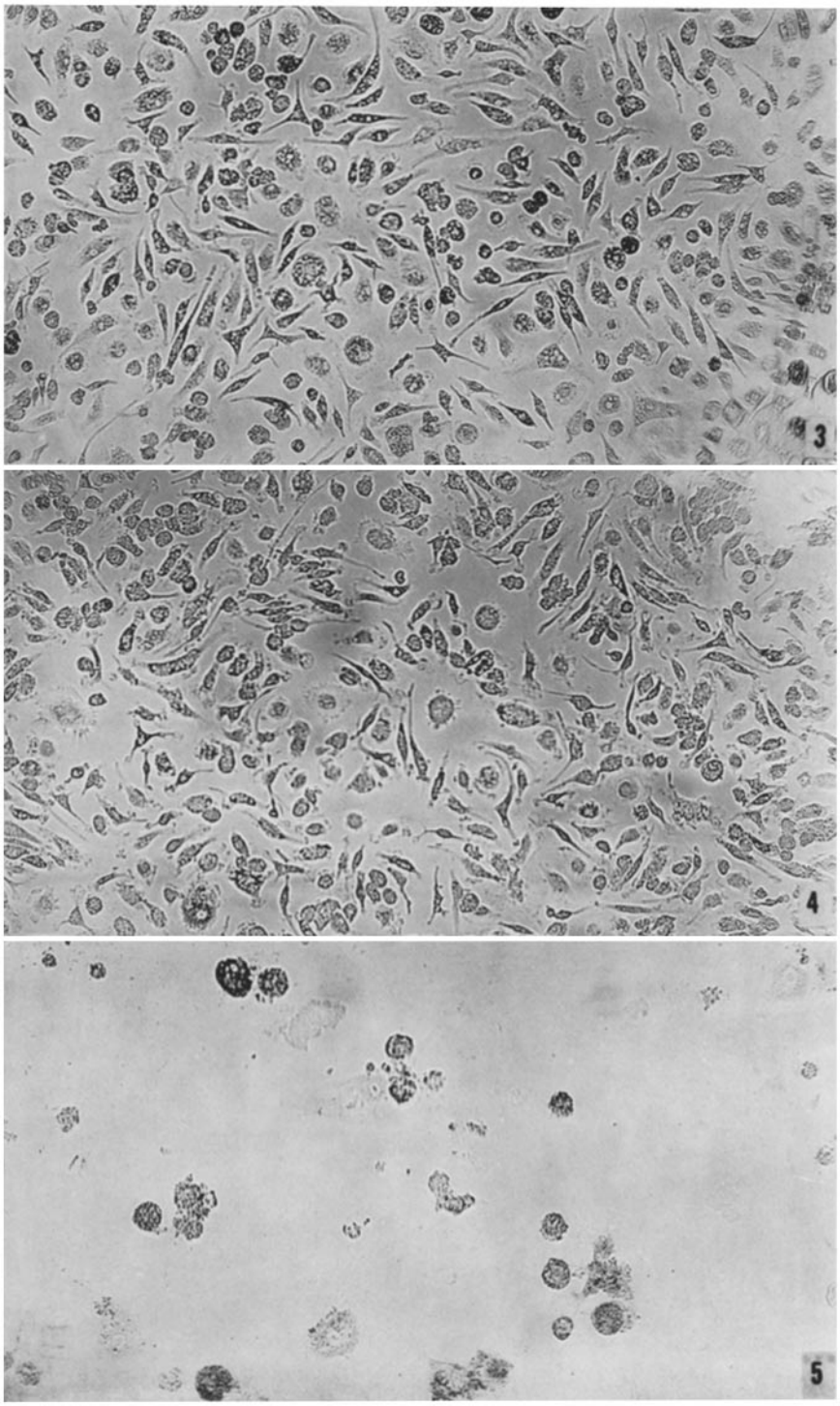
(Kantoch *et al.*: Cellular nature of genetic susceptibility to a virus)

PLATE 46

FIG. 3. C_3H culture, 14 days old, non-infected. × 100.

FIG. 4. C_3H culture, 14 days old, 6 days after infection. No degeneration. × 100.

FIG. 5. C_3H culture, 14 days old, exposed 4 days to extract from PRI macrophages, 6 days after infection. × 100.

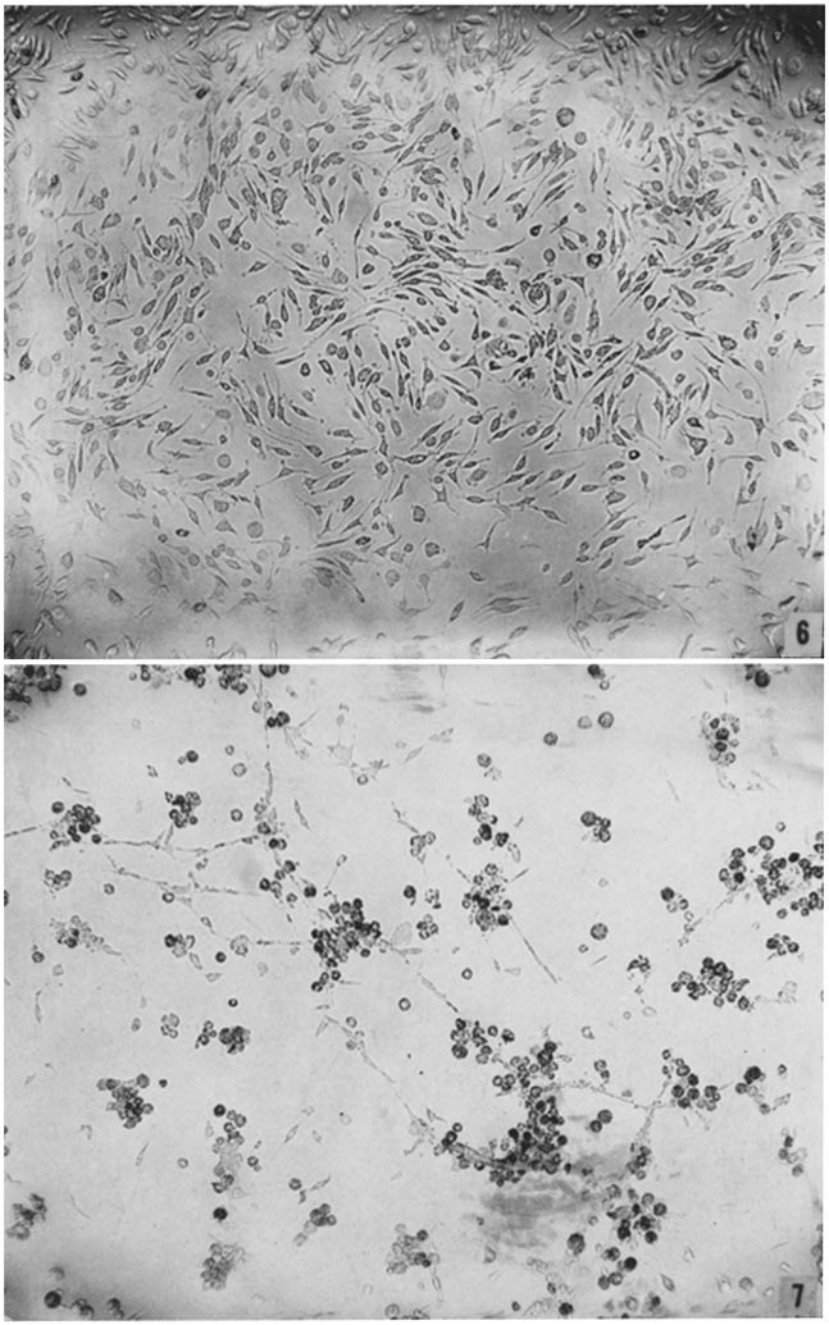


(Kantoch *et al.*: Cellular nature of genetic susceptibility to a virus)

PLATE 47

FIG. 6. F_1 culture, 10 days old, non-infected. × 50.

FIG. 7. F_1 culture, 10 days old, 6 days after infection. × 50.



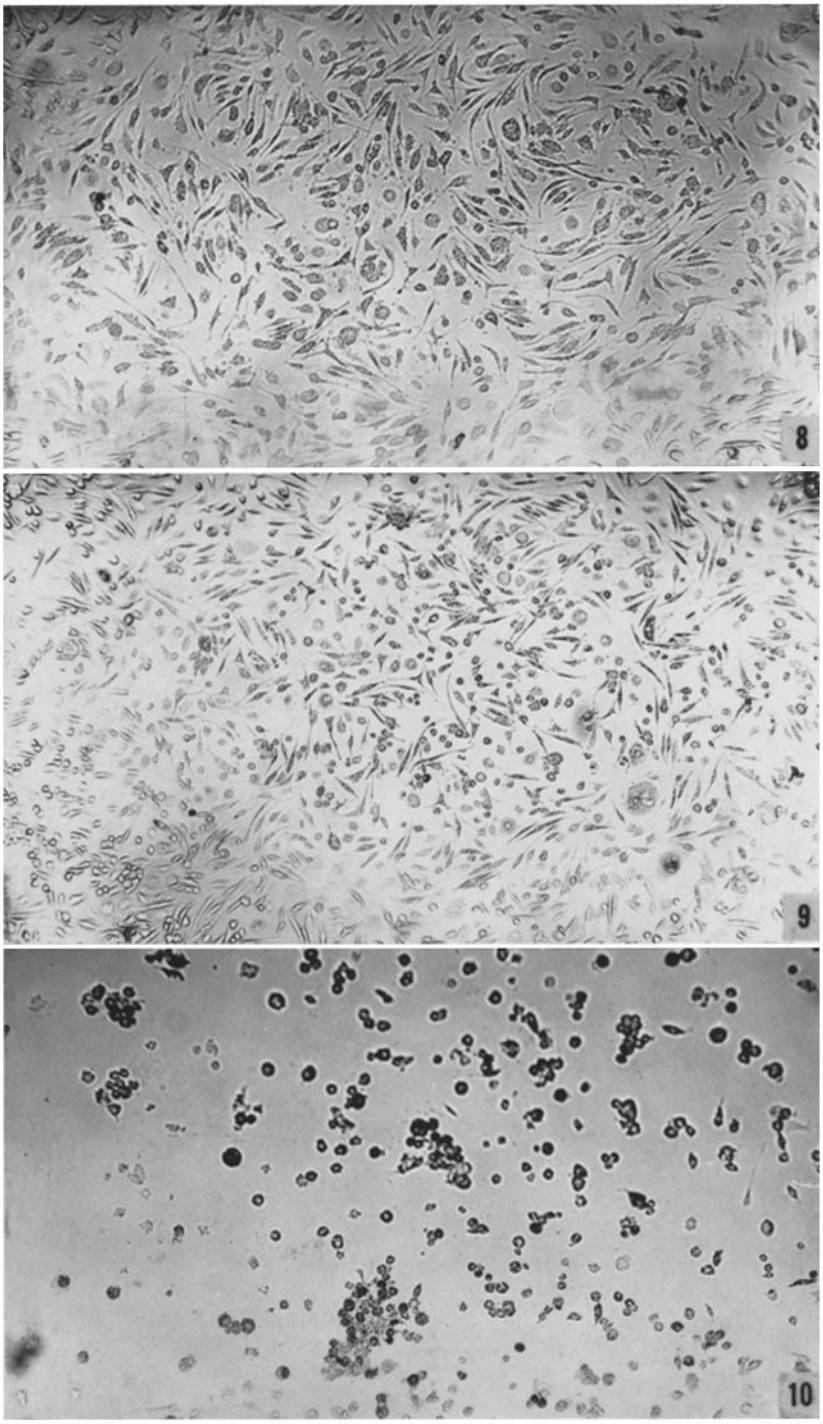
(Kantoch *et al.*: Cellular nature of genetic susceptibility to a virus)

PLATE 48

FIG. 8. BC_1 culture, 10 days old, non-infected. × 50.

FIG. 9. BC_1 culture, 10 days old, 6 days after infection, resistant. × 50.

FIG. 10. BC_1 culture, 10 days old, 6 days after infection, susceptible. × 50.

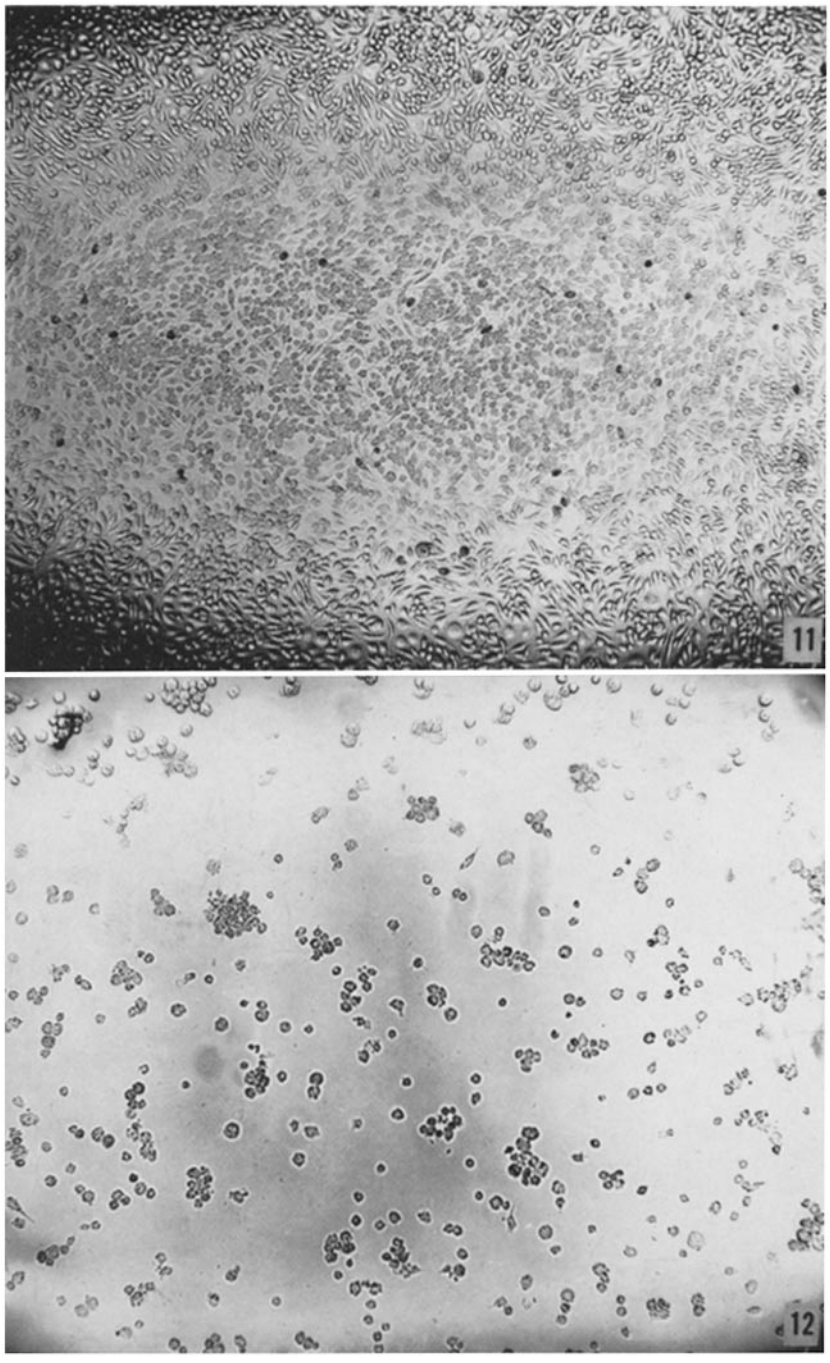


(Kantoch *et al.*: Cellular nature of genetic susceptibility to a virus)

PLATE 49

FIG. 11. BC_2 culture, 10 days old, 6 days after infection, resistant. × 50.

FIG. 12. BC_2 culture, 10 days old, 6 days after infection, susceptible. × 50.



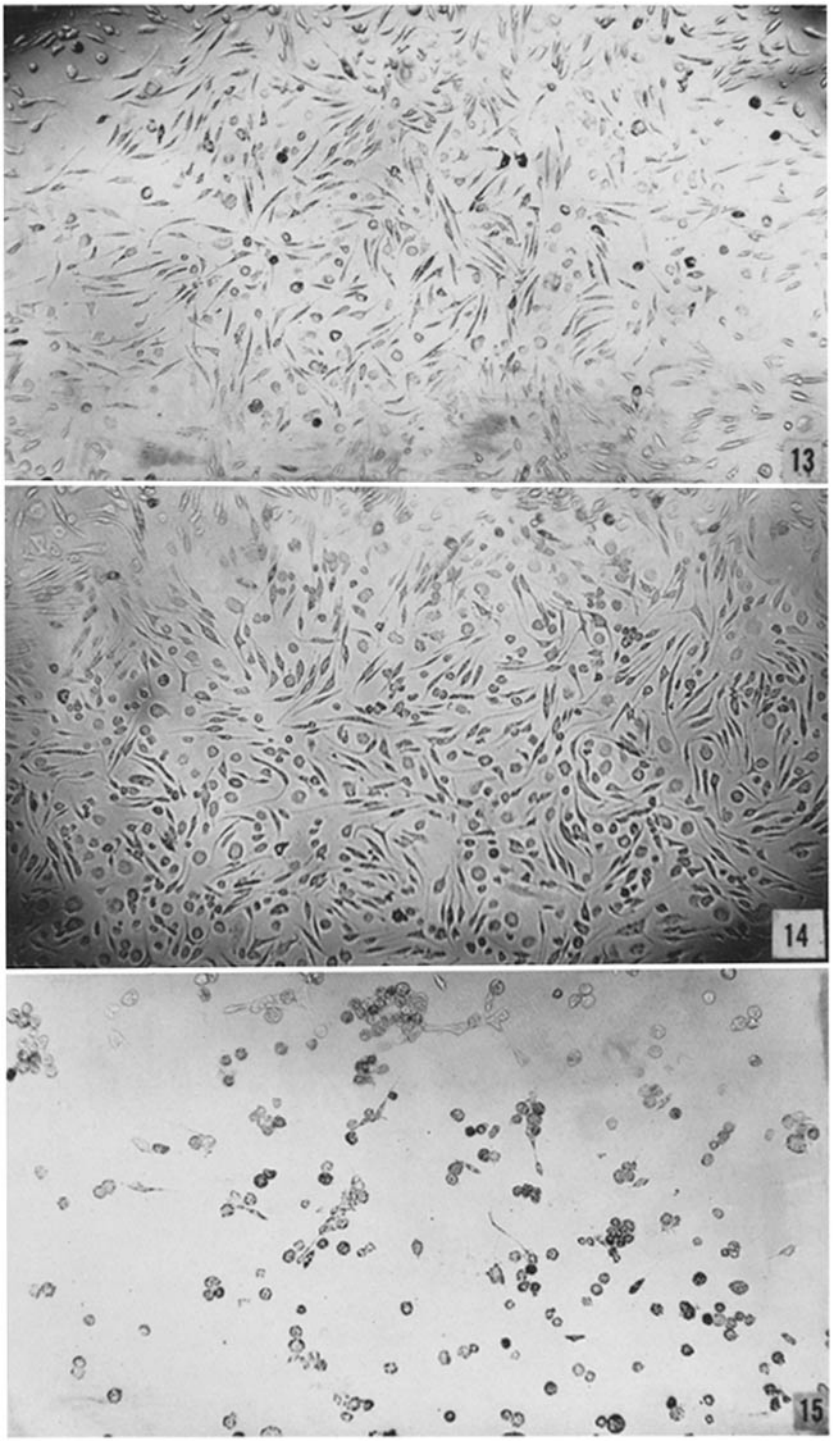
(Kantoch *et al.*: Cellular nature of genetic susceptibility to a virus)

PLATE 50

FIG. 13. BC_3 culture, 10 days old, non-infected. × 50.

FIG. 14. BC_3 culture, 10 days old, 6 days after infection, resistant. × 50.

FIG. 15. BC_3 culture, 10 days old, 6 days after infection, susceptible. × 50.



(Kantoch *et al.*: Cellular nature of genetic susceptibility to a virus)