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*EFFECT OF CORTISONE ON GENETIC RESISTANCE TO MOUSE HEPATITIS VIRUS IN VIVO AND IN VITRO**

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It is known that the genetic resistance of C3H mice to mouse hepatitis virus (MHV) is phenotypically manifested in the macrophage system when tested *in vitro*,[1, 2] and that macrophages obtained from such resistant mice may be converted to temporary susceptibility if exposed to extracts of cells from a susceptible (PRI) strain of mice.[3] The established role of cortisone in altering the susceptibility of various animals to infectious diseases has led us to test the effect of this hormone on the genetic resistance of C3H mice. We have found that C3H mice become highly susceptible to mouse hepatitis virus after administration of cortisone, and that cells from C3H mice also become susceptible *in vitro* if cultures are treated with cortisone. This latter is demonstrable in macrophage cultures obtained from resistant mice, and in cultures of liver from eight-day-old mice, in which liver parenchymal cells as well as macrophages are destroyed.

Materials and Methods.—Inbred strains of C3H (resistant) and PRI (susceptible) mice were used.[2] Mouse hepatitis virus was originally obtained from Dr. J. B. Nelson of The Rockefeller Institute and has been maintained by serial passage in an inbred strain of PRI mice. Frozen stocks were kept at −30°C.

The infected mice were killed in the agonal stage, and 10 per cent liver suspension was prepared in Hanks' balanced salt solution (BSS). The virus was titered by intraperitoneal inoculations of 0.1 ml of serial tenfold dilutions into weanling PRI mice. They were observed for a week, the deaths were recorded, and the LD_{50} was calculated by the Reed-Muench method.

Macrophages were obtained from peritoneal exudate by modification of Barski's method.[4] Each mouse was injected intraperitoneally with 2–3 ml thioglycolate medium. After 24 hr 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. After gently massaging the abdomen, about 3 ml of peritoneal exudate was withdrawn with a hypodermic syringe. The exudate was centrifuged at 800 rpm for 8 min. The packed cells were resuspended in Chang's[5] medium (90% inactivated horse serum, 2% beef embryo extract, and 8% Hanks' balanced salt solution) to yield 1.5×10^6/ml. One ml of cell suspension was inoculated into standard Wasserman tubes (10 × 100 mm). After stationary incubation at 37°C for 12–16 hr the cultures were transferred to a roller drum at the same temperature.

Liver macrophages were obtained by explanting fragments of liver from 8-day-old mice into roller tubes on a reconstituted collagen substrate prepared according to the method of Ehrmann and Gey.[6] Chang's medium was used as the supernate.

In order to test the present resistance of the C3H strain, 10 C3H mice were inoculated intraperitoneally with 0.1 ml of undiluted stock virus which titered $10^{8.5}$. All test mice survived.

Cortisone acetate (cortone acetate, 25 mg/ml, Merck, Sharp and Dohme) was diluted with saline to 10 mg/ml and was injected subcutaneously in the backs of the mice. Hydrocortisone-21-phosphate (hydrocortone phosphate, 100 mg/vial, Merck, Sharp and Dohme) was diluted in Chang's medium before it was added to the tissue cultures.

Results.—Adult C3H mice, 30–40 days old, were treated with 1–3 mg of cortisone per animal two hr before they were injected subcutaneously with 1 ml of a 10^{-2} dilution of MHV. In some of the experiments a second injection of 0.5–1 mg of cortisone was given 24 hr after the virus injection. All cortisone-treated mice died following the inoculation of virus, while all control mice survived (Table 1).

The livers of the cortisone-treated C3H mice which were injected with MHV

TABLE 1

SUSCEPTIBILITY OF ADULT C3H MICE TO MHV AFTER CORTISONE TREATMENT

First cortisone s.c. injection (mg/mouse)	MHV* 1:100 dilution (ml/mouse)	Second cortisone s.c. injection† (mg/mouse)	Mortality	
			No. of animals	Percentage
3.0	0.1	—	5/5	100
1.5	0.1	1.0	15/15	100
1.5	0.1	—	5/5	100
1.0	0.1	1.0	5/5	100
1.0	0.1	0.5	5/5	100
3.0	—	—	0/5	0
1.5	—	1.0	0/18	0
1.5	—	—	0/5	0
—	0.1	—	0/20	0

* The MHV was injected intraperitoneally, 2 hr after the first cortisone injection.
† The second cortisone injection was performed 24 hr after the first cortisone injection.

showed characteristic changes: they were pale, the colors varying from light pink to yellowish white, and there were pin-point hemorrhages on the surface.

The mean survival time of the C3H cortisone-treated mice challenged with MHV was 3.4 days, while the survival time of the normally susceptible PRI strain, injected with the same amount of virus, was 2.2 days. When the relative susceptibilities of the respective strains were determined, the LD_{50} of the virus was found to be about one hundredfold less in the cortisone-treated C3H than in the PRI mice (Table 2); thus, the treated resistant mice did not become as sensitive as the normally susceptible mice.

A stock of virus was prepared from livers of moribund cortisone-treated C3H mice infected with MHV. The LD_{50} of this virus stock, when measured in PRI was $10^{7.5}$. Twenty untreated adult C3H mice were found to be resistant to intraperitoneal inoculation of 1:10 and 1:100 dilutions of this liver preparation. Therefore, growth of the virus in cortisone-treated C3H mice was not accompanied by any marked increase in the capacity of the virus to grow in (adapt to) untreated C3H mice.

TABLE 2

TITER OF MHV IN C3H CORTISONE-TREATED MICE AND PRI MICE (TITER IN LD_{50})

No. of expt.	C3H cortisone-treated mice*	PRI mice
1	$10^{6.5}$	$10^{8.5}$
2	$10^{5.3}$	$10^{7.7}$
3	$10^{6.5}$	$10^{8.0}$

* 1.5 mg/mouse cortisone was inejcted s.c. 2 hr before inoculation of MHV; a second cortisone injection of 1.0 mg/mouse was given s.c., 24 hr after the first cortisone injection.

In our past experience, C3H peritoneal macrophage cultures resisted 10^{-1} and 10^{-2} dilutions of the liver suspension virus, but were destroyed when inoculated with undiluted preparations.[3] Since that time, Huang, in our laboratory, has found[7] that commercial horse sera often contain an inhibitor to MHV. We have subsequently used only horse sera known to be free of this inhibitor. While a 10^{-2} dilution of the virus may occasionally destroy C3H macrophages *in vitro*, the dilutions of 10^{-6} regularly destroy PRI cells.

The susceptibility of the resistant macrophages following treatment with cortisone was next tested. Table 3 summarizes six experiments which show that when cortisone in concentrations of 0.25–10 γ/ml was added to six-day-old macrophage cultures simultaneously with inoculation of 10^{-2} dilution of MHV, and with no subsequent fluid change, the macrophages were destroyed within 5–7 days in 85–100 per cent of the cultures (Figs. 1–4). (C3H macrophage cultures inoculated with

TABLE 3

SUSCEPTIBILITY OF C3H MACROPHAGES TREATED WITH HYDROCORTISONE-21-PHOSPHATE TO MHV*

Hydrocortisone (γ/tube)	MHV 1:100 dilution (ml/tube)	Beginning of destruction	Destruction	
			No. of tubes	Percentage
10	—	—	0/11	0
5	—	—	0/13	0
1	—	—	1/18	0
0.5	—	—	0/7	0
0.25	—	—	0/16	0
—	0.1	—	4/21	19
10	0.1	3–4	15/17	87
5	0.1	3–4	17/17	100
1	0.1	3–4	21/24	85
0.5	0.1	5	7/7	100
0.25	0.1	5	14/16	87

* In three experiments, nos. 5, 6, and 7.

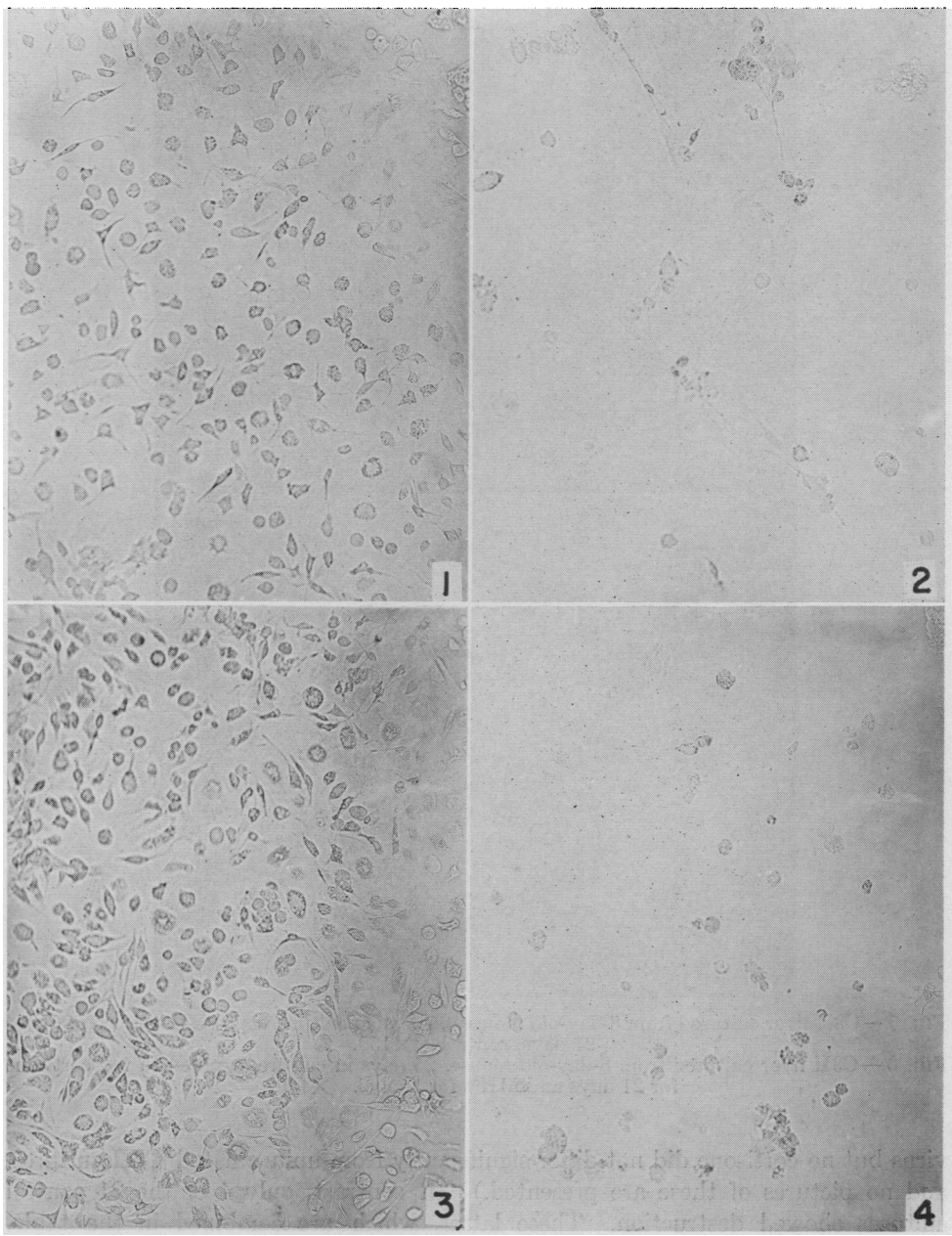

FIG. 1.—C3H peritoneal macrophage culture, 11 days old, exposed 5 days to 1 γ cortisone, control. ×100.

FIG. 2.—C3H peritoneal macrophage culture, 11 days old, exposed 5 days to 1 γ cortisone and MHV. ×100.

FIG. 3.—C3H peritoneal macrophage culture, 11 days old, exposed to 10 γ cortisone, control. ×100.

FIG. 4.—C3H peritoneal macrophage culture, 11 days old, exposed to 10 γ cortisone and MHV. ×100.

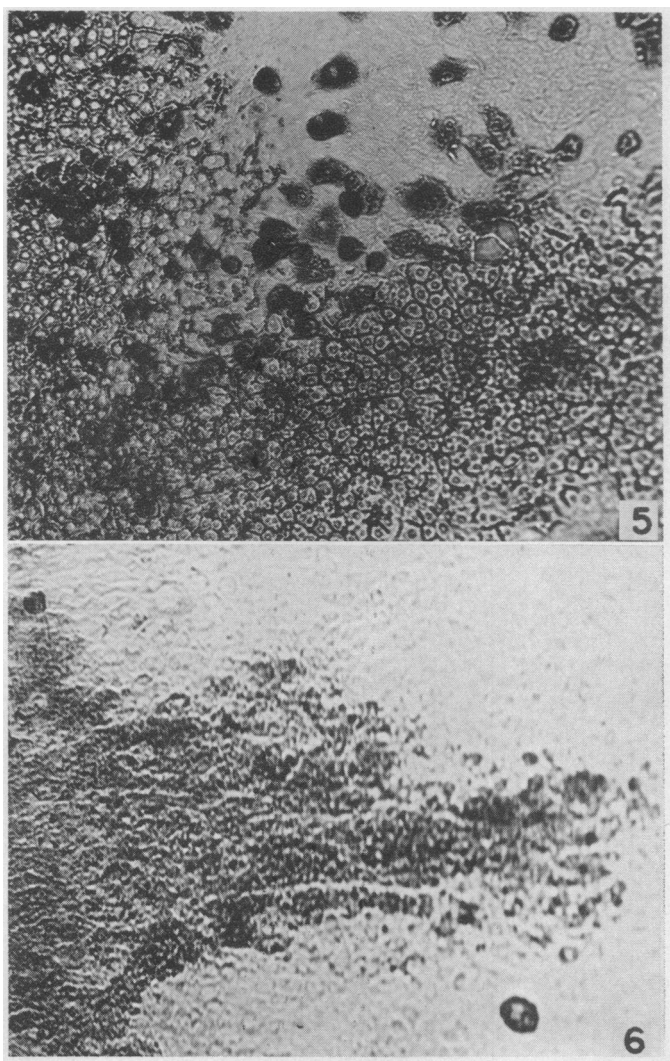

FIG. 5.—C3H liver cultured from 8-day-old mouse, 25 days in culture, exposed to 1 γ cortisone for 21 days, control. ×150.

FIG. 6.—C3H liver cultured from 8-day-old mouse, 25 days in culture, exposed to 1 γ cortisone for 21 days and MHV for 5 days. ×150.

virus but no cortisone did not differ significantly from uninoculated C3H cultures, and no pictures of these are presented.) In contrast, only 4 of the 21 control cultures showed destruction. These latter, which are combined in the table, occurred in different experiments.

The effect of cortisone on the susceptibility of C3H liver macrophages was tested next. The cultures were grown for 4, 7, and 25 days at which time 1 γ of cortisone was added, and the cultures were infected with a 10^{-2} dilution of virus. The cortisone not only produced greater differentiation of the liver as it spread on the collagen, but made both the macrophages and the liver parenchyma susceptible (Figs. 5 and 6). In cultures which did not receive cortisone but did receive virus the

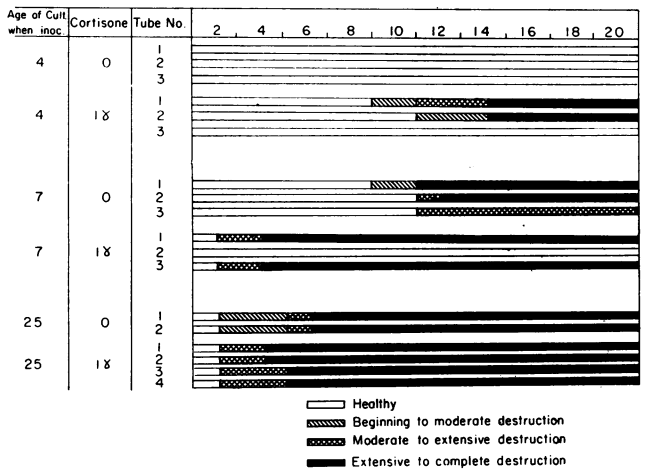

FIG. 7.—Effect of MHV (10^{-2} virus dilution) on cortisone-treated and normal liver macrophages cultured from 8-day-old C3H mice

destruction of the macrophages either did not occur or was delayed for at least one week (see Fig. 7).

It seems increasingly clear that the resistance of C3H cells *in vitro* is a relative matter. Extracts of susceptible cells may convert the resistant cells to temporary susceptibility.[3] Furthermore, our initial observations (1960), which correlated *in vitro* and *in vivo* macrophage susceptibility and resistance, compared the susceptibility of intact weanling mice. Subsequent work has indicated that macrophages obtained from newborn resistant mice show a greatly delayed susceptibility, just as newborn mice do *in vivo*, and that as the mice mature their cells become more resistant.[8]

Discussion.—It is well known that cortisone may alter susceptibility to virus infections. It has been reported that cortisone treatment of influenza virus cultures lowers the yield of interferon,[9] and that cortisone will increase the yield of arboviruses.[10] Little, however, is known of the mechanism of these changes. It is thus interesting that not only will a genetically resistant strain of mice become susceptible upon administration of cortisone, but that genetically resistant cells may be made so susceptible *in vitro* that the "cytopathic" effect of virus is clearly demonstrable. These effects of cortisone offer further means of studying genetic resistance, and in the present model reinforce the correlations between the course of infections in *in vitro* and *in vivo* systems.

Although the important question of whether the cortisone effect is mediated through an indirect effect on the production of interferon by the resistant cells has not been investigated, previous experiments[1] have shown that mixed cultures of resistant and susceptible macrophages were not fully resistant to the effects of the virus.

Summary.—Genetically resistant mice became susceptible to mouse hepatitis virus when treated with cortisone. Macrophage cultures obtained from the resistant mice were resistant *in vitro*, but became susceptible when treated *in vitro* with 0.25 to 10 γ of cortisone. Cultures of liver from genetically resistant mice,

grown in 90 per cent horse serum and then treated with cortisone, showed increased liver cell parenchymal outgrowth and differentiation. The susceptibility of these cultures was greatly increased so that both macrophages and differentiated liver parenchyma were destroyed.

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THE DEVELOPMENTAL SIGNIFICANCE OF ALANINE DEHYDROGENASE IN BACILLUS SUBTILIS

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Genetic functional elements (genes) can be divided into basic and developmental genes, even though these functions may sometimes overlap. Basic genes govern the formation of the basic constituents of all cells, i.e., DNA, RNA, proteins, cell membranes, etc. In contrast, developmental genes are required only for the change of one cell type into another one, that is, for the differentiation of a multicellular organism or for the change into another phase of the life cycle of a unicellular organism. Whereas mutations in basic genes are lethal (if homozygous) unless the missing compound can be fed from without, mutations in developmental genes effect only a specific type of differentiation and are not lethal for individual cells although the organism may not survive in the long run. The biochemical pathway leading toward a specific type of differentiation can be unraveled conclusively by the isolation and biochemical characterization of mutants blocked in the corresponding developmental genes.

A particularly simple system, which lends itself to such a genetic analysis of differentiation, is that of sporulation and germination in bacilli.[1] This paper analyzes in *B. subtilis* the first steps in spore germination, which are specifically initiated by L-alanine.[2, 3]