in embryos. Results of the present experiments also obviously imply that a subcellular factor exists in the blood of patients with neoplastic disease that is involved in the high rate of chick embryo mortality. There is, however, no basis at present for suggesting that a virus is implicated in these phenomena.

The utilization of these techniques, as reported herein, further suggests a possible tool for clinical diagnosis. Experimental data at hand from experiments in progress are insufficient to make a complete analysis possible.

Summary.—Whole blood and blood fractions from patients and animals with neoplastic disease produced a high rate of mortality of chick embryos when inoculated onto the chlorioallantoic membrane. Blood and blood fractions from normal and non-neoplastic sources caused little toxicity and only a low percentage of deaths of the chick embryos. Expanded studies are underway.

The authors wish to express their appreciation to Miss Jo-Anne Hurt for technical assistance.

* This investigation was supported in part by a research grant from the National Cancer Institute, the National Institutes of Health, U.S. Public Health Service, C-6516.

† With the assistance of Theodora G. Sarris.

[1] Handler, A. H., S. I. Magalini, and L. S. Snegireff, *Transpl. Bull.*, **28**, 104 (1961).

[2] Handler, A. H., and J. Cohen, *Proc. Amer. Assoc. Cancer Res.*, **3**, 116 (1960).

[3] Lewis, M. R., and W. H. Cole, *Arch. Surg.*, **77**, 621 (1958).

[4] Handler *et al.*, Unpublished.

[5] Handler, A. H., *Ann. N. Y. Acad. Sci.*, **76**, 775 (1958).

[6] Taylor, A., and N. Carmichael, *Cancer Res.*, **20**, 1636 (1960).

[7] McDuffie, N. G., Jr., B. S. Gibson, and A. Taylor, *Cancer Res.*, **20**, 1631 (1960).

[8] Armstrong, M., and A. Ham, *Cancer Res.*, **7**, 481 (1947).

[9] Heilman, F. R., and J. J. Bittner, *Cancer Res.*, **4**, 578 (1944).

[10] DeRuyck, R., *Bull. du Cancer*, **46**, 391 (1959).

[11] Lasfargues, E. Y., D. H. Moore, M. R. Murray, C. D. Haagensen, and E. C. Pollard, *J. Biophys. and Biochem. Cytol.*, **5**, 93 (1959).

---

*CONVERSION OF GENETIC RESISTANCE OF MAMMALIAN CELLS TO SUSCEPTIBILITY TO A VIRUS INFECTION**

BY M. KAŃTOCH† AND F. B. BANG

DEPARTMENT OF PATHOBIOLOGY, JOHNS HOPKINS SCHOOL OF HYGIENE AND PUBLIC HEALTH

Communicated by Bentley Glass, June 21, 1962

The virulent strain of mouse hepatitis virus has a selective destructive effect for macrophages cultured from the liver of newborn mice of Princeton (PRI) strain.[1] This virus does not destroy cultures of C3H mouse liver macrophages. Hybrids of the resistant and susceptible strains show that susceptibility is dominant, and genetic analysis indicates that segregation of susceptibility and resistance occurs in F2 and backcross-generations.[2] In the present experiments we sought to explain the different reactions of the cells from these two strains of mice to the hepatitis virus.

Materials and Methods.—Macrophages were cultured from peritoneal exudate, a source which yields a more uniform cell type and has the added advantage that

the same mouse may be cultured a number of times. Macrophages were obtained by a modification of Barski's[3] method for acquiring rabbit macrophages. Each mouse was injected intraperitoneally with 2 ml of thioglycollate medium. The following day 5 ml of Hanks' balanced salt solution was injected intraperitoneally. After several minutes' gentle massage of the area, about 3 ml of the exudate was withdrawn and was centrifuged at 1000 rpm for 5 min. The packed cells were then resuspended in Chang's medium (90% inactivated horse serum, 4% beef embryo extract, and 6% Hanks' balanced salt solution)[4] to yield 1×10^6 cells per ml. One ml of cell suspension was then put into each of a series of standard size Wasserman tubes (10 mm × 100 mm). After stationary incubation at 37°C for about 6 hr, the cultures were put in a roller drum at the same temperature.

Under these conditions both PRI and C3H macrophages survived for several weeks and retained their ability to concentrate neutral red. Frequent changes of medium were not necessary. During the first 6 hr most cells attached to the glass surface, and after 24–48 hr began to spread over the surface. Only a few experiments have been done to determine whether macrophages multiply or merely survive in these cultures. Counts of the number of cells have so far failed to show an increase.

Results.—As with previous liver culture experiments, macrophages from PRI mice peritoneal exudates were highly susceptible to hepatitis virus. After 3 days of infection the cells degenerated and virus increased; this was true of cells from both young and old mice (from 4 weeks to 1 year).

On the other hand, C3H macrophages were resistant to the same concentrations of virus preparations. No degeneration of cultures and no multiplication of virus were demonstrated. Undiluted virus (10% liver suspension) caused a partial degeneration of these macrophages and intensive granulation of almost all cells. This effect will be considered in the discussion.

The susceptibility difference was then studied in extracts from the macrophages, obtained by freezing and thawing cells from the peritoneal exudate and resuspending in Hanks' balanced salt solution (BSS). The extracts were centrifuged at 900 rpm for 5 minutes, and the supernatant fluid was removed and used or stored in a refrigerator.

First the PRI (susceptible) macrophages were cultivated in medium containing extract from C3H (resistant) macrophages, and C3H macrophages were cultivated in medium containing extract from PRI macrophages. Extracts were added 3 or 4 days after cultures had been made, and were kept with the culture 4 or 5 days, after which the medium containing the extract was removed and new fluid without extract was added. Selected cultures were then infected at this time. The extracts themselves had no toxic effect on the macrophage cultures.

PRI macrophages exposed to extract from C3H cells remained susceptible to the virus infection and multiplication of virus occurred both in treated and untreated infected macrophages.

Resistant (C3H) macrophages, however, cultivated in a similar way and treated with susceptible (PRI) macrophage extract for 4 or 5 days, became susceptible. After a few days, intensive granulation in infected cultures appeared. Cells became round in shape and tended to fall off the glass into the fluid. The degree of degeneration was variable. In some experiments about 80% to 90% of cells were

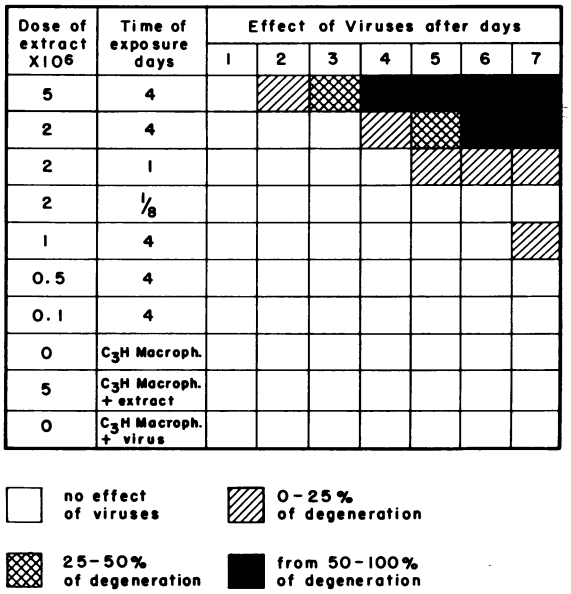

FIG. 1.—Effect of dosage of PRI extract and of incubation time on susceptibility of C3H macrophages to mouse hepatitis virus.

destroyed; in others, the numbers of degenerated cells were smaller. The amount of degeneration in an individual experiment depended upon concentration of extract and upon the time of exposure of cultures to the extract (Fig. 1). It is to be emphasized that in this experiment there was not only intense granulation and degeneration of cells but also multiplication of the virus (Table 1).

TABLE 1

TITER OF VIRUS IN INFECTED C3H MACROPHAGE CULTURES AFTER INCUBATION AT 37°C

Preparations	Titer for Princeton Mice in LD_{50}		
	Expt. I (4 days)	Expt. II (6 days)	Expt. III
Control (virus + medium)	$10^{4.5}$	$<10^{1.50}$	$10^{2.75}$
C3H macrophages (without extract)	$10^{5.0}$	$<10^{1.50}$	$10^{2.57}$
C3H macrophages exposed to PRI extract	$10^{7.16}$	$10^{5.32}$	$10^{5.23}$

Comparison of the results of titrations shows that the virus multiplied only in those C3H cultures which had been previously incubated with extracts from PRI peritoneal cells. The resistant C3H cultures, then, had undergone changes which resulted in infection and multiplication of virus and in cellular destruction which varied in degree but which occurred consistently.

In the next group of experiments a suspension of ground liver from susceptible PRI mice was used instead of macrophages. The results were similar (Fig. 2). In this experiment C3H macrophages were cultivated with medium containing liver suspension in final concentrations of 1.0%, 0.5%, and 0.1%. During the first two days the cultures became very granular but the macrophages kept their normal shape and appearance. In the following few days this granulation gradually disappeared, either completely or almost completely, depending on the concentration of liver suspension used. When these cultures were infected, rapid or moderate degeneration of cells occurred during the next 6 to 8 days, depending again upon the concentration of liver suspension. If 1.0% liver suspension was present in the cul-

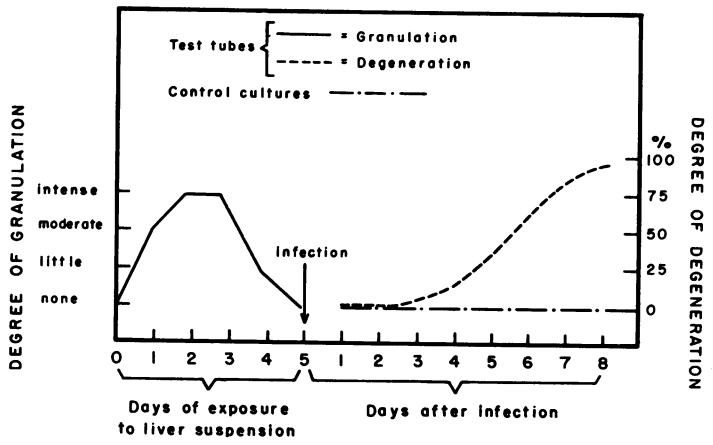

FIG. 2.—Effect of virus infection on C3H macrophage cultures previously cultivated in medium containing 0.5% PRI liver suspension.

ture medium the granulation of cells was intense, and it was difficult to recognize the exact time when degeneration began, but with 0.5% suspension this change could be easily followed. 0.1% concentration of liver suspension did not induce a visible alteration of macrophages and the virus had no apparent effect on these cultures. This second group of experiments provided additional evidence that some factor or factors had affected the resistant macrophages in a way which brought about alteration of their susceptibility to the virus infection. It is evident that this process takes time; it is probably associated with metabolic activity of macrophages. Both in experiments with PRI macrophage extracts and liver suspensions the effects depended upon the concentrations used.

Similar effects have been obtained with heated PRI macrophage extract (56°C/30′, 70°C/10′, and 100°C/10′), so that the conversion factor is apparently heat-stable.

Figures 3–7 are photographs illustrating the differences between infected and non-infected PRI and C3H cultures.

Discussion.—The first clear demonstration of Mendelian inheritance of susceptibility to virus infections was that of Sabin,[5] who showed that resistance to type B arbor viruses is inherited in PRI mice as a dominant factor, and that appropriate F2 generations and backcrosses indicate the likelihood of the unifactorial nature of this resistance. In this work it was also shown that sarcoma 180 when grown in resistant and susceptible mice produced the same amount of virus, thus pointing to the idea that resistance resided in the cells.[6]

Bang and Warwick[2] found a similar distribution of susceptibility to hepatitis virus in crosses between PRI and C3H mice. In this case susceptibility was dominant and segregation of characters occurred in the F2 and backcrosses. In addition it was shown that macrophages obtained from susceptible mice were susceptible and that those from resistant mice were resistant in culture. A similar segregation of characters in the F2 and backcross generations was reported for the cultures. Thus it was shown that tissue susceptibility resided in the macrophage system and, since epithelial and fibroblast cells in these early cultures were not destroyed, that this genetic basis of susceptibility is perhaps limited to this system.

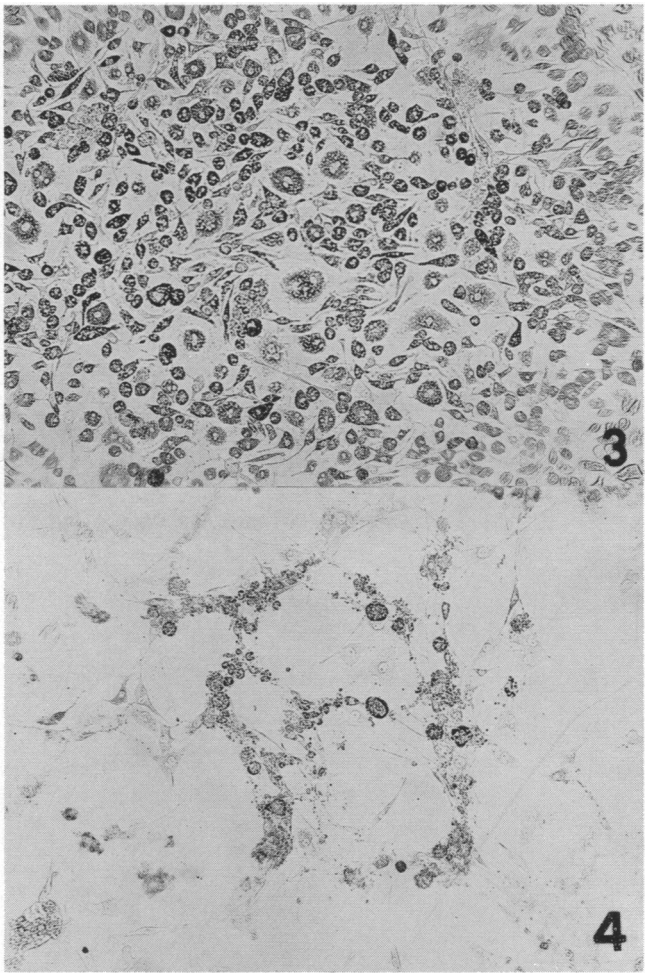

FIG. 3.—Ten-day-old culture of Princeton macrophages. Noninfected culture. × 100.
FIG. 4.—Ten-day-old culture of Princeton macrophages. Six days after virus infection. × 100.

Goodman and Koprowski[7] have confirmed the fact that macrophages from the appropriate mice reflect the susceptibility of the parent strain, by testing peritoneal exudate macrophages against the 17D strain of yellow fever and other type B arbor viruses. This conclusion was highlighted by a study of co-isogenic strains in which the difference between the two strains in the eighth backcross would be limited largely to the character for resistance.

In the present paper it has been demonstrated that cultivation of C3H resistant macrophages in medium containing an extract from susceptible Princeton macrophages changes them to susceptible cells.[8] On the other hand, cultivation of susceptible Princeton macrophages in medium containing extracts from resistant cells had no effect on their susceptibility to the virus infection. Extracts of C3H macrophages, furthermore, had no effect on the resistance of the C3H macrophages. There is thus some specific factor in the Princeton mouse macrophage.

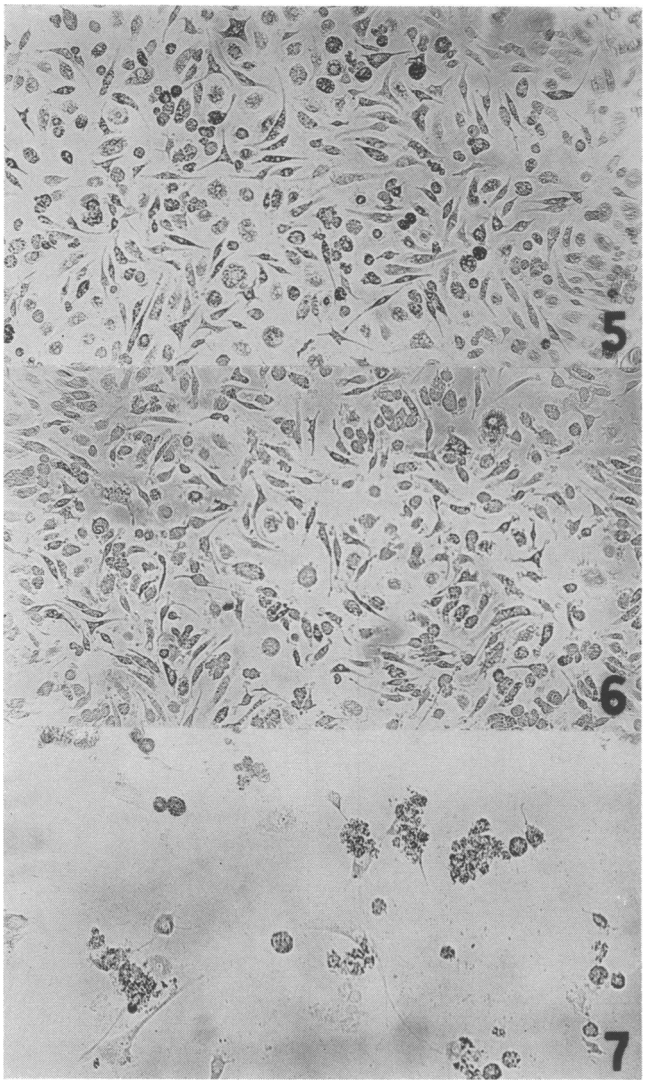

FIG. 5.—Fourteen-day-old culture of C3H macrophages. Noninfected culture. × 100.
FIG. 6.—Fourteen-day-old culture of C3H macrophages. Six days after virus infection. No destructive effect of virus visible. × 100.
FIG. 7.—Fourteen-day-old culture of C3H macrophages cultivated four days in medium containing extract from 5 × 10^6 Princeton macrophages. Six days after virus infection. Degeneration of macrophages visible. × 100.

In these experiments it has been demonstrated that the factor (or factors) responsible for alteration of C3H macrophages can be quantitated. The effect of the extract is dependent upon the concentration of extract, and the factor withstands boiling for 10 minutes.

Several kinds of reactions might account for the alteration:

(a) Transformation of some genetic factor responsible for resistance into a state of susceptibility.

(b) Alteration of the surface of the resistant macrophages in such a manner that

they can be infected by the virus. If this latter hypothesis is tenable, one must admit that C3H macrophages are potentially able to support virus growth. The experiments with undiluted virus preparations might support this.

(c) Dependence of virus growth on the factor present in the susceptible extract. This would mean that the susceptibility factor can be adsorbed on the surfaces of the cells.

Preliminary experiments show that the change in susceptibility is altered if the extract is treated with deoxyribonuclease (DNAse), but these are too limited to have established whether this is an effect of DNAse on the extract or an effect of residual DNAse on the cells.

Summary.—Macrophages obtained from peritoneal washings of mice which are genetically resistant to mouse hepatitis are resistant to this virus in culture. Similar cells from susceptible mice are susceptible in culture. Susceptibility can be conferred on the resistant cells by treatment of these cells with a heat-stable factor extracted from the susceptible cells. This factor must be given in sufficient concentration to produce the conversion to susceptibility. The nature of the factor is under investigation.

* 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.

[1] Bang, F. B., and A. Warwick, *Virology*, **9**, 715–717 (1959).

[2] Bang, F. B., and A. Warwick, these PROCEEDINGS, **46**, 1065–1075 (1960).

[3] Barski, G., G. Messore, and P. Lepine, *Ann. Inst. Pasteur*, **89**, 366 (1955).

[4] Chang, Y. T., *Transactions of the Symposium on Research in Leprosy*, **1961**, sponsored by The Leonard Wood Memorial (American Leprosy Foundation) and The Johns Hopkins University, School of Hygiene and Public Health.

[5] Sabin, A. B., these PROCEEDINGS, **38**, 540–546 (1952).

[6] Sabin, A. B., in *Genetics and the Inheritance of Integrated Neurological and Psychiatric Patterns*, Proceedings of the Association for Research in Nervous and Mental Disease (Baltimore, Md.: The Williams & Wilkins Co., 1954), vol. 33, pp. 57–66.

[7] Goodman, G. T., and H. Koprowski, these PROCEEDINGS, **48**, 160–165 (1962).

[8] Presented to American Society for Microbiology, May 6–10, 1962; *Bacteriological Proceedings* (1962).

RELATIVISTIC QUANTUM THEORY OF PARTICLES WITH VARIABLE MASS, I

BY H. C. CORBEN

SPACE TECHNOLOGY LABORATORIES, INC., LOS ANGELES

Communicated by L. B. Slichter, July 27, 1962

1. *Introduction.*—It is the purpose of this paper to present a relativistic quantum theory of particles with spin 0, 1/2, 1, 3/2, 2 which has as its classical limit the theory of a particle which possesses moments of inertia about more than one axis. The simplest classical theory is known to correspond to the equations of Dirac, and Kemmer and Duffin, for particles of spin 1/2 or of spin 0, 1 respectively. In these