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STUDIES ON THE INHERITANCE OF THE SUSCEPTIBILITY TO POLIOVIRUS OF PHYTOHAEMAGGLUTININ-TRANSFORMED MACROPHAGES

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Summary. — The Brunhilde strain of poliovirus was found to replicate in phytohaemagglutinin-transformed macrophages of CF inbred mice, but not in such macrophages of C_3H mice. This phenomenon is genetically conditioned. After inbred crossing of the two lines, segregation according to two genes theory of inheritance occurred in the F2 mouse generation.

The experiments reported below concern the susceptibility of inbred mouse lines to viral infection. The peritoneal macrophages and enteroviruses were used in the study.

Since the elegant studies on mitotic stimulating activity of phytohaemagglutinin published by Nowel in 1960, reports concerning the influence of phytohaemagglutinin on the reproduction of herpes simplex (Nahmias *et al.*, 1964), mumps (Duc-Nguyen and Henle, 1966) and vesicular stomatitis (Edelman and Wheelock, 1966) viruses have been published.

In our experiments we used inbred CF and C_3H mice. The peritoneal macrophage cultures were settled using about 1.8×10^6 cells per tube (Kańtoch *et al.*, 1963). Difco M phytohaemagglutinin was used in a concentration of 0.2 mg per ml. Increased mitotic activity began 3 days after introduction of phytohaemagglutinin. At the 6th day the number of cells usually doubled. At the begining of the increase of mitotic activity, phytohaemagglutinin-treated and untreated cultures were inoculated with poliomyelitis type 1 (strains Brunhilde and Mahoney), Coxsackie B3 and ECHO 9 viruses. The fluids from the test cultures were harvested 3 and 6 days after infection and the virus titres measured.

We found only the increase of poliovirus titres in CF macrophages treated with phytohaemagglutinin (Table 1). The increase depended on the infective dose. After introduction of small virus doses (10^2 $TCID_{50}$ or less), no virus reproduction was observed. In controls, the virus titres markedly dropped. Here we would like to call attention to studies with mouse hepatitis virus (Bang and Warwick, 1960; Kańtoch *et al.*, 1963) and arboviruses (Goodman and Koprowski, 1962) both in vivo and in macrophage cultures, and to studies with leukaemia (Odaka and Jamamoto, 1962), polyoma (Jahkola, 1965) and influenza (Lindenmann, 1964) viruses. It was demonstrated that susceptibility or resistance might be manifested as a dominant or recessive trait depending on the virus, and in the case of macrophages a correlation between susceptibility in vivo and in vitro was reported.

After we found susceptibility of macrophages from inbred CF mice and resistance of macrophages from C_3H mice, we crossed the two mouse lines:

Table 1. The reproduction of enteroviruses in CF and C_3H mouse macrophage cultures

Virus strain	Mouse strain	Infective doses (log $TCID_{50}$)	Virus titre (log $TCID_{50}$) in				
			phytohaemagglutinin-treated cultures		untreated cultures		medium without cells after 6 days
			3 d*	6 d*	3 d	6 d	
Polio							
Brunhilde	C_3H	5	3.8	3,6	4.1	4.4	2.9
	CF	5	4.9	7.2	3.0	2.6	2.9
		4	4.2	6.6	3.2	3.2	
		3	2.6	4.4	2.9	2.6	
		2	2.6	2.4	<2	<2	
		1	<2	<2	<2	<2	
Mahoney	CF	5	4.2	6.4	3.2	3.4	3.2
	C_3H		5.4	5.0	4.2	4.2	
ECHO 9	CF	5	5.8	5.8	5.6	5.6	3.2
		3	2.5	2.2	2.3	2.0	2.6
Coxsackie B3	CF	5	4.0	3.0	4.3	3.8	0
		3	3.6	3.7	3.4	3.0	1.5

* 3 d, 6 d = 3 and 6 days after inoculation, respectively.
0 means no virus demonstrated.

a) CF × C_3H = F1, and b) F1 × F1 = F2. The macrophages of F1 and F2 mice were tested. The results are presented in Table 2.

One can see that F1 hybrid macrophages reproduced the virus in statistically insignificant degree, although always some titre increase was observed. In F2 macrophage cultures there appeared segregation according to two genes theory of inheritance of susceptibility to virus infection. The lower degree of virus reproduction by hybrids could be explained by insufficient influence of one gene. Similar situation was observed in studies with mouse hepatitis virus (Kańtoch *et al.*, 1963) when a slower cytopathic effect in hybrid macrophages took place, and with polyoma virus (Jahkola, 1965) when F1 mouse showed a longer survival time. Macrophages from 25 per cent of F2 mice showed virus reproduction similar to that in CF macrophages.

Summarizing we can say that the phytohaemagglutinin-transformed mouse macrophages of some inbred strains are able to reproduce poliovirus. This ability is inherited and can be transferred through F1 hybrids to F2 homozygotes.

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Table 2. Reproduction of poliovirus (Brunhilde strain) in macrophages of F1 and F2 mouse generations

Mouse	Parents		Increase of virus titre (log units)
	Female	Male	
CF female	CF	CF	**2.5**
C_3H male	C_3H	C_3H	0
F1 (A) male	CF	C_3H	1.0
F1 (A) female	CF	C_3H	1.6
F2 (1A)	F1 (A)	F1 (A)	**2.2**
F2 (2A)	F1 (A)	F1 (A)	1.4
F2 (3A)	F1 (A)	F1 (A)	2.6
F2 (4A)	F1 (A)	F1 (A)	1.0
F2 (5A)	F1 (A)	F1 (A)	1.8
F2 (6A)	F1 (A)	F1 (A)	1.2
F1 (G) male	CF	C_3H	0.6
F1 (G) female	CF	C_3H	1.2
F2 (6G)	F1 (G)	F1 (G)	0.2
F2 (7G)	F1 (G)	F1 (G)	1.4
F2 (8G)	F1 (G)	F1 (G)	1.2
F2 (9G)	F1 (G)	F1 (G)	1.4
F2 (10G)	F1 (G)	F1 (G)	**2.0**
F2 (11G)	F1 (G)	F1 (G)	0
F1 (L) male	CF	C_3H	1.0
F1 (L) female	CF	C_3H	1.4
F2 (L13)	F1 (L)	F1 (L)	**2.0**
F2 (L14)	F1 (L)	F1 (L)	0.4
F2 (L15)	F1 (L)	F1 (L)	1.8
F2 (L16)	F1 (L)	F1 (L)	1.4

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