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INACTIVATION OF VIRUSES BY PHENETHYL ALCOHOL AT TEMPERATURES BELOW AND ABOVE 0° C

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Summary. — All of the tested myxoviruses, arboviruses and poxviruses proved to be highly sensitive, while enteroviruses, adenoviruses and papovaviruses were completely resistant to the action of phenethyl alcohol (PEA). The virucidal action of PEA was manifested at —20° C, but not at 37° C.

PEA has been recently added to the numerous compounds inhibiting the multiplication of DNA- and RNA-containing viruses (Konetzka and Berrah, 1962; Roizman, 1963; Nonoyama and Ikeda, 1964; Bowen *et al.*, 1966; Roihel and Zeitlenok, 1966). Data on the virucidal activity of PEA are presented below.

PEA purified by distillation at 219° C was used. The mixture of the virus with PEA (0.3% final concentration) was kept at 37° C for 3 hours and then at —20° C for at least 48 hours until titrated. A mixture of the virus with culture medium used as diluent of PEA kept under the same conditions served as control. Under these conditions poliovirus type 1, ECHO 7 virus, adenoviruses 3 and 5 and SV 40 proved to be insensitive to the action of PEA, while the infectivities of influenza A, Sendai, Newcastle disease (NDV), western equine encephalomyelitis (WEE), Semliki forest, Chikungunya and vaccinia viruses were completely inactivated.

The role of temperature on the action of PEA was tested as follows: 1) the virus — PEA mixtures were kept at 37° C and immediately titrated; and 2) the viruses were mixed with PEA, frozen at —20° C and kept at this temperature until titrated. The results presented in Table 1 showed that the sensitive viruses were completely inactivated only when their contact with PEA proceeded at —20° C.

Some features of the virucidal action of PEA were elucidated on the model of vaccinia virus. Freezing of the virus — PEA mixture for 1 hour already caused some decrease in the virus titre. Prolonged storage of the mixture at —20° C led to a progressive drop in titre until complete inactivation of virus was reached after 48 hours. The duration of contact itself was of no importance, since keeping the virus — PEA mixture for 48 hours at 4° C caused not even partial inactivation.

We also attempted to determine the influence of pH on the inactivating ability of PEA at —20° C. It was not changed within a pH range from 6 to 10.

The inactivating effect of PEA at —20° C was not changed when the PEA concentration was reduced from 0.3 to 0.03%; it decreased somewhat

Table 1. The virucidal activity of phenethyl alcohol at different temperatures

Viruses	Activity units per ml	Conditions of contact of PEA with virus			
		−20° C		+37° C	
		Control	Test	Control	Test
Vaccinia	PFU	1.8×10^4	0	—	2.0×10^5
Adeno	log TCD_{50}	2.44	2.09	3.20	3.18
SV40	log TCD_{50}	5.70	4.94	6.09	5.36
Influenza A PR8	log ID_{50}	7.36	0	6.70	6.99
	HAU	256	<2	256	256
Parainfluenza, Sendai	log ID_{50}	5.36	0	—	—
NDV	PFU	1.8×10^6	0	—	—
WEE	PFU	2.0×10^3	0	—	—
Semliki forest	PFU	1.2×10^5	0	1.0×10^5	1.0×10^5
Chikungunya	PFU	2.5×10^2	0	—	—
Poliovirus 1					
Mahoney	PFU	5.5×10^5	4.9×10^5	—	—
LSc2ab	PFU	3.0×10^5	3.5×10^5	—	—
ECHO 7	PFU	1.7×10^5	1.7×10^5	1.9×10^5	1.6×10^5
	HAU	64	64	64	64

PFU = plaque forming units; HAU = haemagglutinating units.
0 = no virus detected; — = not done.

on further reduction to 0.006%. At a concentration of 0.003%, no inactivating effect was observed. On increasing the concentration of PEA to 10%, it appeared to inactivate influenza virus haemagglutinin even at room temperature.

PEA-inactivated influenza virus failed to interact with specific anti-haemagglutinins since its addition to antiserum affected the activity of the latter not more than dilution of the serum with a corresponding volume of saline.

In the available literature we found no data on the ability of PEA to exert a direct virucidal effect as described above. This effect was characterized by two features: 1) it was manifested only when keeping the virus — PEA mixtures frozen at −20° C; and 2) among the viruses tested, a group selectivity was clearly revealed. Members of picornaviruses, papovaviruses and adenoviruses, the virions of which possess no supercapsid membrane, were insensitive to the action of PEA; on the other hand, viruses possessing a supercapsid membrane, namely members of myxoviruses, arboviruses and poxviruses proved to be sensitive to PEA. The action of PEA was selectively directed, evidently to the structure of supercapsid membranes of the viruses as reported for other phenol derivatives (Klein and Deforest, 1965).

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