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Correlation between Heat-Resistance of Polioviruses and Other Genetic Markers.* (26979)

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The study of the genetic markers of poliovirus has assumed added importance as a result of the wide use of attenuated viruses as vaccine. The association of certain tissue culture markers with the presence or absence of neurovirulence and the stability of these traits has been investigated intensively. The present study deals with the resistance of polioviruses to heating at 50°C and correlation of this property with 2 other widely used markers, efficiency of plating at low bicarbonate concentrations(1) and capacity to multiply at 40°C(2).

Materials and methods. Viruses. Sixteen strains representing the 3 immunologic types of poliovirus were studied. Three strains (SI, SII, SIII) were provided by Dr. Joel Warren and 10 strains were obtained through the courtesy of Dr. J. L. Melnick (PII, PIII, MIA, MIB, MIC, MID, MIIIA, MIIIB, MIIIC, MIIID). The other 3 strains (Mah, MEF-1, Sau) were carried in this laboratory.

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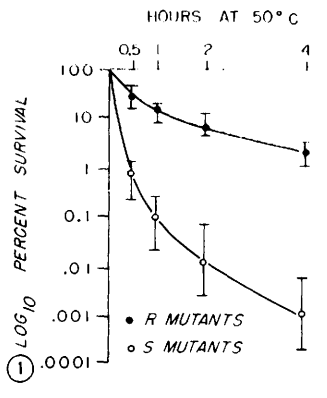

FIG. 1. Inactivation curves at 50°C of S and R mutants of Type 1 poliovirus strains. Range of survival and mean values for six strains.

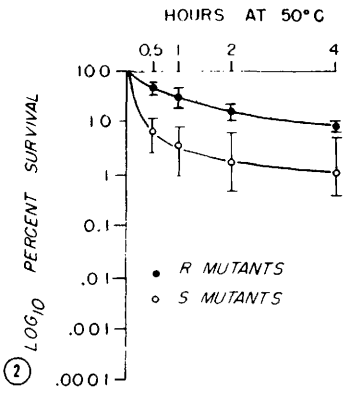

FIG. 2. Inactivation curves at 50°C of S and R mutants of Type 2 poliovirus strains. Range of survival and mean values for three strains.

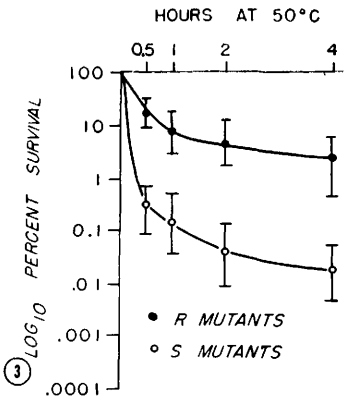

FIG. 3. Inactivation curves at 50°C of S and R mutants of Type 3 poliovirus strains. Range of survival and mean values for seven strains.

Virus cultivation and plaque assays were done in primary monkey kidney cultures as previously described(3,4). Single plaque progeny lines of all virus strains were used in these experiments.

Thermal inactivation of virus at 50°C was carried out as previously described(5). Thermal resistant (R) mutants were selected from sensitive (S) parent virus by 4 cycles of alternate heating at 50°C for 8 hours and propagation of the survivors in monkey kidney cell cultures.

Tissue culture markers were studied by methods which have been previously reported. The *T marker,* capacity to grow at 40°C, was determined by the method of Lwoff and Lwoff(2). The *d marker,* efficiency of plating at low bicarbonate concentration, was measured by the technique of Hsiung and Melnick(6). As control, known d^+T^+ and d^-T^- strains were included in all tests.

Results. The rates of inactivation at 50°C of parent viruses and corresponding R mutants of 16 strains representing the 3 immunologic types of poliovirus were studied. Comparison of the inactivation curves (Fig. 1,2,3) of the parent viruses shows that distinct differences can be found in rates of inactivation of the 3 immunologic types. Type 1 strains are most sensitive to 50°C inactivation, type 2 strains are least sensitive, and type 3 strains occupy an intermediate position. These results confirm data obtained previously with 16 other strains(5). It was possible to select 50°C-resistant (R) mutants from all parent viruses. The parent viruses, therefore, are referred to as sensitive (S) mutants. The data illustrated in Fig. 1, 2, and 3 show that the inactivation curves of the R mutants are similar for all 3 types of poliovirus.

The results in Table I illustrate 2 important points. First, parent (S) viruses which have different d and T markers show inactivation curves characteristic of their immunologic type. For example, of the 6 type 1 parent viruses studied, 3 were d^+T^+ and 3 were d^-T^-. It can be seen from Fig. 1 that all 6 showed essentially the same inactivation characteristics when heated at 50°C. This is also true of the type 2 and type 3 strains studied. Second, Table I shows that when R mutants from all 16 strains were selected, the d and T markers did not change.

Discussion. The susceptibility to inactivation of polioviruses at 50°C has been designated by Dulbecco(7) as either t^s (sensitive mutant) or t^r (resistant mutant). It was suggested(7) that there is an extensive degree of covariation of this marker with the d and T, as well as some other markers. The data presented here indicate that there is no correlation between rate of inactivation at 50°C of the 16 strains studied and their d and T markers. The susceptibility to inacti-

TABLE I. Comparison of d and T markers of S and R mutants of 16 strains of poliovirus.

	Type 1							Type 2						Type 3							
Strain	Mah		SI		MIA		MIB	MIC	MID	MEFI		SII	PII	Sau	SIII	PIII	MIIIA	MIIIB	MIIIC	MIIID	
Mutant (50°C)	S	R	S	R	S	R	S R	S R	S R	S	R	S R	S R	S R	S R	S R	S R	S R	S R	S	R
d marker	+							+	+				+	±		+					+
T "	+							+	+				+	+		+				+	+

vation at 50°C of polioviruses is more related to their immunologic type than to the other markers. For example, the inactivation rate of type 2 viruses is significantly slower than the inactivation rates obtained with type 1 and type 3 viruses. Furthermore, the resistant mutants showed the same d and T markers as the sensitive parent viruses from which they were selected. These results clearly support the conclusion that resistance to inactivation at 50°C varies independently from the d and T markers.

It is interesting to note that all 16 parent virus strains studied were clearly t^s in regard to their inactivation at 50°C while differing in their d and T characters. From all strains, t^r mutants could be selected which did not differ significantly in their inactivation rates. Since the selection of these t^r mutants did not alter the other characteristics measured, it is suggested that t^r mutants could be used as an additional criterion for identifying and studying the stability of attenuated poliovirus strains. It would also be of interest to determine whether there is any correlation between the degree of neurovirulence and the $t^s \rightarrow t^r$ mutation.

Summary. The genetic marker involving resistance of polioviruses to heating at 50°C is independent of the capacity of mutants to multiply at 40°C or their plating efficiency at low bicarbonate concentrations. The possible usefulness of heat-resistant mutants for the study of attenuated poliovirus strains is discussed.

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