Differences in Thermostability of Antigenically Related Strains of Poliomyelitis Virus.* (21106)

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Recent studies have shown that related strains of a virus may have different sensitivities to heat(1,2), and previous work from this laboratory has suggested that this might also be true for poliomyelitis strains(3). The earlier experiments have been extended in the present study which shows that 3 strains of poliomyelitis virus, all of the same immunologic type, differ in their susceptibility to heat. Because the differences are slight, a method (3,4) for their accentuation was used, *viz.*, the viruses were suspended in heavy cream or ice cream mix and heated in these media. A report on the pasteurization of milk and cream artificially contaminated with poliomyelitis virus has appeared elsewhere(3).

Materials and methods. Viruses. 1) *Lansing strain.* This poliomyelitis strain, received from Dr. Charles Armstrong, had been passed 275 times in mice and then 10 times in cotton rats. The latter material was used in these experiments. 2) *Y-SK strain.* This strain was isolated in this laboratory by Trask and Paul. After 21 passages in monkeys, it was adapted to cotton rats and mice. The strain used in these experiments had been passed 5 times in cotton rats. 3) *MEFl strain.* This strain was isolated by Dr. C. E. Van Rooyen and obtained from Drs. Jordi Casals and Peter K. Olitsky who adapted it to infant mice. The MEFl strain had been passed 86 times in infant mice and once in cotton rats, the latter being the source of virus for the heating experiments. *Preparation of virus suspensions.* For each strain the brains and cords of 20 infected cotton rats were thoroughly ground without abrasive until a homogenous mass resulted. To one portion, enough sterile distilled water was added to make a 10% suspension; to the other portions cream and ice cream mix were added in the place of water. The suspensions were centrifuged sufficiently to sediment tissue debris, but not at forces great enough to break the cream emulsion. *Suspending media.* The pasteurized heavy cream for these experiments was purchased on the open market. The ice cream mix (nonaerated) had been pasteurized before being used for the heating experiments. *Method of heating*(3). The virus suspensions were drawn into thin, U-shaped glass capillaries with an average outside diameter of 1.71 mm and with a length of 40 cm, and then both ends were sealed. These capillaries were tied with cotton thread, by means of which they were lowered until completely immersed into a water bath maintained at the desired temperature; immediately after heating for 15 or 25 seconds, the capillaries were dropped in ice water. The water bath employed was accurate to 0.05°C. *Determination of virus activity.* The rodent strains of poliomyelitis virus were inoculated intracerebrally in 0.03 ml amounts into 4-8-week-old Swiss mice and 4-6-week-old cotton rats. The animals were observed for a period of 4 weeks. Deaths which occurred within 2 days after inoculation were considered nonspecific. Passage was made of brain and spinal cord from those animals exhibiting typical signs as well as atypical ones, particularly those coming down following inoculation of material which had been heated at high temperature. A special point was made to pass material where only a small number in a test group developed disease or died without signs being observed.

Results. Effect of cream on the thermostability of virus. None of the 3 strains, Lansing, Y-SK, or MEF1 suspended in *water* withstood inactivation by heating at 71.1°C for 15 seconds. This was true regardless of whether the heated viruses were inoculated into cotton rats or mice (Table I). When the Lansing strain was suspended in *cream,* however, a small amount of virus resisted inactivation at this temperature. Increasing the temperature

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TABLE I. Effect of Cream on Thermal Stability of 3 Strains of Type 2 Poliomyelitis Virus.*

Strain of virus	Heated in water 71.1°	Heated in cream 71.1°	73.9°	76.7°	79.5°C	Original virus titer
		A. Tests in cotton rats				
Lansing	0/11	2/11†	0/12	0/11	0/12	$10^{-4.0}$
Y-SK	0/12	6/11	7/10	8/12	10/12	$10^{-3.3}$
MEF1	0/11	3/12	6/11	4/11	2/10	$10^{-2.8}$
		B. Tests in mice				
Lansing	0/36	9/48	2/46	2/29	2/43	$10^{-3.5}$
Y-SK	0/24	16/24	9/24	8/17	18/39	$10^{-2.5}$
MEF1	0/36	22/36	15/40	12/38	10/39	$10^{-3.0}$

* 10% spinal cords of infected cotton rats heated 15 sec. at indicated temperatures.
† 2/11 indicates that of 11 animals inoculated, 2 came down with disease.

to 73.9°C sufficed to inactivate the virus to the point where none of the inoculated cotton rats came down. As the results in Table I show, Y-SK proved to be more thermostable than did Lansing, and the virus persisted, despite an increase of the temperature to 79.5°. Ten cotton rats developed experimental poliomyelitis out of the 12 inoculated with Y-SK heated at this temperature. The difference between Lansing and MEF1 was less marked, but the latter strain also remained active even at this high temperature, 2 out of 11 inoculated cotton rats being infected.

The results were less striking, but nevertheless comparable with those obtained with cotton rats, when mice were used as the test animals. Thus traces of Lansing virus suspended in cream persisted even when the suspension had been heated at 79.5°C for 15 seconds. However, of 43 mice inoculated with Lansing, only 2 showed signs of infection. In contrast to this, 18 of the 39 mice inoculated with Y-SK heated under the same conditions came down. The MEF1 strain behaved intermediately between the other two.

Effect of ice cream mix on thermostability of virus. Lansing virus suspended in *ice cream* was heated for 25 seconds with the result that none of the cotton rats came down after an inoculation with virus held at 71.1°C. Despite the 10 second increase in the heating time, a similar increased resistance of Y-SK was observed. Again, as the results in Table II indicate, Y-SK virus persisted up to a temperature of 79.5°C with more than half (7 of 11) of the cotton rats inoculated coming down with disease. Raising the temperature to 82.2, however, sufficed to inactivate the virus to such an extent that it was no longer detectable in cotton rats. The MEF1 strain showed a slightly greater resistance than Lansing to heat in that 2 of the 11 cotton rats inoculated with virus heated at 71.1°C were paralyzed, but at 73.9°C virus could no longer be detected.

The observation that mice were more susceptible to the 3 strains after heating in cream was made again with virus suspended in ice cream (see bottom half of Table II). Lansing virus suspended in ice cream remained viable even though the temperature was elevated to 82.2°C: 3 of 42 mice became paralyzed following inoculation. The Y-SK strain remained infective at this temperature and as in all the previous experiments, more of the animals (10 of 29) came down than in the case of the Lansing strain. The MEF1 strain appeared to be as resistant as Y-SK until the temperature of 82.2°C was reached; it was inactivated at this point to about the same extent as Lansing virus.

Discussion. Although the 3 rodent-adapted strains of Type 2 poliomyelitis virus, Lansing, Y-SK, and MEF1, are immunologically indistinguishable, certain differences have been noted in the past to exist between at least the first 2 strains. Monkeys could be infected when given the Y-SK strain of virus orally, but they were not susceptible to infection with Lansing by this route of inoculation(5). In the growth of Lansing and Y-SK viruses in monkey testicular tissue culture(6), only the latter strain proved cytopathogenic even though both viruses multiplied to the same

TABLE II.
Effect of Ice Cream on Thermal Stability of 3 Strains of Type 2 Poliomyelitis Virus.*

Strain of virus	Heated in water 71.1°	Heated in ice cream 71.1°	73.9°	76.7°	79.5°	82.2°C	Original virus titer
		A. Tests in cotton rats					
Lansing	0/11	0/11	0/11	0/10	0/10	0/12	$10^{-4.0}$
Y-SK	0/12	5/12	8/12	6/12	7/11	0/12	$10^{-3.8}$
MEF1	0/11	2/11	0/11	0/10	0/11	0/10	$10^{-2.8}$
		B. Tests in mice					
Lansing	0/35	9/38	10/40	7/40	5/45	3/42	$10^{-3.5}$
Y-SK	0/24	12/21	8/22	6/19	12/27	10/29	$10^{-2.5}$
MEF1	0/37	18/42	13/31	16/41	15/41	2/36	$10^{-3.0}$

* 10% spinal cords of infected cotton rats heated 25 sec.

degree, according to titrations of infectious culture fluid in mice.‡ To these differences between the strains, one may now add the greater thermal resistance of the Y-SK virus. To detect this difference in response to heat by the 3 strains, it is apparently necessary to suspend them in a protective medium such as cream or ice cream mix(3), for in no case did any of the strains prepared in water suspension resist thermal inactivation at 71.1°. The cream probably slows the rate of inactivation sufficiently to magnify the differences between the strains. The differences in the thermostability between the 3 strains did not appear to be due to a greater concentration of one over the other in the suspensions being heated. This is emphasized by the fact that in every one of the experiments reported here the titer of Lansing was from 0.7 to 1.0 log higher than that of Y-SK.

The present results confirm and extend earlier observations on differences in the thermostability of these strains(3): Lansing strain suspended in milk was inactivated by heating at 50°C for 30 minutes, whereas Y-SK proved resistant to this treatment. When suspended in cream, and heated at 71.1°C, the Lansing strain was inactivated after holding time of 45 seconds, whereas the Y-SK strain remained infectious until it had been held at this temperature for 180 seconds.

As far as we are aware, this is the first reported instance of a difference in the response of 2 related poliomyelitis viruses to a physical agent like heat. The fact that 2 such closely related strains can differ in their thermostability is, however, not a new observation in the field of virology. Burnet and Lind(1) have found that the 3 virus types derived from the classical WS strain of influenza differ in their heat resistance, and they have used these differences as genetic markers. A similar phenomenon has been reported by Adams(2) for a heat resistant mutant of coliphage T5 which appears to be identical to the heat susceptible phage in all of its other properties. Thus, we now have a means for the characterization of Lansing and Y-SK strains of poliomyelitis virus which perhaps may be used as a marker in studies of viral genetics.

Previous work(3) has shown that when human fecal strains of poliomyelitis virus were suspended in milk or cream, they were destroyed by pasteurization. It is highly unlikely that dairy products in nature would ever be contaminated so heavily that they would contain the concentration of virus used in our experiments, but if so, and if the contaminating viruses were as stable as Y-SK, the present conditions of high-temperature short-time pasteurization would be inadequate for thermal inactivation.

Summary. Three rodent-adapted strains of poliomyelitis virus, all belonging to the same immunologic type, were found to differ in their thermostability; these were, in decreasing order of resistance, Y-SK, MEF1, and Lansing. The differences between these strains became evident only when they were

‡ Infected mouse cords were used as inoculum in these experiments(6). Phillips has shown that about 30 serial passages in tissue culture were required of this Lansing strain before it became cytopathogenic (7).

suspended in cream or ice cream before being heated. All three strains suspended in water were inactivated by heating at 71.1°C for 15 seconds. When heated in cream the viruses withstood 79.5°C for 15 seconds and in ice cream they withstood 82.2°C for 25 seconds, with Y-SK resisting inactivation to the greatest extent.

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Effects of Plastic and Steel Surfaces on Clotting Time of Human Blood.* (21107)

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Studies in our laboratories using a mechanical heart pump(1) and intravascular prosthetic devices(2) have stimulated an investigation of the effects of various plastic and steel surfaces on the clotting of human blood. Only a few relatively limited comparative studies of surface effects on clotting have been reported previously(3-6).

Materials and methods. A method for the determination of clotting time on various types of surfaces was devised in which all observations were made by one individual. Materials studied were identically shaped into biconcave receptacles or open-end curved tubes with radii of ½ inch and 2 inches. Prior to each measurement, the surfaces under study were thoroughly cleaned with an alkali detergent, rinsed in distilled water, and oven dried. A plastic box with a removable transparent cover was suspended in a constant (37 ± 1°C) temperature water bath and continually rocked by a slow speed motor through an arc of 70° at a rate of 15 cycles per minute. Within the box, 2 test surfaces were placed simultaneously. Blood was obtained from normal individuals with no clotting defects. For each pair of determinations, 10 cc of blood were drawn from an antecubital vein, through a sharp 18-gauge needle into a siliconed syringe. Blood was drawn immediately after placement of a tourniquet, and samples were discarded if the vein was not immediately entered. Two cc of blood were then placed on each of the 2 surfaces to be tested and the first 6 cc drawn into the syringe were discarded. Thus, the blood tested contained a low tissue thromboplastin concentration(7). Time was measured with a stop-watch. Initially, 2 times were recorded; the onset of clot formation, and the time of completion of the clot. It was found

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