Thermal-Resistant Populations of Foot-and-Mouth Disease Virus. (25588)

H. L. Bachrach, R. E. Patty and R. A. Pledger
Plum Island Animal Disease Lab., U. S. Dept. of Agriculture, Greenport, L. I., N. Y.

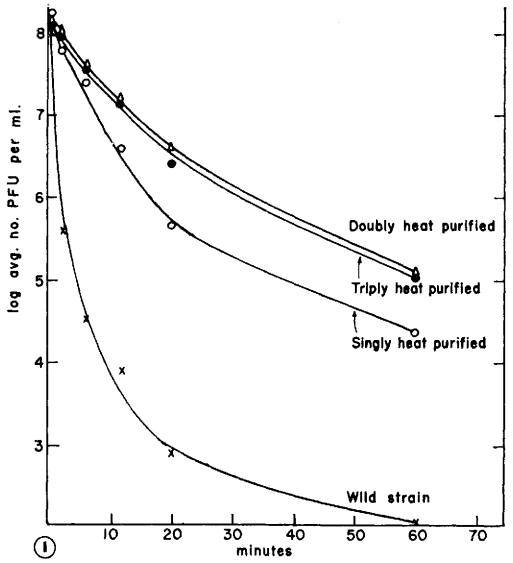
Bachrach *et al.* reported thermal resistance of a small fraction (0.001) of a wild strain of foot-and-mouth disease virus (FMDV) grown in tissue culture(1). The present communication deals with the genetic homogeneity of this strain. The study shows that the thermal-resistant virus fraction, after subsequent passages in tissue culture under continuing heat selection, yields virus populations containing a much higher proportion of thermal resistant particles than were present in the wild population. However, further passages of such populations, made without selecting out the thermal-resistant fraction, yield virus populations which have partially reverted toward the heat sensitivity of the wild strain.

Material and methods. Wild virus was FMDV, type A, strain 119, (FMDV-A119) passaged 76 times in calf-kidney cultures as previously reported(2). Infectious fluids were harvested, pooled, and stored at –40°C. As needed frozen virus was thawed to 4°C and centrifuged at 6,400 x g for 30 minutes to remove cell debris. *Cultures* as confluent sheets of cells were produced in 4-oz prescription bottles and Roux flasks from trypsin-dispersed fragments of calf kidney. *Virus infectivity measurements* were made by plaque assay as described elsewhere(2). One-tenth-ml volumes of properly diluted virus were plated in duplicate onto calf-kidney cultures in prescription bottles. After 90 minutes incubation at 37°C, the cultures were overlaid with agar medium. Plaques were read at 72 hours. A few virus titrations were also made in guinea pig foot pads and in cattle tongues. *A thermal inactivation procedure* was used which changed the temperature of the virus quickly. That is, one part of buffered virus at 20°C was mixed with 9 parts of veronal-acetate buffer held in a water bath at one-half degree higher than the temperature desired. A nearly instantaneous adjustment of temperature was thereby effected. After the desired interval of exposure, the thermal reaction was stopped by diluting the heated virus sample with an equal volume of ice-cold buffer.

Results. As previously reported, wild virus populations heated at 55°C yield, within a few minutes, a residual fraction amounting to 10^{-3} of the original virus. This fraction is much more resistant to heat than the bulk population. A similar relationship with a surviving fraction of only 10^{-5} is given in Fig. 1. The 60-min. survivors were then grown out to a high population in tissue culture and the new population was tested for its heat resistance. Fig. 1 shows that the new population (single heat purification) contains a much higher surviving fraction, *i.e.*, approximately 10^{-2} of heat-resistant virus particles than the wild parent. Survivors of this second heat treatment were again grown to a high population and again heated. This process was repeated a third time with results presented in Fig. 1. Populations from doubly and triply heat-purified viruses appear more stable to heat than progeny from the heat residuum of wild virus. Furthermore, their curves, which approach straight lines, indicate that at least 10% of the virus particles are of the heat-resistant kind.

Differences in heat stability of wild parent virus and of progeny obtained after heat purification were thought possibly due to a sensitization of the wild parent during its freezing and thawing—a process to which progeny were not subjected. Therefore, wild parent virus and its progeny obtained after heat purification were each examined as to rate of thermal inactivation with and without prior freezing. The respective curves were identical to those in Fig. 1, indicating that freezing and thawing of heat-sensitive or heat-resistant populations have no effect on their inactivation at 55°C.

Experiments were next carried out to determine if populations with increased thermal resistance could be maintained without heating for selection. The triply heat-purified virus (Fig. 1) was subcultured several times without heat treatment and then tested for



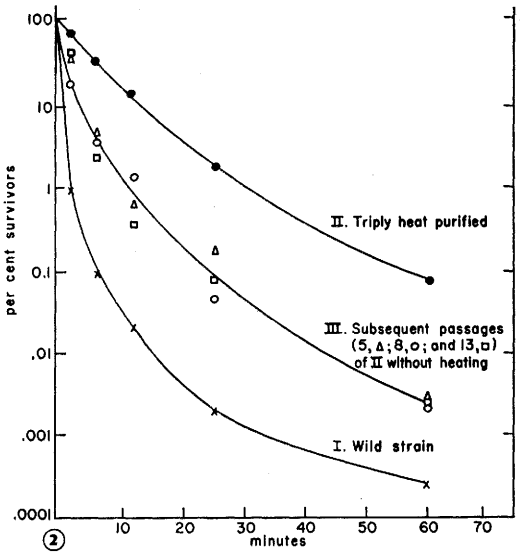


FIG. 1. Inactivation curves at 55°C for wild foot-and-mouth disease virus and of virus derived from it by single, double, and triple heat purifications and by passages in calf-kidney cultures.

FIG. 2. Inactivation curves at 55°C for wild foot-and-mouth disease virus and of various heat-resistant subcultures derived from it.

thermal resistance. After 5 such passages, (Fig. 2) partial reversion toward the heat sensitivity of the wild strain occurred with 90% inactivation at 55°C taking place 3 times faster. Further passages of virus in culture, at least through the eighth and thirteenth, caused no further change in heat resistance.

No additional differences between wild and thermal-resistant populations were detected. There were no changes in size of plaques or in their rates of development at 37°C. Growth curves in calf-kidney cultures of 2 heat-resistant populations were compared with that of the wild virus (Table I). Peak titers appeared to be reached 2.5 hours earlier when inoculated with thermal-resistant virus and there was a slightly higher liberation of progeny per cell. This was not unexpected because greater thermal inactivation during culturing would tend to spread out the logarithmic growth phase of the thermal-sensitive strain and to decrease its peak titer. Also, no significant differences in infectiousness have been demonstrated between the two kinds of populations in any of the hosts tested, *i.e.*,

TABLE I. Wild and Heat-Resistant Populations of Foot-and-Mouth Disease Virus: Growth and Comparative Infectivity.

			Population	
		Wild virus	No. 1*	No. 2†
Growth:	Time for 1 log increase in titer (min.)‡	42	35	35
	Peak titer (PFU/ml)	5.7×10^7	6.2×10^7	6.2×10^7
	Time of peak titer (hr)	15	12.5	12.5
	Avg yield (PFU/cell)	102	118	113
Infectivity tests in:	Calf-kidney culture (log PFU/ml)	8.2	7.8	8.0
	Guinea pigs (log ID_{50}/ml)	4.9	5.7	5.2
	Cattle (" ")	6.7	5.7	6.5

PFU—Plaque forming units.

* Inoculum for this population derived from triply heat-purified virus (II in Fig. 2) subcultured 14 times without heating.

† Inoculum for this population derived from triply heat-purified virus (II in Fig. 2) subcultured 13 times without heating followed by one passage after heating.

‡ During logarithmic growth phase.

calf-kidney cultures, guinea pigs, and cattle (Table I).

Discussion. Since it is assumed that suspensions of genetically reproducible units are variable in a number of characteristics, it is not surprising to find that FMDV is variable in its sensitivity to heat. The application of heat to the wild strain of FMDV appears to destroy primarily the heat-sensitive particles. Thus, by the process of selection, heat-resistant particles remain. However, in the competitive reproduction of both, heat-sensitive particles appear to have a selective advantage in their replication for they tend to predominate in progeny. If this reversion process were to continue indefinitely in absence of heat treatment, it is expected that a complete reversion to the level of heat sensitivity of the wild type would be effected. Since the isolated resistant fraction—after 13 passages without heat—retains a significant resistance to the selecting agent, the heat treatment may effect a physical change within the virus particle itself. From work being reported elsewhere(Bachrach,3) it is clear that heating at 55°C denatures an infection-initiating property of the protein coat of FMDV without appreciable effect on its ribonucleic acid core.

Summary. A small thermal-resistant fraction (10^{-3} to 10^{-5} at 55°C) of foot-and-mouth disease virus, type A, has been isolated from a wild population. Under continuing thermal selection, it breeds populations which contain much higher fractions, at least 10^{-1}, of thermal-resistant virus particles. Such populations revert partially, however, toward the sensitivity of the wild strain if not subjected to heat.

1. Bachrach, H. L., Breese, S. S., Jr., Callis, J. J., Hess, W. R., Patty, R. E., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 147.

2. Bachrach, H. L., Callis, J. J., Hess, W. R., Patty, R. E., *Virology,* 1957, v4, 224.

3. Bachrach, H. L., *Biochem. Biophys. Res. Commun.,* 1959, v1, 356.

Received January 6, 1960. P.S.E.B.M., 1960, v103.

Detection of Insulin-Binding Antibodies and Separation of Free and Antibody-Bound Insulin by Rapid Chemical Procedure. (25589)

ENOCH GORDIS* (Introduced by S. A. Berson)
Radioisotope Service, Vet. Admin. Hospital, Bronx, N. Y.

The presence of insulin-binding antibodies in serums of insulin-treated patients has been demonstrated by paper strip chromato-electrophoresis, starch block electrophoresis and ultracentrifugation of insulin-I^{131} plasma mixtures(1), by cellulose column chromatography (2) and by precipitation of insulin-antibody complexes with rabbit antihuman globulin serum(3). These technics are all somewhat time consuming, and are not suitable for analysis of large volumes of material with low concentrations of radioactivity. It seemed worthwhile to attempt rapid separation of free and bound antibody-insulin by chemical procedure. Bates *et al.*(4,5) showed that in 76% ethanol saturated with NaCl most plasma proteins are precipitated at room temperature, while insulin remains largely soluble. The present paper describes adaptation of a modification of this procedure to separate free insulin from antibody-bound insulin.

Materials and methods. Control (non-immune) serums were obtained from subjects who had never been treated with insulin. Antiserums were obtained from insulin-treated patients. Donors of "immune" serums were not clinically insulin-resistant and required less than 75 units of insulin daily to control their diabetes. "Resistant" serums came from donors who required at least 150 units of insulin daily at the time their blood was drawn. All "immune" and "resistant" serums had been studied in detail previously(6). Insulin-I^{131} (specific activity, 38 - 73 mc I^{131}/mg insulin) and gamma globulin-I^{131} were prepared from

* Fellow of Dazian Fn., 1958-1959.