FOOT-AND-MOUTH DISEASE VIRUS: STABILITY OF ITS RIBONUCLEIC ACID CORE TO ACID AND TO HEAT

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Introduction. In previous research with foot-and-mouth disease virus (FMDV), infectious ribonucleic acid (RNA) was extracted with phenol (Mussgay and Strohmeier, 1958; and Brown et al., 1958). Evidence has also been presented by Mussgay (1959) that FMDV dissociates under acid conditions into nucleic acid and protein subunits.

The research reported here compares the stability to acid and to heat of the infectivity of FMDV with that of its RNA before and after release with phenol.

Materials and Methods. FMDV, type A, strain 119 was used. It had been propagated about 100 times in calf-kidney cultures. The virus was concentrated from tissue culture fluid by precipitation at 0°C with 20% methanol (Bachrach and Breese, 1958). The precipitate was resuspended at 100-fold concentration in 1:1 tris-Hanks' salt solution containing 0.5% bovine lactalbumin hydrolyzate (BLH), dialyzed against this same medium to remove methanol, and clarified at 15,000 rpm for 10 minutes. Before use in experiments, the virus concentrate was diluted 1:6 in 0.02M phosphate buffer of pH 7.6 containing 0.01% sodium ethylenediamine tetraacetate and 0.1% sodium dodecylsulfate. Infectious RNA was produced by two extractions with water-saturated phenol (Gierer and Schramm, 1956) from virus before and after its treatment with acid or heat. All RNA preparations were tested against ribonuclease (RNase) for the presence of intact infectious virus, but none was ever detected. Acidification of virus or RNA to pH 5.0 for 5 minutes at 20°C was carried out with 1N HCl. Dilute NaOH was then used for adjustments to pH 7.5. Heating of virus or RNA at temperatures up to and including 100°C was carried out

in a water bath. Two ml of virus or 0.7 ml of RNA was introduced into a vessel already at temperature. The time required for virus or RNA to reach bath temperature was usually about 2 minutes. Heating was then continued for a 5-minute period which was terminated by cooling the vessel in ice water. Virus was also autoclaved at 123°C. After a 4-minute period required to reach temperature, autoclaving was continued for 5 minutes. Virus and RNA infectivity measurements were made by plaque assay (Bachrach et al., 1957b) in calf-kidney cultures grown in 4-oz prescription bottles. Dilutions of virus for assay were made in Hanks' fluid containing 2% bovine serum and 0.5% BLH. Because of its RNase content, serum was omitted for making dilutions of RNA; moreover, before plating RNA, cultures were triply washed with serum-free fluid to remove RNase. One-tenth ml of decimally-diluted infectious entities were plated, and plaques were counted after 3 days' incubation at 37°C. Counts were converted to plaque-forming units per ml (PFU/ml) of undiluted sample.

Experiments and Results. Experiments with acid-treated virus. A comparison was made of the yields of infectious RNA extractable by phenol from FMDV before and after its treatment at pH 5.0 for 5 minutes. Table I presents the experimental data. Unexposed virus with titers approaching 10^9 PFU/ml yielded RNA containing about 10^3 PFU/ml. The pre-acidified virus, although 10^4 times less infectious for bovine cultures than untreated virus, gave fractional yields of RNA by phenol treatment 500 to 2,100 times higher than were obtained from the original virus.

TABLE I

Fractional Yield of Infectious RNA from Foot-and-Mouth Disease Virus Before and After Treatment with Acid

Expt. no.	Virus conc.	Acid treatment	Virus conc. after acidification	RNA conc.	Fract. yield of RNA	Approx. increase in fract. yield
	PFU/ml		PFU/ml	PFU/ml		
1	7.0×10^8	none	-----	1.5×10^3	2.1×10^{-6}	
	7.0×10^8	pH 5.0,5 min	1.6×10^4	7.0×10^1	4.4×10^{-3}	2100 X
2	4.5×10^8	none	-----	1.7×10^3	3.8×10^{-6}	
	4.5×10^8	pH 5.0,5 min	2.8×10^4	5.5×10^1	2.0×10^{-3}	500 X

Additional data have aided in the interpretation of a change in fractional yield of such magnitude. That is,(1) infectious RNA could not be obtained by phenol from unacidified virus diluted to a concentration of 1.0×10^5 PFU/ml or less, (2) the infectivity of free RNA was more stable to acid treatment than that of intact virus; RNA containing 2.1×10^3 PFU/ml still possessed 6.7×10^2 PFU/ml after exposure to pH 5.0 for 5 minutes, and(3) infectious RNA was not present in virus suspensions after acid treatment and reneutralization. This was considered to be due to the presence of pH 5-stable RNase in the virus (Mussgay, 1959, and McDonald, 1955).

Experiments with heat-treated virus. The thermal stability of the infectivity of whole virus was compared with that of its RNA before and after release by phenol extraction. The whole virus and free RNA had only to be heated and assayed for infectivity. Thermal resistance of RNA *in situ* was determined by comparing the amounts of infectious RNA which could be extracted from heated and unheated virus. That is, virus held at 4°C as well as virus samples which had been heated for 5 minutes at 55°,61°,85°,100° and 123°C were assayed for infectivity and were also stripped with phenol to yield RNA. The resulting RNA from each virus sample was assayed before and after the addition of RNase (ca. 5 μg/ml). Results of a typical experiment are recorded in Table II.

TABLE II

Thermal Stability of the Infectivity of Foot-and-Mouth Disease Virus and of Its RNA, *In Situ* and Free

5-min heat treatment	Yield of RNA from heated and unheated virus[a]			Stability of free RNA from unheated virus[b]	
	Virus	RNA[c]	RNA survival	RNA[c]	RNA survival
	PFU/ml	PFU/ml	%	PFU/ml	%
none	5.2×10^8	4.6×10^3	100	1.8×10^3	100
25°C	-----	-----	---	1.8×10^3	100
37	-----	-----	---	1.9×10^3	100
55	1.0×10^1	4.0×10^3	87	1.3×10^3	72
61	0	3.5×10^3	76	6.0×10^2	33
85	0	1.5×10^3	33	2.3×10^2	13
100	0	2.3×10^2	5	0	0
123	0	0	0	-----	---

a,b. These were different lots of tissue culture FMDV, type A, strain 119.
c. In separate tests, each RNA preparation was found to be completely inactivated by RNase acting at 4°C for 5 minutes.

The data show that the ability of intact virus to infect calf-kidney cultures is essentially lost at 55°C compared with a reduction of only 13% in the amount of extractable infectious RNA. After heating at higher temperatures, 61°,85°, 100° and 123°C, the infection-initiating capacity of the virus appears to be destroyed, whereas infectious RNA extractable from the heated virus amounts to 76, 33, 5 and 0% respectively, of that obtained from unheated virus. Also, survival of free RNA infectivity much more closely approximates that of *in situ* RNA than of whole virus. Free RNA did not, however, survive heating at 100°C.

Discussion. The data show that there are two factors which participate in infection of susceptible cells by intact FMDV. One is an acid- and heat-labile infection-initiating factor in the coat protein, and the other is its acid- and heat-stable internal RNA. If the coat factor is damaged by acid or by heat, then the infectious RNA cannot act unless first freed by phenol extraction. Since infectious RNA was not obtained from unacidified virus at a concentration of 1.0×10^5 PFU/ml or less and since acidified virus did not contain free RNA, it is likely that most, if not all, of the infectious RNA extractable from acid-treated virus is derived from damaged virus particles. RNA which would possess greater infectiousness than its source, the acid-treated virus, could not be obtained by phenol treatment. Such results have been attained however, by Ada and Anderson (1959) with Murray Valley encephalitis virus. The heat lability of the infectivity of the whole virus at 55° and 61°C was already known from thermal inactivation rate studies reported earlier (Bachrach *et al.*, 1957a). Since the thermal stability of free RNA infectivity was nearly as high as that of RNA *in situ*, it appears that the protein coat of FMDV stabilizes its internal RNA not so much against thermal inactivation as it does against the action of RNase.

RNA extracted from virus heated at 100°C not only initiated plaques in tissue cultures, but also produced foot-and-mouth disease in an inoculated steer. In one experiment virus was actually placed in a flask with attached condenser and refluxed vigorously at 100°C for 5 minutes with the open flame of a Bunsen burner. RNA extracted from this virus still had 1% of the infectivity of RNA obtained from unheated virus.

Summary. The infectivity of the RNA core within FMDV, type A, was found to be very stable. Virus which was pre-acidified at pH 5.0 to destroy 99.99% of its infectivity for calf-kidney cultures gave fractional yields of RNA, after extraction with phenol, that were 500 to 2,100 times higher than were obtained from untreated virus. Moreover, RNA in situ was not completely inactivated when the virus was heated at 100°C for 5 minutes, even though the infectivity of the intact virus was entirely lost at 61°C and higher temperatures. It is concluded, therefore, that an acid- and thermal-labile property of the protein coat is required for the initiation of infection by whole virus. When the coat factor is damaged by acid or by heat, the resistant internal RNA cannot act unless first freed by phenol extraction.

The infectivity of free RNA was only slightly less stable to heat than that of RNA in situ, and it was more stable to acid than the infectivity of intact virus.

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ERRATUM

Volume 1, Number 4, October 1959 in the article entitled "Influence of Vitamin A Deficiency on the Biosynthesis of Cholesterol, Squalene and Ubiquinone" by U. Gloor and O. Wiss (pages 182 - 185):

Page 183, Table I, heading of Column 1 should read

Days of vitamin A-depletion

Page 184, in line 23 the reference should read

Linn et al. (1959)

Page 184, the first author's name in the first entry in the Bibliography list should read

Linn, B. O.