mechanism of the cross-protection in animals. therefore, is lacking.

Summary. Mice were capable of withstanding a large challenge dose of virulent Venezuelan equine encephalomyelitis virus and a substantial challenge dose of eastern equine encephalomyelitis virus after immunization with an attenuated 9t strain of VEE virus. This resistance was elicited after administration of the 9t strain by the intraperitoneal, subcutaneous, and respiratory routes, the latter being accomplished by short exposures to aerosols of the attenuated strain. Rabbits and guinea pigs, which were refractory to infections with EEE virus, were protected against lethal doses of the heterologous virulent VEE virus.

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Thermal Degradation of Foot-and-Mouth Disease Virus Into Infectious Ribonucleic Acid.* (26703)

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Infectious ribonucleic acid (RNA) was first obtained from foot-and-mouth disease virus (FMDV) by phenol extraction(1,2). Mussgay(3) presented evidence that FMDV dissociates upon acidification at pH 5.0 into RNA and protein subunits. Bachrach(4) reported that FMDV, which appeared to be completely inactivated by boiling at 100°C for 5 minutes, still yielded infectious RNA upon extraction with phenol; phenol-derived RNA was not completely inactivated after heating at 85°C for 5 minutes; and (5) FMDV-RNA has a wider pH stability spectrum than FMDV preparations that contain ribonuclease (RNase).

The research reported here shows that heating degrades FMDV into infectious RNA whenever environmental RNase is simultaneously inactivated with hot sodium dodecylsulfate (SDS) or when Rnase has been previously removed.

Materials and methods. FMDV, type A, strain 119, which had been passaged in calf-kidney cultures 100 times, was used. Virus was concentrated 100-fold from tissue culture fluid containing 2% bovine serum by precipitation with 20% methanol at 0°C, resuspension in 1:1 Tris-Hanks' solution containing 0.5% bovine lactalbumin hydrolyzate (BLH), dialysis to remove methanol, and clarification at 15,000 rpm for 15 minutes. Such concentrates still contained RNase(6). Virus from which RNase was to be quantitatively removed was propa-

* A preliminary report of this work was presented at the Primer Congreso Panamericano de Biol. y Patol. Exp., Caracas, Venezuela, Sept. 24-Oct. 1, 1960.

TABLE I. Effect of Heating FMDV Concentrates Containing Environmental Ribonuclease with and without Sodium Dodecylsulfate.

Exp. No.	Virus treatment: SDS conc.	Virus treatment: 5-min. heating	Virus treatment: RNase* added	Plating of treated virus (PFU/ml): Before phenol ext'ns	Plating of treated virus (PFU/ml): After phenol ext'ns
1 a	.1%	none	none	2.2×10^7	7.0×10^2
	"	"	.5 μg	2.2×10^7	
b	.1%	61°C	none	3.2×10^2	8.5×10^2
	"	"	.5 μg	0	
c	none	61°C	none	0	0
	"	"	.5 μg	0	
2 a	.1%	none	none	3.2×10^7	6.0×10^2
b	.1%	61°C	none	2.0×10^3	1.5×10^3
	"	"	.5 μg	0	
c	.1%	85°C	none	7.0×10^2	5.5×10^2
	"	"	.5 μg	0	

* Infectious RNA was identified by its sensitivity to pancreatic ribonuclease (Worthington Biochemical Corp.) at 25°C for 15 min.

gated in cultures devoid of serum. Cell-derived RNase was removed by methanol precipitation in the presence of 0.03% Armour bovine serum albumin, extractions with 1:1 BuOH-$CHCl_3$ mixtures, and differential centrifugation in which virus was precipitated at 40,000 rpm for 90 minutes before resuspension in 0.01M phosphate-0.11M NaCl buffer at pH 7.5. *Infectious RNA* was produced by a modification of the phenol procedure(5), and also, as this report will show, by heating FMDV in the presence of 0.1% SDS or by heating FMDV that has been freed of RNase. *Virus was heated* in water baths. Temperatures of 61° and 85°C were reached in about 2 minutes, and heating was continued for 5 minutes longer. The tubes were then cooled quickly by immersion in an ice bath. *RNA and FMDV plaque assays* have been described previously(5,7). Dilutions for assay were made in Hanks' fluid-0.5% BLH for both virus and RNA. All platings were made on calf-kidney cultures previously washed 3 times with Hanks' fluid-0.5% BLH to remove RNase. One-tenth ml of decimally-diluted test fractions were plated, the cultures incubated at 37°C for 90 minutes, agar nutrient medium applied, and plaque-forming units (PFU) counted after 3 days' incubation at 37°C.

Results. Direct comparisons were first made of heating RNase-contaminated FMDV concentrates with and without 0.1% SDS (Table I). The data show that FMDV yields infectious RNA after phenol extraction (Exp. 1a, 2a); virus heated in 0.1% SDS contains infectious RNA even before phenol treatment (Exp. 1b, 2b-c); phenol treatment does not produce additional RNA in heated virus preparations (Exp. 1b, 2b-c); and virus heated without detergent does not yield infectious RNA even if subsequently treated with phenol (Exp. 1c).

It next appeared important to examine the thermal behavior of FMDV which had been processed to eliminate RNase. Its degradation by heat is summarized in Table II.

The data in Table II show that purified FMDV concentrates produced from serum-free cultures contain little or no RNase activity before (Exp. 1a) or after (Exp. 2b-c) heating and yielded infectious RNA after heating at 61° or 85°C for 5 minutes in the absence of SDS (Exp. 1b, 2b-c). The heat-derived RNA was not increased in quantity by subsequent extractions with phenol (Exp. 1b).

Discussion. The data show that FMDV degrades upon heating at 61° or 85°C into infectious RNA provided that environmental RNase is either inactivated by hot SDS or is removed before heating. This negates the earlier hypothesis(4) that heating FMDV at 61° to 100°C in the presence of 0.1% SDS only inactivates an infection-initiating property of its protein coat, leaving a phenol-extractable infectious core. The present studies show that it was not the intact virus

TABLE II. Effect of Heating Ribonuclease-Free FMDV Concentrates in the Absence of Sodium Dodecylsulfate.

	Virus treatment*			Plating of treated virus (PFU/ml)	
Exp. No.	5-min. heating	Acidification	RNA added (PFU/ml)	Before Phenol ext'nst	After Phenol ext'ns
1 a	none	none	none	2.0×10^7	1.7×10^2
	"	pH 3.2	"	0	
	"	"	1.2×10^3	1.6×10^2	
b	none	none	none	4.0×10^7	2.7×10^2
	61°C	"	"	6.0×10^2	3.0×10^2
	85°C	"	"	1.3×10^2	6.0×10^1
2 a	none	none	none	4.5×10^7	
b	61°C	none	none	5.5×10^2	
	"	"	1.8×10^2	8.0×10^2	
c	85°C	none	none	5.0×10^1	
	"	"	1.8×10^2	3.0×10^2	

* Acidification at pH 3.2 for 3 min. and readjustment to pH 7.5 completely inactivates FMDV and RNA without inactivating RNase(5,8). RNA added was made by the phenol method.

† The entities produced from heated virus yielded no plaques after incubation with 0.1 μg pancreatic RNase at 25°C for 15 min.

which survived boiling at 100°C for 5 minutes but rather the thermally released RNA. The fact that FMDV-RNA prepared by the phenol method can withstand such boiling in 1 M NaCl without complete inactivation has been confirmed by Thomas and Leclerc (9). In addition, thermal disruption of the protein coat allowing digestion of the exposed RNA by environmental RNase tends to reveal the mechanism of the high-temperature, rapid inactivation of FMDV with its associated high energy of activation, approximately 120,600 calories per mole(10).

Heat alone degrades FMDV without the action of SDS as evidenced from the fact that FMDV without RNase present yielded infectious RNA. Moreover, FMDV is completely resistant to 2 to 5% SDS at 25° and 37°C for at least 48 and 0.5 hours, respectively(11). Also, hot SDS is a protein denaturant capable of inactivating RNase during extraction of sodium ribonucleate from tissues(12).

The thermal degradation of FMDV by heat is quite analogous to its splitting upon acidification at pH 5.0(3) in an RNase-free environment into infectious RNA and protein subunits. Consistent with Mussgay's finding is the wider pH-stability spectrum of infectious RNA, pH 4-11.5, than of FMDV containing RNase, pH 7-10(5).

Summary. Foot-and-mouth disease virus (FMDV) heated at 61° to 85°C yields infectious ribonucleic acid (RNA) when either sodium dodecylsulfate (SDS) is present to inactivate environmental ribonuclease (RNase) or when this enzyme is removed before heating the virus. Heat alone degrades FMDV into infectious RNA, and hot SDS only serves to inactivate RNase. Subsequent phenol treatments of heat-derived infectious RNA preparations did not significantly add to the amount of infectious RNA present.

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Isocarboxazid Metabolism in Rat Liver Homogenate. (26704)

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Isocarboxazid (Marplan®), 1-benzyl-2-(5-methyl-3-isoxazolylcarbonyl) hydrazine is a potent monoamine oxidase inhibitor(1) being used in treatment of various depressions(2,3, 4) and in management of angina pectoris(5, 6). In rats 75% of the radioactivity of an intraperitoneal dose of C^{14}-isocarboxazid labeled in the benzyl moiety was recovered in the urine in 24 hours(7). By reverse isotope dilution, virtually all radioactivity was found to reside in hippurate indicating that the benzyl moiety of isocarboxazid was oxidized to benzoate prior to formation and excretion of hippurate. The present study was designed to gain information concerning this biological conversion of the benzyl moiety to benzoate.

Methods. All incubations of liver homogenates were performed in a Dubnoff metabolic shaker at 38°C. Isocarboxazid was solubilized in an aqueous medium *via* Tween 80 as described by Meier *et al.*(8). For its determination, isocarboxazid was quantitatively extracted from an aliquot of the incubation medium by 2 volumes followed by 1 volume of chloroform. The combined chloroform extracts were backwashed with ½ volume of water and evaporated to dryness. The residue was then dissolved in acetone and the isocarboxazid content measured in a Beckman DU spectrophotometer by the method of Colarusso *et al.*(9). Synthesis of C^{14}-isocarboxazid with the methylene carbon of the benzyl moiety labeled has been described(7). Radioactivity was measured in a Nuclear of Chicago Corp. D-47 flow gas counter and corrected for self-absorption.

A solvent system of tertiary butanol:methanol:water (50:40:10) run by the ascending method on Whatman #1 paper pretreated with McIlvaine's phosphate-citrate buffer, pH 3.7, was employed for separation of labeled benzylhydrazine from other labeled components in extracts of the incubation medium. "Carrier" benzylhydrazine (technical grade containing 2% benzylamine) was added to the extract before it was applied to the paper and its position was determined by spraying the paper with a 1% solution of p-dimethylaminobenzaldehyde in 1N HCl. Radioactivity on the chromatograms was located by putting the papers through a Nuclear of Chicago Actigraph II chromatogram scanner.

Labeled benzylhydrazine was determined by a reverse isotope dilution technic involving the synthesis of benzylhydrazine monooxalate. For this purpose, an extract of the incubated material was prepared by adding an equal volume of absolute ethanol to an aliquot of incubation medium and filtering off the proteins after 15 minutes in an ice bath. Benzylhydrazine hydrochloride (300 mg as carrier) was dissolved in an aliquot of this extract which was then made alkaline with dilute NaOH solution, and following the addition of anhydrous sodium sulfate was extracted with 2 volumes of chloroform. The chloroform layer was concentrated to an oil after the complete removal of water by further addition of sodium sulfate. Oxalic acid (200 mg) in alcohol was added to precipitate benzylhydrazine monooxalate and this product was recrystallized from ethanol-water to constant specific activity. Furthermore, the final product caused no depression in the melting point of authentic benzylhydrazine monooxalate.

Results. 1. Enzymatic destruction of isocarboxazid. The aerobic disappearance of isocarboxazid in presence of rat liver homo-