masked by heat treatment previous to electrophoresis. 3) Both hemagglutinins migrated with the same speed slightly faster than rabbit hemoglobin when tested after an electrophoresis for 3 hours at pH 8.6. 4) Support for the presence of an inhibitor of hemagglutinin in the masked strain 8844 was gained. 5) It appeared that a hemagglutinin-inhibitor separation could be achieved when previously unmasked hemagglutinin was purified by electrophoresis.

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Demonstration of Immune Response to Foot-and-Mouth Disease Vaccine In Protection Test in Young Adult Mice. (25640)

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Since its development in 1938, aluminum hydroxide adsorbed foot-and-mouth disease virus vaccine(1,2) has gained wide acceptance throughout the world. Potency tests of this vaccine are generally conducted in cattle, the number used varying according to the quality of the test(3,4,5). The high cost of the test and the difficulty in infected countries of obtaining susceptible cattle generally make impracticable the potency testing of each batch of vaccine. It would be highly desirable, therefore, to find a laboratory animal that could be used as a substitute for cattle in such tests. Recent publications show that this problem is being investigated elsewhere. Uhlmann and Traub(6) refer to use of suckling mice to determine immune response of vaccinated adult mice and Skinner(7) refers to use of adult mice, the immunity of which is tested by antibody titration in suckling mice (as cited by Henderson,5). The experience that foot-and-mouth disease virus could be adapted to adult mice(8), led to investigation of the immune response of these animals to aluminum hydroxide adsorbed vaccines, assessing the immunity established by response to inoculation of the vaccinated mice with adapted strains of virus. Brief reference to this work has already been made(9) and in the present paper a description is given of experiments performed to evaluate the protection produced in young adult mice by foot-and-mouth disease vaccines prepared from virus of different sources and to determine if the protection afforded was type specific.

Materials and methods. A Rockefeller Institute strain of Swiss mice was used. Those referred to as suckling mice were 6 to 8 days old. The young adult mice were weaned mice, 3 to 6 weeks old, weighing 9 to 13 g at beginning of experiments. Older mice, up to 8 weeks and 21 g weight, were also used for certain preliminary experiments with type O vaccines. Aluminum hydroxide formalised vaccines were used that had been prepared in the State Veterinary Research Inst., Amsterdam, Holland; the Veterinary Research Inst., Maracay, Venezuela; the Animal Biol. Inst., Rio de Janeiro, Brazil or in Pan American Foot-and-Mouth Disease Center. The virus for vaccine preparation was obtained by the Frenkel method of culture(10), by inoculation of the tongue of cattle or by infection of

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TABLE I. Inoculation of Young Adult Mice with Foot-and-Mouth Disease Vaccine and Challenge of Immunity Produced with a Virus Strain Adapted to Young Adult Mice.

Vaccine		Mortality of mice inoculated with dilutions of type A adapted virus								
Type and batch	Dose, ml	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	Virus 50% end-point	Protection afforded in ID_{50}
A 4C*	.05	6/8†	5/8	7/8	4/8	3/7	0/7		$10^{-3.7}$	$10^{2.1}$
	.1	7/8	"	6/8	2/8	1/7	"		$10^{-3.3}$	$10^{2.5}$
	.2	5/8	"	5/8	3/8	2/8	2/8		"	"
	.4	3/8	0/8	2/8	3/8	1/8	1/8		$10^{-1.5}$	$10^{4.3}$
	Nil	8/8	8/8	8/8	7/8	7/8	4/8	0/8	$10^{-5.8}$	

10^{-1}, 10^{-2}, 10^{-3} dilutions of virus infected all mice in control groups, data from corresponding vaccinated groups are used to calculate 50% protection dose of vaccine, thus

Dose of vaccine	Log dose of vaccine	Protected	Not protected
.4	1.6	19	5
.2	1.3	9	15
.1	1.0	6	18
.05	2.7	6	18

By method of Reed and Muench 50% Protection Dose (PD_{50}) is calculated to be 0.2 ml.

* C—monolayer culture virus. † No. dead/No. inoculated.

monolayers of bovine kidney cells (see Tables). Mice were vaccinated by the subcutaneous route in groups of 50 for each dose. An equal group of unvaccinated mice was set apart in each experiment for later use as controls. Twenty-one days later, 6 dilutions, 10^{-1} to 10^{-6}, of the challenge virus were inoculated intraperitoneally, 8 mice of the group of 50 being used for each dilution. Control groups received dilutions from 10^{-1} or 10^{-2} to 10^{-7}. The challenge virus was also titrated in suckling mice as a control of the infectivity of the preparation. These adapted viruses were from the 37th to the 49th passage of a type Vallée O strain, from the 89th to the 92nd passage of a Vallée A strain and from the 28th to the 34th passage of a type Waldmann C strain of foot-and-mouth disease virus in young adult mice. Adaptation was achieved by use of mice previously treated with 5 mg cortisone acetate for the earlier passages. The O and A strains were inoculated in doses of 0.25 ml and the C strain, 0.5 ml except for titration in suckling mice when for all strains the dose was 0.05 ml. The mice were observed for a period of 15 days, titration end-points were calculated by the method of Reed and Muench(11) and the difference between end-points obtained in vaccinated and in control mice provided an indication of degree of protection produced. The data of these titration results were also used to calculate the 50% protection dose (PD_{50}) of the vaccines by the accumulation method of Reed and Muench(11). This was done by noting the mice "protected" and "not protected" in the vaccinated groups inoculated with the dilutions of virus that had infected all the control mice. The result of a typical experiment is given in detail in Table I as an example of the methods employed and to facilitate interpretation of the subsequent tables.

Results. Preliminary experiments were performed with type O vaccines at a time when only the type O strain of virus had been adapted to adult mice. Two vaccines from the Animal Biol. Inst., Rio de Janeiro, and 2 from the State Vet. Research Inst., Amsterdam, were tested in a total of 7 experiments using mice from 5 to 8 weeks old. The results of these tests showed that foot-and-mouth disease vaccine, inoculated by the subcutaneous route in mice, conferred protection against the challenge virus and that this protection increased with the dose of vaccine. With the largest doses tested of these vaccines, 0.5 ml, virus titration end-points were lowered by 4 log units.

Subsequently, after type A and type C viruses were adapted to young adult mice, the investigation was extended to cover monovalent vaccines of the 3 virus types occurring in South America. The specificity was also in-

TABLE II. Inoculation of Young Adult Mice with Foot-and-Mouth Disease Vaccine and Challenge of Immunity Produced with Virus Strains Adapted to Young Adult Mice. Summary of results.

Vaccine		Titer* of challenge virus in control mice	Protection afforded in log ID_{50} to mice inoculated with vaccine doses (ml)				PD_{50} calculated as in Table I, ml
Type and batch	Log ID_{50} per ml		.05	.1	.2	.4	
O 1F†	5.3	5.0	Nil	Nil	Nil	1.5	.38
2F	7.2	4.6	"	"	"	2.1	.29
3F	7.6	4.5	1.7	.9	2.8	2.5	.27
VE‡		4.5	1.4	2.0	3.5	>3.5	.13
A 2F	6.5	4.6		2.0	3.2	3.4	.16
3F	6.5	6.6	3.9	4.2	3.7	5.0	.17
4F	6.3	6.1	2.9	2.9	3.9	3.1	.14
5F	7.0	5.3	2.5	3.3	2.7	4.5	.10
6F	7.1	5.3	1.8	.7	1.9	2.7	.34
VE		4.5	1.9	2.5	3.2	3.3	.14
C 1F	7.1	5.2	3.9	3.4	>4.2	>4.2	.13
3F	7.5	4.0	2.6	2.6	3.0	>3.0	.07
10§	7.4	4.0	>3.0	>3.0	>3.0	"	<.05

* Negative powers of 10. † F—Frenkel culture virus. ‡ VE—Tongue epithelium virus, Venezuelan vaccine. § C—Monolayer culture virus.

vestigated of the immunity conferred by a monovalent vaccine. Experiments were performed with monovalent vaccines of types O, A and C by vaccinating groups of young adult mice with doses of 0.05 ml, 0.1 ml, 0.2 ml and 0.4 ml. Except for 2 vaccines received from the Vet. Research Inst., Maracay, all the remainder were prepared in this Center's Laboratory for other experimental purposes and the number of ID_{50} of virus contained in each ml of vaccine was known (Table II). These data were obtained by titration of virus suspensions in suckling mice, except for vaccine A 4C (Table I), the titration for which was done in bovine kidney monolayer tubes. Table II summarizes results obtained with types O, A and C vaccines against virus of the homologous immunological type.

To check that protection induced by a monovalent vaccine was specific only to the homologous type of virus, 5 experiments were carried out with vaccines of different types. Only one dose of vaccine was used but 3 groups of 50 mice were inoculated, one group for each type of challenge virus. The results obtained are shown in Table III, and indicate that the protection induced by a monovalent vaccine is type specific.

In Table III the results have been included of titration of the challenge virus in suckling mice. These results are similar to those obtained in corresponding titrations performed in connection with the experiments recorded in Table II. Although the strains of virus adapted to adult mice regularly produce infection in this host over a sufficient range of dilutions to make their use practicable in this type of experiment, infectivity of the strains is still much greater for suckling mice.

Discussion. The present experiments have shown that foot-and-mouth disease vaccines induce immunity in young adult mice, this immunity being checked by direct inoculation of vaccinated mice with virus strains adapted to adult mice. There is no doubt that this protection test is more practicable than those technics(6,7) proposed in which the additional use of suckling mice is required. The results obtained by comparing the differences in virus titration end-points in vaccinated and in control mice showed considerable irregularity in correlation between dose of vaccine and grade of immunity although there was a definite trend towards larger doses giving better immunity. Application of accumulation technic of Reed and Muench in assessing the 50% protection dose had the effect of removing much of this irregularity of response. The experiments were not originally designed for interpretation of the results in this qualitative manner. Further investigation of this point will be necessary before deciding whether potency of the vaccine should be expressed in terms of ID_{50} protection in the manner of the Habel test of rabies vaccine(12) or in terms of the dose of vaccine protecting 50% of the

TABLE III. Demonstration of Immunological Type Specificity of Monovalent Foot-and-Mouth Disease Vaccines in Young Adult Mice.

Vaccine			Challenge virus				
				50% end-point*			
Type and batch	Log ID_{50} per ml	Dose, ml	Type	Vaccinated mice	Control mice	Protection afforded in log ID_{50}	Titer* of challenge virus in suckling mice
O V187E†		.4	O	<1.0	4.5	>3.5	7.6
			A	4.3	4.7	.4	7.5
			C	4.1	"	.6	6.6
A 3F‡	6.5	"	O	5.6	5.4	Nil	7.5
			A	2.1	5.1	3.0	7.8
			C	4.0	4.2	.2	6.4
A 8F		"	O	5.5	5.3	Nil	7.5
			A	2.6	5.6	3.0	8.0
			C	5.1	5.0	Nil	8.2
C 1F	6.8	.2	O	5.4	5.8	.4	7.5
			A	4.3	4.8	.5	7.6
			C	<1.0	5.1	>4.1	7.0
C 2F	6.6	"	O	5.6	5.6	Nil	7.3
			A	5.3	5.5	.2	7.2
			C	1.8	4.3	2.5	7.5

* Negative powers of 10.
† E—Tongue epithelium virus (Venezuelan vaccine).
‡ F—Frenkel culture virus.

vaccinated mice.

The results of testing vaccines in mice must also be correlated with the results of testing the same vaccines in cattle but the experience gained in the experiments described is sufficiently promising to suggest that further work may show that mice can be used as substitutes for cattle in testing the potency of foot-and-mouth disease vaccines.

Summary. Foot-and-mouth disease vaccines inoculated by the subcutaneous route in young adult mice conferred protection against challenge by inoculation of virus strains adapted to young adult mice. These mice were 3 to 6 weeks old weighing 9 to 13 g at time of vaccination.

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