Antibody Response of Adult Mice to Virus of Foot-and-Mouth Disease. (24913)

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An immune response to foot-and-mouth disease virus (FMDV) in adult mice was reported by Skinner *et al.*(1), who observed that offspring of mother mice inoculated during pregnancy were resistant to virus challenge. Although suckling mice are susceptible, adult mice are usually considered resistant. In studies, normal adult mice were unaffected clinically after inoculation with FMDV unless some process of virus selection or host treatment was utilized(2-4). Because of advantages of adult mice, the present experiments study neutralizing antibody response of such animals to FMDV to learn when antibody appears and when highest antibody level is reached.

Materials and methods. Adult female Rockefeller H strain mice, 120 days old at time of inoculation, were used for antibody production. Litters of 10 suckling mice of same strain, 5-10 days of age, were used in neutralization tests for detection of antibody produced in adult mice(1). Foot-and-mouth disease *virus*, type A, strain 119, produced in Roux flask cultures of bovine kidney cells(5) was the stock virus preparation. Nutrient fluid from cultures of infected cells was centrifuged in Spinco Model L centrifuge (No. 30 rotor) at 10,000 rpm for 30 minutes to remove cellular debris. The supernatant fluid containing the virus was stored in sealed ampoules at -40°C. This material was considered as undiluted stock virus. Dilutions of stock virus were prepared in tryptose phosphate broth (pH 7.4). Two experiments were performed, the first with 40 and the second with 38 adult mice. Mice were inoculated intraperitoneally (IP) with 0.1 ml of undiluted stock FMDV. At intervals of approximately 24 hours after inoculation in first experiment, blood was obtained and pooled from 2 randomly selected mice by incision in axillary region after ether anesthesia. In the second experiment, blood was obtained at intervals of approximately 24 hours for first 2 weeks and 7-day intervals thereafter. Serums collected from pooled blood were placed in rubber-capped vaccine vials and stored at -40°C until all serums from a given experiment were available for testing at the same time. Antibody content of adult mouse serums was studied by neutralization tests in suckling mice, 5-10 days of age, using dilutions of FMDV mixed with constant amount of serum previously heated at 56°C for 30 minutes. Beginning with 1:5 dilution of stock virus, with a titer of approximately $10^{6.6}$ LD_{50}/ml in suckling mice, serial 10-fold dilutions were prepared; and to 0.5 ml of each dilution, 0.5 ml of serum was added. Final virus dilutions were 10^{-1} through 10^{-7}. To induce death in all mice in at least the 10^{-1} dilution so that calculations of neutralizing indices could be made, serums collected to approximately 10 days postinoculation were diluted initially 1:20. Serums collected subsequently were diluted initially 1:1000. Allowance for these dilution factors was made in calculations for various serums by expressing LD_{50} neutralization indices on the basis of ml of undiluted serum. Virus-test serum mixtures and controls consisting of virus-normal mouse serum and virus broth mixtures were incubated in water bath at 37°C for 30 minutes, after which each mixture was inoculated into a litter of 10 suckling mice. Each mouse was inoculated IP with 0.05 ml. The mice were observed daily for 10 days after inoculation. Viral end points were determined by method of Reed and Muench(6).

Results of neutralization tests are summarized in Tables I and II. In Exp. 1, observations were made periodically from 3rd through 32nd day after inoculation, and in Exp. 2,

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from 1st through 84th day after inoculation. Serums collected on first and second day after inoculation had no demonstrable antibody. On 3rd day after inoculation, however, Exp. 1 sample had a virus-neutralizing index of 2.48 logs/ml of undiluted serum, and Exp. 2 sample had a virus-neutralizing index of 3.61 logs/ml of undiluted serum. A gradual increase in neutralization indices of serums in each experiment occurred thereafter until they leveled off at between $10^{6.0}$ and $10^{7.0}$ LD_{50}/ml, approximately 20 days after mice had been inoculated.

Discussion. Antibody response of adult mice inoculated with FMDV indicated that after IP inoculation of 0.1 ml of tissue culture fluid containing approximately $10^{6.6}$ suckling mouse LD_{50} of FMD/ml, adult mice produced neutralizing antibody detectable within 3 days after inoculation. In general, the antibody reached a maximum between 20 and 30 days after inoculation and remained at high level throughout the 84 days of Exp. 2. An exception can be found in neutralizing indices of serums collected on 56th and 63rd days of Exp. 2 (Table II), which were greater than those of serums collected on 21st and 28th days. It was not our purpose to determine persistence of antibody in adult mice. The experiments indicate, however, that a significant level of neutralizing antibody persists for at least 3 months after inoculation.

TABLE I. Neutralizing Capacities of Serums Collected at Varying Intervals from Adult Mice Inoculated with Foot-and-Mouth Disease Virus.

Exp. I

Serum sample D.P.I.*	LD_{50} neutral. index uncorrected for ser. dil.	Final serum dilution (Log, base 10)	LD_{50} neutral. index/ml of undil. ser.
0	.15†	−1.6	
3	.88	"	2.48
4	2.14	"	3.74
5	2.60	"	4.20
6	1.10	−3.3	4.40
7	1.93	"	5.23
10	1.31	"	4.61
11	1.79	"	5.09
12	2.50	"	5.80
14	1.87	"	5.17
18	2.66	"	5.96
20	3.41	"	6.71
24	3.26	"	6.56
26	2.79	"	6.09
31	2.94	"	6.24
32	2.00	"	5.30

* D.P.I. = Days postinoculation.
† Not considered as evidence of neutralization.

TABLE II. Neutralizing Capacities of Serums Collected at Varying Intervals from Adult Mice Inoculated with Foot-and-Mouth Disease Virus.

Exp. II

Serum sample D.P.I.*	LD_{50} neutral. index uncorrected for ser. dil.	Final serum dilution (Log, base 10)	LD_{50} neutral. index/ml of undil. ser.
0	.09†	−1.6	
1	.00†	"	
2	.30†	"	
3	2.01	"	3.61
4	2.90	"	4.50
8	1.88	"	3.48
10	1.09	−3.3	4.39
11	.90	"	4.20
14	1.44	"	4.74
21	2.71	"	6.01
28	2.85	"	6.15
35	2.24	"	5.54
42	2.33	"	5.56
56	2.92	"	6.22
63	3.07	"	6.37
70	.64	"	3.94
78	1.79	"	5.09
84	1.49	"	4.74

* D.P.I. = Days postinoculation.
† Not considered as evidence of neutralization.

Seventy-eight adult mice were inoculated with FMDV. In addition, 24 adults were inoculated in a preliminary experiment not included. In this group of 102 mice plus approximately 50 adult mice used as controls in other experiments now in progress, no normal adult mouse died because of infection with FMDV or revealed any other symptoms of infection. This lack of signs of illness in adult mice is in accordance with previous observations(2-5).

Summary. Normal adult female mice were inoculated with the virus of foot-and-mouth disease, and serums obtained from these animals at intervals up to 3 months after inoculation were tested for their neutralizing capacities. Neutralizing antibody was detected as early as 3 days after inoculation and increased gradually until a maximum was reached at 20-30 days. The antibody persisted at a high level throughout remainder of 84-day period.

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Direct Evidence for Fatty Acid Mobilization in Response to Growth Hormone Administration in Rat.* (24914)

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It has long been known that anterior pituitary gland extracts and purified growth hormone preparations, when administered to experimental animals, increase the concentration of lipids in plasma and the liver while decreasing amount of total body fat (see 1 and 2 for review). More recently, it has been demonstrated that small doses of growth hormone cause a rapid increase in the plasma nonesterified fatty acid (NEFA) concentration in man(3), rhesus monkey(4) and rat (5). These effects of growth hormone on levels of circulating lipid have been interpreted in terms of an increase in mobilization of lipids from adipose tissue but direct evidence for such an interpretation has been lacking. The following experiments were performed to establish the effect of prior growth hormone administration on release of NEFA from isolated adipose tissue *in vitro*.

Material and methods. Male rats of the Charles River strain weighing between 100 and 150 g were used. Hypophysectomies were performed 4 weeks prior to experiment. All animals were fasted 24 hours before autopsy. In the "acute" experiments a single intraperitoneal injection of bovine growth hormone† (3 mg) was administered three hours before sacrifice. In the "chronic" experiments bovine growth hormone (3 mg per rat per day) was given intraperitoneally for 3 days and the rats sacrificed on the day following last injection. Control animals were injected with equal volumes of normal saline. All animals were anesthetized with 30 mg sodium pentobarbital given intraperitoneally. The epididymal fat bodies were removed and incubated individually (2 for each animal) in a system previously described(6). NEFA released into the incubating medium was determined by the method of Dole(7) and expressed as μeq. NEFA released per g wet weight of tissue per hour. Blood glucose concentrations were determined by the method of Nelson(8) and plasma NEFA concentrations by the method of Dole(7).

Results. Hypophysectomy reduced release of NEFA from adipose tissue when compared with normal control animals fasted for the same length of time (Table I). Administration of a single 3 mg dose of growth hormone to hypophysectomized animals 3 hours before sacrifice significantly ($P<.01$) increased NEFA release from adipose tissue *in vitro* (Table I). No difference in blood glucose

TABLE I. Effect of Hypophysectomy and Growth Hormone Treatment on NEFA Release by Adipose Tissue. Ten animals/series; 2 observations/animal.

Rats	NEFA release, μeq/g/hr
Normal control	5.79 ± .44‡
Hypox. + saline	.90 ± .16
" + G.H. (acute)*	2.30 ± .37
" + " (chronic)†	3.40 ± .66

* 3 mg/rat 3 hr before sacrifice.
† 3 mg/rat/day for 3 days.
‡ Mean ± S.E.

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