Serologic Studies of Theiler's Mouse Encephalomyelitis Virus. (22487)

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Identification of TO(1) strains of mouse encephalomyelitis has generally been based on the signs of disease in mice and on histopathologic evidence, since neutralization tests in mice injected by the intracerebral route have been unsatisfactory. Theiler(2,3), by cross immunity tests, demonstrated an immunologic relationship between his TO viruses and the higher titered GD VII and FA strains. Strain variation among the TO viruses is suggested by differences in localization of paralysis in mice(4) and in their adaptation to growth in eggs and tissue culture (5).

This report presents a summary of neutralization tests using Theiler-free suckling mice, susceptible to infection by peripheral routes, and hyperimmune hamster sera produced with different Theiler strains. They demonstrate the group relationship and, in addition, serologic differences.

Methods and materials. Virus strains. The TO strains Nos. 4727, 4771, and 47218 were isolated in this laboratory from the intestines of albino mice(4). Strain No. 52102 was received from the American Type Culture Collection as a representative strain of TO virus. Its relationship to our strain No. 4727 has been shown(5) by tests in which mice injected with No. 4727 egg or tissue culture virus resisted challenge with No. 52102. GD VII Strain No. 51200 was received from Dr. Peter Olitsky. Virus stocks were stored in the dry ice chest as 20% suspensions of mouse brain and cords, or of chick embryo legs and wings. *Antisera* to strains Nos. 4727 and 52102 were prepared in hamsters by intraperitoneal injection of egg passage virus. An initial dose of 0.5 ml and 2 subsequent doses of 1 ml were given at from 3- to 4-day intervals. For certain lots of sera a preliminary intracerebral dose was given; to others a booster dose was given one month after third injection. Animals were bled 10-18 days after final dose. Antiserum to GD VII virus was prepared by Dean using strain received from Dr. Gilbert Dalldorf. Hamsters were immunized with 6 graduated doses of 10% suspension of infected mouse brains (0.125 to 1.0 ml) given over a period of 3 weeks. *In neutralization tests* a constant virus dose was combined with 5-fold serial dilutions of serum. Dilutions were made in 0.85% saline solution containing 10% beef infusion broth. Mixtures of virus and serum were held one hour at room temperature before injecting intraperitoneally in 0.05-ml doses into suckling mice from a Theiler-free colony. Usually the mice were from 3-6 days old; occasionally it was necessary to include those slightly younger or older. Viruses Nos. 4727 and 52102 were tested both as suspension of mouse brain and cord and of chick embryo legs and wings. The usual dosage represented approximately 100 mouse ID_{50}. Other amounts were also used. The effect on the serum titer is shown in the tables. Animals were observed daily for paralysis for a period of three weeks. Neutralization was considered definite if 60%, partial if 45-60% of the mice were protected.

Results. Until recently, because of the low peripheral infectivity of the Theiler viruses for standard laboratory mice, and the poor results obtained by intracerebral neutralization tests, identification of Theiler (TO) viruses by serologic methods has been impracticable and has depended on histopathology and signs of disease in mice. The complement-fixation test has been used for the GD VII and FA strains(6,7) and the hemagglutination test for GD VII(8). The work of von Magnus (9,10), who developed a Theiler-free mouse colony and established that such mice were susceptible to infection with TO virus by several peripheral routes, permitted a new approach to a serologic investigation of these low titered viruses. She used Theiler-free mice to evaluate hyperimmune cotton rat sera prepared with a Theiler TO strain and also presented data from other tests carried out by

TABLE I. Neutralization of Theiler Viruses by Homologous and Heterologous Hyperimmune Hamster Sera.

Sera		Virus suspension*							
		No. 4727			No. 4771	No. 47218	No. 52102		GD VII No. 51200
Strain	Dilution	E	E(x)	M	M	M	M	E	M
TO No. 4727	5	3	3	3	3	3	3	3	3
	25	3	3	3	3	3	3	3	3
	125	3	3	2	3	0	1	3	3
	625	2	0	0	1	0	0	0	0
	3125	0	—	—	—	—	0	—	—
TO No. 52102	5	3	1	3	3	3	3	3	1
	25	3	0	1	3	2	3	3	1
	125	1	0	0	2	0	3	3	1
	625	0	0	0	0	0	2	2	0
	3125	—	—	—	—	—	0	0	
GD VII No. 51200	5	3	1	3	3	3	3	3	3
	25	3	3	3	3	1	3	3	3
	125	3	0	3	3	0	1	1	3
	625	3	0	0	0	0	0	0	1
	3125	0	—	—	—	—	—	0	2†

* Suspension used in dilutions which represented approximately 100 mouse ID_{50} except No. 4727 E(x). This suspension = 1000 mouse ID_{50}.

† With approximately 1/10 the dose of virus, this serum protected at a dilution of 15,625.

E = Egg passage virus, suspension of embryo legs and wings. M = Mouse passage virus, suspension of brains and cords.

3 = 60-100% infected mice survived for 21 days.
2 = 45- 60% *Idem*
1 = 30- 45% "
0 = 0- 30% "
— = Not done.

the intracerebral technic, stressing the superiority of the peripheral route for neutralization tests with neurotropic viruses. Dean(11), in this laboratory, also developed a colony of Theiler-free mice and showed that by whatever route tested they were more susceptible than latently infected animals to infection with the TO strain. They were also more susceptible to GD VII and FA viruses.

The availability of Theiler-free suckling mice permitted us to evaluate our antisera by the intraperitoneal neutralization technic. Table I incorporates the results of tests with three immune sera and five virus strains. In other experiments these strains were shown not to be neutralized by antisera for MM virus, Type II poliovirus strain Y-SK, or Coxsackie Group A, Type 1. None of the Theiler antisera had any protective effect against MM virus. Serum from control uninoculated hamsters had no neutralizing activity. The relationship between serum titer and the dosage of virus is indicated in Table I where 2 dosage levels of No. 4727 egg-passage virus are shown; and in Table II in which the results with 3 antisera on 2 different doses of strain No. 4771 have been summarized.

The TO strains isolated in this laboratory, the ATCC strain, and GD VII virus gave reciprocal cross reactions. However, the titer of each serum was higher for its homologous strain than for any other. This specificity is particularly noticeable in comparing the antisera for No. 52102 and GD VII. The latter had a high titer against its homologous strain and considerable neutralizing activity against all the strains. Antiserum to No. 52102 was

TABLE II. Influence of Virus Dose on Titer of Antisera.

Serum dilution	Antisera					
	No. 4727		No. 52102		GD VII No. 51200	
	100	1000*	100	1000	100	1000
5	3	3	3	3	3	3
25	3	3	3	2	3	3
125	3	1	2	0	3	0
625	1	0	0	0	0	0

* 100 and 1000 = No. of mouse ID_{50} of virus No. 4771, suspension of mouse brains and cords.

in general less reactive; but against virus No. 52102 it was definitely more active than was the GD VII antiserum.

All the antisera had a lower titer against strain No. 47218. This strain differs from the others in that it did not adapt to egg or tissue culture while strains Nos. 4727, 4771, and 52102 readily did so.

Summary. Satisfactory antisera to various Theiler strains were prepared in hamsters. Using Theiler-free suckling mice in neutralization tests, reciprocal cross reactions between GD VII and TO strains were demonstrated. Serologic differences within the TO group were also noted, the titer of a serum being in each case higher for the homologous than the heterologous strains.

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Occurrence of Melanocyte Stimulating Hormone (MSH) in a Transplantable Pituitary Tumor. (22488)

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Transplantable adrenocorticotropin (ACTH) secreting tumors have been produced in LAF_1 mice by Furth *et al.*(1,2,3) by ionizing irradiation. There was some indication that they released ACTH. To date, there has been no report on the ACTH content of the tumors. In the case of thyroid stimulating hormone (TSH) secreting tumors (3) an extremely high content of TSH has been reported. In view of many reports on the relationship of melanocyte stimulating hormone (MSH) and ACTH, it was of interest to assay the ACTH tumors for MSH, ACTH and vasopressin. It was found that the concentration of MSH was extremely high while the ACTH and vasopressin were relatively low.

Methods. Tumor Transplants. Several LAF_1 mice bearing Strain 2 adrenotropic tumor were sacrificed, the tumors were removed aseptically and homogenized with 5 volumes of isotonic saline. One-tenth ml of this suspension was injected intramuscularly into male LAF_1 mice weighing 22 to 28 g. Three weeks after implantation they were bilaterally adrenalectomized and injected subcutaneously with one mg each of desoxycorticosterone acetate (DOCA). The tumors were palpable about 8 weeks following implantation. Once palpable, they reached the size of 1-6 g within a few weeks. All the reported experiments were carried out with tumors which had been transplanted twice following receipt in our laboratory. *Assays.* ACTH assays were conducted by the intravenous technic described by Munson, Barry and Koch(4). Vasopressin determinations were by the standard U.S.P. XV method. All samples for ACTH and vasopressin were prepared by homogenization with 0.1N hydrochloric acid followed by dilution with isotonic saline. MSH determinations were made using the *in vitro* method of Shizume and Lerner(5). Samples were extracted with 10