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Certain Properties of Theiler's Virus, Especially in Relation to Its Use as Model for Poliomyelitis.

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Theiler[1] and the writer[2] have stated that in view of the close resemblance between the properties of Theiler's virus and that of human poliomyelitis, and the similarity of certain clinical, pathological, and epidemiological features of Theiler's disease (spontaneous mouse encephalomyelitis; mouse poliomyelitis) with human poliomyelitis, the murine virus and malady might be employed as a model for problems of the human disease. This is especially true since the former is a natural disease, arising spontaneously in mice, and not an experimentally induced infection, as is the case when the virus of human poliomyelitis is transmitted to monkeys. Theiler based his opinion on the reactions of the first 5 viruses (later called "Strain 1") which he isolated first in 1933,[3] from the central nervous system of a mouse with spontaneous encephalomyelitis. These viruses he designated as "strains of low virulence"; hereafter in this paper they will be referred to as TO virus (*i.e.*, Theiler's original virus). The writer,[4] on the other hand, affirmed the similarity between the murine and human viruses, and the diseases induced by them, from his experiences chiefly with TO virus isolated in 1939 from the intestinal contents of normal stock mice.[4]

Theiler and Gard[5] later recovered by accident two highly virulent "strains," the GD VII and the FA, from mice that became ill during experiments with yellow fever virus. They were characterized by producing shorter incubation periods and higher mortality rates; they showed greater invasiveness by peripheral routes (intranasal; intraäbdominal) of inoculation but were immunologically related to, though not identical with each other and the earlier TO virus. Although the GD VII, FA, and TO viruses had certain features in common, *e.g.*, size of their active particles, the reactions of the first two in animals were so different from the TO virus as to raise the question of whether they could serve as models for the study of human poliomyelitis.

The present paper will concern itself with (a) certain properties of Theiler's virus to supplement others already reported and thus to enhance its value as a model for research on poliomyelitis problems; (b) the selection of the strain that represents best this function of the virus.

Susceptibility of Mouse Strains. Webster,[6] by inbreeding from a hybrid stock of albino mice, developed a W-Swiss strain particularly susceptible to louping-ill and St. Louis encephalitis viruses, and more recently Casals and Schneider[7] found this stock highly responsive to Russian tick-borne encephalitis. The Rockefeller Institute (R.I.) strain of mice, on the other hand, are resistant to certain neurotropic viruses to a degree which prevents their experimental use. Although other viruses (rabies,[6] lymphocytic choriomeningitis,[8] and vesicular stomatitis[9]) have been shown to be uniformly infective in both varieties of mice, investigators regard the Swiss mouse as especially susceptible to neurotropic viruses in general; at least, no instance has been hitherto noted in which a response to a virus was

* With the technical assistance of Betty Lee Wilde and Mary Halsey.

1 Theiler, M., *Medicine*, 1941, **20**, 443.

2 Olitsky, P. K., *J. Exp. Med.*, 1940, **72**, 113.

3 Theiler, M., *J. Exp. Med.*, 1937, **65**, 705; *Science*, 1934, **80**, 122.

4 Olitsky, P. K., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 434.

5 Theiler, M., and Gard, S., *J. Exp. Med.*, 1940, **72**, 49.

6 Webster, L. T., *J. Exp. Med.*, 1937, **65**, 261.

7 Casals, J., and Schneider, H. A., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 201.

8 Casals, J., unpublished data.

9 Olitsky, P. K., Sabin, A. B., and Cox, H. R., *J. Exp. Med.*, 1936, **64**, 723.

TABLE I.
Comparison of Response of One-Month-Old Swiss and Rockefeller Institute Strains of Mice to Injection of Theiler's Virus.

Strain of mouse	Virus	Dose	Route	No. used	Results No. dead or with signs	Survivors
R.I.	GD-VII	10^{-1}	iper.	25	1 D-3;* 5 D-4; 4 D-5; 2 Pr-5;† 1 Ci-6; 1 D-7; 2 D-8; 1 D-15; 1 D-16	7 normal‡
W-Swiss	"	10^{-1}	"	25	2 D-8; 1 D-8; 1 D-9	21 normal
R.I.	"	10^{-2}	icer.	15	1 Co-1; 5 D-2; 1 Pr-2; 2 Co-2; 6 W-2	0
W-Swiss	"	10^{-2}	"	15	1 D-1; 3 D-2; 1 Pr-2; 2 Co-2; 8 W-2	0
R.I.	"	10^{-6}	"	15	3 D-3; 1 LAP-3; 4 D-4; 2 Co-4; 5 W-4	0
W-Swiss	"	10^{-6}	"	15	2 D-3; 1 AP-3; 1 LAP-3; 1 RAP-3; 1 W-3; 2 D-4; 1 Co-4; 1 W-4; 2 D-5; 1 D-6	2 normal
R.I.	TO	10^{-1}	iper.	12	1 RPP	1 paralyzed; 11 normal
W-Swiss	"	10^{-1}	"	12		12 normal
R.I.	"	10^{-2}	icer.	20	2 APP-6 (D8);§ 2 D-7; 1 PP-7 (D8); 2 AP-7 (D9); 1 AP-7 (D10); 2 APP-7 (D13); 1 AP-8 (D9); 1 PP-9 (D13); 1 AP-10 (D11); 1 AP-10 (D22); 2 AP-10; 1 D-13; 1 AP-13 (D15); 2 APP-13	4 paralyzed
W-Swiss	"	10^{-2}	"	20	1 AP-6 (D13); 3 AP-8 (D13-14); 1 PP-9 (D15); 1 AP-10 (D16); 2 APP-11 (D17-18); 2 APP-11; 6 APP-13; 2 PP-14	10 paralyzed; 2 normal

* 1 D-3—1 mouse found dead on 3rd day after inoculation.
† 2 Pr-5 = 2 mice prostrate on 5th day; Ci = circling; Co = convulsive; W = generalized weakness.
‡ Normal = not at all affected.
§ 2 APP-6 (D8) = 2 mice, anterior limbs partially paralyzed on 6th day, died on 8th day. 1 PP = 1 mouse, posterior limb paralysis; AP, anterior limb paralysis; L = left and R = right. In the GD VII strain, signs were preagonal, the mice dying in a few hours after their first appearance.

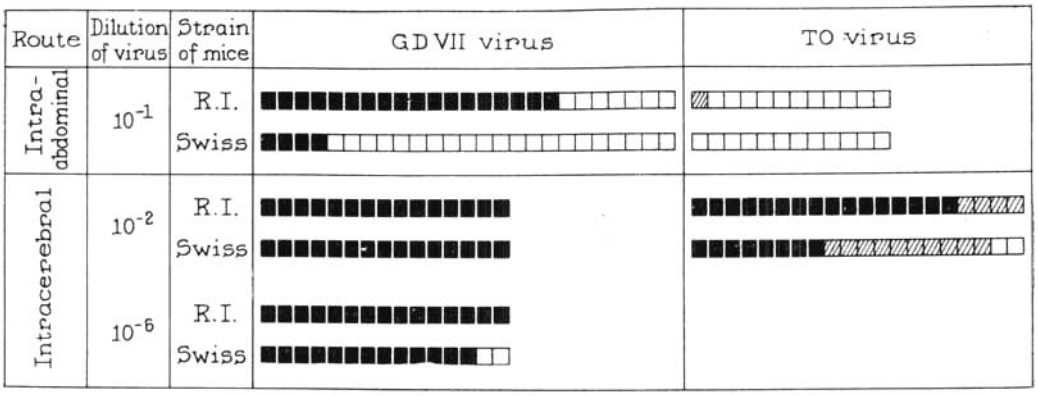
greater in the R.I. stocks of mice than in the Swiss. How this applies to the strains of Theiler's virus will be revealed from a typical experiment shown in Table I which presents details, and its graph.

It is clear that both the GD VII and TO active agents are more "virulent" in the Institute than in the Swiss variety of albino mice, as manifested by higher incidence and mortality rates and smaller number of normal or paralyzed survivors among the Institute stock. The effects follow central (CNS) introduction of TO and peripheral (intraäbdominal) injection of GD VII virus.

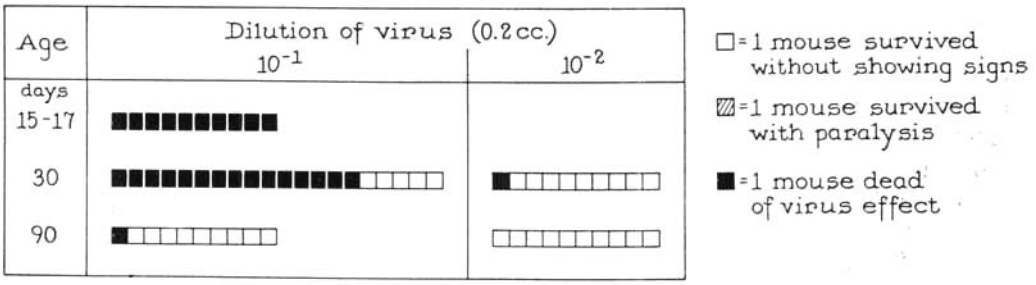
The finding that the W-Swiss mice are less susceptible to Theiler's virus than the R.I. is unexpected since it is a reversal of the hitherto accepted greater responsiveness of the Swiss mice to certain neurotropic viruses in general. The question now is, What relationship exists between selective susceptibility and the virus carrier state of normal animals?[4] Both normal stocks have been found to harbor TO Theiler's virus in the intestinal tract.[4,5] In tests performed concomitantly with the present series, the R.I. and W-Swiss mice, kept in separate animal houses, were again positive. The quantitative difference in individual incidence of carrier state and in amount of virus harbored by each carrier between the two strains of mice is yet to be determined. Care should therefore be taken to avoid discrepancies in results by selecting only single stocks of mice and not mixing strains in the same test.

The results were observed in mice of the same age,† one month old, for, as will be discussed in the next section, age is a factor determining natural susceptibility to Theiler's virus.

† Age of animals is commonly used in comparative tests, such as the present ones, rather than weight. The weight of the W-Swiss mice of this study averaged 11.7 g and of the R.I., 17.4 g at one month of age. At all comparable ages the weight of the former is considerably below that of the R.I. mice; the latter are never overtaken in weight by the W-Swiss.



Graph of Table I



Graph of Table II

FIG. 1.

TABLE II.
Influence of Age on Intraäbdominal Inoculation of GD VII Strain of Theiler's Virus in R. I. Stock Mice.

Age (days)	Dose (0.2 cc)	No. used	Results No. dead or with signs	Survivors
90	10^{-1}	10	1 LAP-7 (D7)	9 normal
90	10^{-2}	10	None reacted	10 "
30	10^{-1}	20	2 D-4; 2 Pr-4 (D4-5); 4 D-5; 1 Ci-5 (D 5); 1 D-6; 1 D-7; 1 LPP-7 (D8); 2 D-8; 1 D-9	5 "
30	10^{-2}	10	1 D-7	9 "
15-17	10^{-1}	10	7 D-3; 1 Ci-4 (D4); 1 D-7; 1 D-10	0

Age Factor. Theiler[3] has previously demonstrated the influence of age on resistance to TO virus injected intracerebrally and intranasally; the resistance develops rapidly in Swiss mice from birth to 6 or 7 weeks of age. The following results (Table II) were obtained when the more susceptible R.I. mice were given GD VII material intraäbdominally. The resistance shown by various strains of mice of differing age against peripheral administration of the TO Theiler's virus has already been reported by others[3,10,11] and the writer[2] (see also Table I).

It is thus clear that the degree of invasiveness of GD VII virus from a peripheral site, in relation to age of the host, corresponds more generally with that of the encephalitis viruses,[12] while the resistance shown against the TO virus given by such routes compares with that of the rodent strains (Lansing type) of polio-

[10] Gildemeister, E., and Ahlfeld, I., *Centr. Bakt.*, I Abt., *Orig.*, 1938, **142**, 144.

[11] Iguchi, M., *Kitasato Arch. Exp. Med.*, 1939, **16**, 56.

[12] Lennette, E. H., and Koprowski, H., J. *Immunol.*, 1944, **49**, 175.

myelitis virus and with other neurotropic viruses such as vesicular stomatitis in mice.[13]

Signs of Infection with GD VII Virus. Whether the GD VII virus is introduced into the CNS or intraäbdominally, its effects have been reported to be different from the TO virus in that most animals succumbed promptly after showing signs—the course of the disease was therefore shorter, as was also the incubation period, and the first sign was often hyperexcitability followed by spasmodic or convulsive movements.[5]

In the present study it was found that after intracerebral or intraäbdominal injection of GD VII virus, the incubation period in young mice (15 to 17 days old) is usually 3 days, and in older ones (one month or older), from 4 to 9 days. The signs are referable to meningeal and encephalitic involvement: hyperexcitability, circling, spasmodic and convulsive movements, and only occasionally weakness or paralysis of one or more limbs. Signs invariably precede death by a few hours. In most instances mice are found dead before any disturbance is noticeable. The signs here are thus different from the poliomyelitis-like syndrome which develops after TO virus is inoculated: the longer incubation period; the development of flaccid paralysis of various portions of the limbs, the rapidly developing muscular atrophy; the deformities; the general well-being of the host during most of the course of the disease, and the survival, through good nursing, of the majority of the paralyzed.

Pathology. An examination of several thousand sections of the CNS of mice which had succumbed to GD VII and to TO virus reveals that in the former the lesions are referable to a meningoencephalitis comparable to that produced in the mouse by the so-called virus encephalitides, such as Russian tick-borne, St. Louis, Japanese B, and West Nile. The chief features are massing of leucocytes and plasma cells in the meninges, in the choroid plexus and ependyma, and of leucocytes and glial cells about blood vessels, the walls of certain ones showing degeneration and, beyond, occasional perivascular hemorrhage. Degeneration and necrosis of neurons predominate and are present throughout the brain; areas of necrosis and gliosis and hemorrhage are also seen. Important to record is that the spinal cord is, as a rule, free from any noteworthy lesions. This picture is in sharp contrast to that found in mice that succumbed to TO virus infection, as previously described.[14] Here the changes induced are present caudally from the level of the thalamus through the spinal cord. In the brain neuronal degeneration and necrosis, along with neuronophagia, are not as conspicuous as the mesodermal-glial lesions but this distribution is reversed in the spinal cord, where neuronal damage is outstanding. From the viewpoint of pathology, GD VII-virus infection is seen as a meningoencephalitis, TO as an encephalomyelitis or a poliomyelitis.

Serum-Virus Neutralization Tests. Theiler and Gard[5] and Theiler[1] have already demonstrated the insusceptibility of mice, infected first with TO virus, to GD VII virus given intracerebrally but the resistance developed was shown to be relative, not uniform or complete: the two were not found to be immunologically identical. Moreover, the possibility was mentioned[5] that the resistance may have been only a reflection of the "interference" phenomenon.

In serum-virus neutralization tests by the intracerebral method in R.I. mice,‡ a certain relationship, but not identity, between TO and GD VII viruses could be affirmed. Thus a serum deriving from rabbits repeatedly injected with active GD VII virus neutralized 100 LD_{50} of the homologous virus. When mixed with TO virus and incubated for one hour at room temperature, it prolonged the incubation period (*e.g.*, with normal monkey serum plus 10^{-3} virus dilution the period averaged 12 days, as against 19 when the rabbit antisera were used). Little or no delay was observed when antiserum to TO virus, deriving from convalescent cotton rats, was added to GD VII virus. Protection from disease by

[13] Sabin, A. B., and Olitsky, P. K., *J. Exp. Med.*, 1937, **66**, 15, 35; 1938, **67**, 201.

[14] Olitsky, P. K., and Schlesinger, R. W., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 79.

‡ Thanks are due Dr. Max Theiler for his cooperation and generous gift of anti-TO and anti-GD VII sera, and to Dr. I. M. Morgan for performing these tests, reported here with permission.

means of both antisera could not be shown by statistical analysis.

Discussion. The term "strain" as applied to GD VII virus when it was first recovered, and later,[5] was not intended to indicate biological taxonomy, that is, as representing descendants from a common stock; it was so called for convenience only, meaning sample of virus isolated. Indeed, the malady from which it was recovered was not clearly one of the original Theiler's disease.[5] It is suggested that it be designated henceforth as Theiler GD VII virus. But this question of classification is not particularly important; what is significant is that the GD VII virus and the disease it induces have features not in common with the original TO Theiler's virus and its disease. The characteristics as here described, when added to others already recorded,[5] such as activity in high dilution (10^{-7}) and greater invasiveness into the host's tissues constitute elements of a picture not that of mouse poliomyelitis—natural or experimental—or its virus.

Conclusion. Additional properties of Theiler's virus—both the original and the GD VII—are described. The GD VII virus§ has been found to differ in several characteristics from the original Theiler's virus; the differences are great enough to make it unusable as a "model" for the study of human poliomyelitis.

§ What applies to the GD VII virus may or may not apply to the FA virus which was not available for study (*Cf.* reference 5).

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Comparative Effects of Thromboplastin, Lecithin, and Saline on Mortality of Mice Following Experimental Burn Shock.*

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Recent investigations on the systemic chemotherapy of shock resulting from standardized experimental burns have disclosed that isotonic saline solution[1,2] and a fraction from commercial liver extract[2] will significantly increase the mean survival time of groups of treated mice over control groups.

The purpose of this report is to present evidence for the existence of a principle in a commercial cattle brain extract (Thromboplastin, USP) which is effective in prolonging the survival time and in decreasing the mortality of mice which have been subjected to experimental burns.

Method. White male mice ranging in weight from 20 to 25 g were divided, at random, into 3 groups each of which was injected intraperitoneally (approximately one-half hour prior to the application of trauma) in doses of 0.8 cc per 100 g as follows: (1) saline, 0.9% NaCl solution; (2) lecithin, 1% suspension made by homogenizing soybean lecithin with physiological saline solution in a Waring blendor; and (3) thromboplastin (Local-Cutter) diluted to a 1% homogeneous suspension with isotonic saline.

The mice were fasted 12 hours preceding the experiment, and neither food nor water was given once the test was started. This was done in order to minimize the number of variables since nutrition might conceivably be a factor and administration of food or water might influence survival time. A uniform degree of trauma in the mice was attained by immersing each etherized animal completely up to the

* Part of the experiments were carried out at the University of California through the courtesy of Dr. Ernest W. Page.

1 Rosenthal, S. M., *U. S. Pub. Health Rep.*, 1943, **58**, 513.

2 Prinzmetal, M., Hechter, O., Margoles, C., and Feigen, G., *J. Am. Med. Assn.*, 1943, **122**, 720.