ployed by Sanders *et al.*[1] Groups of 25 to 28 mice, weighing 16-20 g each, were inoculated intraperitoneally with 0.06 ml each of graded doses of SK virus ranging from 2×10^{-4} to 2×10^{-8}. Treatment was initiated 24 hours after virus inoculation and continued for 5 consecutive days. Group 1 mice received 1 ml of the drug intraperitoneally (8.33 mg phenosulfazole per ml) at 8 a.m. and 6 p.m. Group 2 mice were injected in similar manner with sterile saline solution, while Group 3 mice received no treatment. The LD_{50} titers were as follows: Group 1 (phenosulfazole treated) $10^{-6.3}$, Group 2 (saline treated) $10^{-6.6}$, Group 3 (no treatment) $10^{-6.1}$.

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Therapeutic Failure of Phenosulfazole (Darvisul) in Mice Infected with EMC or with MM Viruses.

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Phenosulfazole (Darvisul) has been reported by Sanders, SubbaRow, and Alexander[1] as having a therapeutic effect in mice infected with "a mouse virus isolated in 1940." Elsewhere in their report[1] the authors mention "SK mouse poliomyelitis," hence they presumably worked with the Columbia-SK virus which is a neurotropic agent immunologically indistinguishable from the encephalomyocarditis (EMC) and MM viruses.[2] The lack of effect of this drug on the course of the disease in mice experimentally infected with EMC and MM viruses is reported here. The phenosulfazole used in the present work was supplied by Dr. H. R. Cox of the Lederle Laboratories.

In Exp. 1, groups of 30 Swiss mice (Bagg strain) weighing 12 g each were infected with varying amounts of EMC virus intraperitoneally and treated with phenosulfazole by the same route. The drug was diluted to contain 6.25 mg/cc and given at 8 and 11 a. m. and 2 and 5 p. m. in doses of 0.5, 0.25, 0.25 and 1.0 cc respectively. Treatment was begun 17 hours after infection and continued for seven days with a total daily dose of 12.5 mg. Control mice were injected in the same manner as the treated group except that normal saline was used. Following infection the mice were observed for 17 days when the death ratios were calculated. It should be noted that few deaths occurred subsequent to the ninth day after infection. Selected animals in the treated and control groups were autopsied and the virus recovered by passage of brain tissue into normal mice.

In Exp. 2, groups of 16 mice weighing about 20 g each were infected and treated in a manner similar to that employed in Exp. 1, except that MM virus was used and the schedule of therapy was varied somewhat. Therapy was initiated in the 3 groups of treated mice as follows: (a) 2 hours before infection (b) 2 hours after infection and (c) 19 hours after infection. In each instance the initial dose was 8.33 mg followed by a course of treatment similar to that described above to give 16.7 mg daily. Because of evidence of toxicity the daily dosage was reduced to 12.5 mg per mouse on the second day and was maintained at this level until stopped 5 days after infection. It should be noted that in this experiment the titer of the MM virus in the control mice was at least $10^{-7.9}$. That the upper limit of infectivity could not have been much beyond this range is indicated by the

[1] Sanders, M., SubbaRow, Y., and Alexander, R. C., *Texas Rep. Biol. and Med.*, 1948, **6**, 385.

[2] Warren, J., and Smadel, J. E., *Fed. Proc.*, 1948, **7**, 311.

TABLE I.
Phenosulfazole in the Treatment of Mice Infected with EMC or with MM Viruses.

Virus	Therapy: Initial dose (hr)	Therapy: mg/day	Dilution of infectious material: $10^{-5.0}$	$10^{-6.0}$	$10^{-7.0}$	$10^{-8.0}$	MLD 50%
EMC	0 (control)		30/30*	21/29	17/30	5/30	$10^{-7.0}$
	17 post infection	12.5	30/30	29/30	23/30	—	$10^{-7.3}$†
MM	0 (control)		16/16	12/16	12/16	11/16	$10^{-7.9}$†
	2 pre inf.	12.5‡	13/14	14/15	11/14	—	$10^{-7.3}$†
	2 post inf.	12.5‡	14/14	14/16	10/14	—	$10^{-7.2}$†
	19 post inf.	12.5‡	16/16	15/16	9/16	—	$10^{-7.1}$†

* Numerator = No. of mice dying; denominator = No. of mice in group.
† These figures represent minimal values. The exact titers are at least this great.
‡ Initial dose 8.33 mg/mouse. These mice received 16.7 mg drug per day during the first 24 hours and 12.5 mg per day subsequently. See text.

fact that this same preparation of frozen stored virus when titrated five days later had a titer of $10^{-7.5}$.

The results of the two experiments are summarized in Table I. These data indicate that treatment with phenosulfazole produced no reduction in mortality among the mice infected with either EMC or MM viruses. Furthermore, there was no indication that it delayed the onset or duration of the disease.

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Evaluation of the Effect of Darvisul upon Infection with SK Strain of Virus in Mice.*

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Darvisul, N-(2-thiazolyl)-phenol sulfonamide, or phenosulfazole was reported by Sanders, SubbaRow, and Alexander[1] to be effective against as many as 100 LD_{50} of a mouse virus, SK, when administration was begun 24 hours after intraperitoneal infection.

Through the courtesy of Dr. Sanders the virus was received in the form of two infected mouse brains in glycerine. The drug was received in ampules of 25% solution of lot No. 7-8522, through the kindness of Dr. Sanders and Dr. Leo Rane of the Lederle Laboratories. The plan of testing the drug was that outlined by Dr. Sanders and was rigidly observed.

The original brain material was made into a 10% suspension in 10% horse serum physiological salt solution. A titration was done by intraperitoneal inoculations of groups of 5 mice and found to be approximately 2×10^{-7}. A pool was made from the brains of mice sacrificed when moribund and the 10% suspension of this material served as the material with which the therapeutic effects of the compound were tested.

Albino mice of 20 g weight of a single strain were used. As shown in the table 50 mice were inoculated intraperitoneally with the respective concentrations of virus and 24 hours later administration of the chemical was begun by the same route. The stock solutions of compound were diluted 1:31 in physiological salt solution so that each ml contained 8 mg. The material was given

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1 Sanders, M., SubbaRow, Y., and Alexander, R. C., *Texas Rep. Biol. and Med.*, 1948, **6**, 385.