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Failure of Phenosulfazole (Darvisul) in Treating Experimental Viral Infections.

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Recently Sanders, SubbaRow and Alexander[1] reported sodium phenosulfazole (Darvisul), N-(2-thiazolyl)-phenol sulfonamide, to be effective against at least as many as 100 LD_{50} doses of the Columbia SK virus[2,3] for mice when intraperitoneal treatment was instituted 24 hours after intraperitoneal infection.

The purpose of this communication is to report the failure of phenosulfazole to protect mice and monkeys against experimental infection with the Columbia SK virus, and the Brunhilde strain of poliomyelitis virus, respectively, as well as its lack of effect against certain other viral infections in mice and in chick embryos.

A. Experiments with Columbia SK Virus. Exp. 1. Groups of 15 mice (Rockland Swiss albino), weighing 20 to 24 g each, were inoculated intraperitoneally with graded doses of Columbia SK virus (a frozen pool of infected mouse brains received through the courtesy of Dr. Murray Sanders) and treated with phenosulfazole by the same route. Treatment was started 24 hours after virus inoculation and continued for 6 consecutive days. The phenosulfazole used in this experiment was obtained from Dr. Sanders as a 25% solution and represented a portion of the same preparation that was used in his laboratory. The drug was diluted with sterile saline solution to give a concentration of 8.26 mg per ml and given at 8 and 11:30 a. m., and 3 and 5 or 6 p. m. in doses of 0.5, 0.25, 0.25 and 1.0 ml respectively. Thus, the dose per day per mouse was 16.5 mg. Control mice were injected in a manner similar to the treated group except that sterile physiological saline solution was used. The mice were observed for 21 days at which time the mortality ratios were calculated.

Exp. 2 This experiment was similar to Exp. 1 except that slightly smaller mice (Rockland Swiss albino), about 18 to 20 g in weight, were used. Dilutions of the Columbia SK virus, 10^{-4} through 10^{-7}, were used with 20 mice per each dilution. Group 1 mice received 22 mg phenosulfazole* per mouse per day. Group 2 mice received 32 mg of drug per mouse per day. Group 3 mice served as virus controls and received injections of sterile saline solution in place of the drug. Group 4 mice received no virus but served as drug controls and received 22 mg phenosulfazole per day. Group 5 (only 10 mice in this group) likewise received no virus but received 32 mg phenosulfazole per day as drug controls.

Treatment was started 20 hours after infection and continued for 12 days. The drug was used in a concentration of 20 mg per ml. The mice received 4 injections (intraperitoneally) per day. Those of Group 1 and 4 received 6 mg at 8 a. m., 4 mg at 11:30 a. m., and 3 p. m., and 8 mg at 5 p. m., whereas Groups 2 and 5 received 8 mg each at 8 and 11:30 a. m., and 3 and 5 p. m. The mice were

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

[2] Jungeblut, C. W., and Sanders, M., *J. Exp. Med.*, 1940, **72**, 407.

[3] Committee on Nomenclature of the National Foundation for Infantile Paralysis, A Proposed Provisional Definition of Poliomyelitis Virus, *Science*, 1948, **108**, 701.

* The phenosulfazole used in this and most of the subsequent experiments was received from Dr. M. C. Lockhart, Director of the Pharmaceutical Section, Lederle Laboratories Division, American Cyanamide Company, as a sterile 25% solution in sealed glass ampoules.

TABLE I.
Results Obtained with Phenosulfazole in the Treatment of Mice Infected with Columbia SK Virus.

Exp. No.	Wt of mice, g	Drug per day, mg	Mortality ratio† of mice infected with virus dilutions				LD_{50} titer
			10^{-4}	10^{-5}	10^{-6}	10^{-7}	
1	20-24	16.5	15/15	13/15	9/15	12/15	$10^{-7.0}$
		0* (Virus control)	12/15	12/15	9/15	11/15	$10^{-6.6}$
2	18-20	22	16/20	12/20	4/20	7/20	$10^{-5.4}$
		32	14/19	12/20	7/20	9/20	$10^{-5.6}$
		0* (Virus control)	10/19	9/20	8/20	4/20	$10^{-5.0}$
		22 (No virus drug control)		0/20			
		32 (No virus drug control)		0/10			

* Received injections of buffered saline solution instead of drug.
† Numerator = No. of mice dying; denominator = No. of mice in group.

observed for 16 days at which time the mortality ratios were calculated.

The results of the first two experiments are summarized in Table I. The data show that phenosulfazole had no beneficial therapeutic effect in mice infected with the Columbia SK virus and treated as described above. In addition there was no evidence to suggest that the drug affected the onset or duration of the infection.

B. Experiments with Poliomyelitis Virus in Monkeys. Exp. 3. Five monkeys (*M. rhesus*) were infected intranasally under ether anesthesia with 4 ml each of a 10% suspension of a frozen pool of monkey cords infected with the Brunhilde strain of poliomyelitis virus (3rd passage in this laboratory of material received from Dr. Isabel Morgan, Baltimore, Md.). Three monkeys were treated with phenosulfazole whereas 2 served as virus controls and received sterile saline solution in place of the drug. Treatment was started 24 hours after infection and consisted of 200 to 300 mg intravenously at 8 a. m., 300 mg intraperitoneally (intravenously on one occasion) at 12 noon, and 500 mg intraperitoneally at 5 p. m. Treatment was continued for 11 days. The results may be summarized by stating that no evidence was obtained to indicate that treatment with phenosulfazole showed any beneficial effect.

The 3 treated monkeys reacted as follows: No. 26 showed definite weakness of both arms and legs on the 5th day, complete paralysis of both legs on the 8th day, was prostrate on the 9th day, and was sacrificed in a moribund condition on the 10th day. No. 34 showed tremors and weakness of both legs and arms on the 10th and 11th day, paralysis of the left arm and leg on the 12th and 13th day, extreme weakness with inability to move from the 14th to 23rd day but from thereon gradually improved and eventually recovered with some residual paralysis. No. 43 showed progressive weakness from the 11th through the 13th day, was paralyzed in both arms and legs on the 14th day and was sacrificed in a moribund condition on the 15th day.

The 2 untreated monkeys reacted as follows: No. 9 showed no signs of illness throughout the 28 days of observation, whereas No. 42 developed paralysis of both legs and arms on the 15th day and was sacrificed in a moribund condition on the 16th day.

C. Experiments with some Neurotropic Viruses and Influenza Virus in Mice. Three experiments were carried out with the MEF-1 strain of poliomyelitis virus (obtained through the courtesy of Dr. Peter K. Olitsky of the Rockefeller Institute for Medical Research, New York, N. Y.), using 50 to 500 LD_{50} doses inoculated intracerebrally into Rockland Swiss albino mice. In Exp. 1 treatment of the infected animals was initiated 24 hours after infection. In Exp. 2, in which 2 separate groups were treated, drug therapy was

started 24 hours after infection in one group, and 6 hours after infection in the other. In Exp. 3, two groups of treated animals were again employed with the drug being administered 6 hours after infection in one group, and 1½ hours after infection in the other.

Two experiments were carried out with Eastern equine encephalomyelitis virus using Tumblebrook Swiss albino mice. In one case the mice were inoculated by the intracerebral route and in the other by the intraperitoneal route. In both cases drug therapy was initiated 24 hours following infection.

Two experiments were carried out using Venezuelan equine encephalomyelitis virus inoculated subcutaneously into Rockland Swiss albino mice. In both experiments drug treatment was started 24 hours following infection.

A single experiment was carried out with Russian Spring-Summer encephalitis virus inoculated subcutaneously into Carworth Swiss albino mice. Drug therapy was started 24 hours following infection.

Two experiments were carried out with the PR8 strain of influenza virus (Type A) inoculated intranasally into Rockland Swiss albino mice. In the first experiment drug therapy was started 24 hours following infection whereas in the second it was started 1½ hours following infection.

The essential data pertaining to these experiments are shown in Table II. The results may be summarized by stating that again no evidence was obtained to show that phenosulfazole had any beneficial therapeutic effect in mice infected with any of the above named viral infections.

D. Experiments in Chick Embryos. A number of experiments were carried out to determine the effect of phenosulfazole upon chick embryos infected with Newcastle disease virus, the Lee strain (Type B) of influenza virus, the Bitter Root strain of Rocky Mountain spotted fever (*R. rickettsii*), the Breinl strain of epidemic or louse-borne typhus (*R. prowazekii*) and the 6 BC strain of psittacosis (*M. psittacii*). The experiments may be summarized by stating that only in the case of psittacosis, in which relatively large doses of the drug were employed (25 mg per embryo), was there any suggestive evidence that phenosulfazole had a protective effect. This was demonstrated by the fact that drug-treated embryos showed a slight increase in their survival time and a reduction in the number of elementary bodies observed by microscopic examination of yolk-sac smears, as compared to infected control eggs treated with saline in a similar manner. This was not an unexpected finding, however, because other sulfonamides, particularly sulfadiazine, have been shown to be active against psittacosis.[4-6] It should be pointed out, however, that the results obtained with phenosulfazole in the treatment of chick embryos infected with *M. psittacii* were not nearly as satisfactory as those reported recently for the antibiotic Aureomycin.[7]

Conclusion. Phenosulfazole (Darvisul) showed no beneficial effect in the treatment of mice infected with the Columbia SK virus and other neurotropic viruses, including a rodent adapted strain of poliomyelitis (MEF-1 strain). The lack of therapeutic effectiveness of the drug was also observed in monkeys infected intranasally with the Brunhilde strain of poliomyelitis virus. In other viral and rickettsial infections of mice and of developing chick embryos treatment with phenosulfazole was of no therapeutic value, with the possible exception of psittacosis in which a slight inhibition of growth in the developing chick embryos was observed. However, even in the latter case the effectiveness of phenosulfazole therapy was of much lower magnitude than that observed with other sulfonamides,[4-6] or antibiotics.[7]

ADDENDUM

Since this paper was written an additional experiment was carried out using Columbia SK virus in Carworth Swiss albino mice in a manner as identical as possible to that em-

[4] Meiklejohn, G., Wagner, J. C., and Beveridge, G. W., *J. Immunol.*, 1946, **54**, 1.

[5] Wiseman, R. W., Meiklejohn, G., Lackman, D. B., Wagner, J. C., and Beveridge, G. W., *J. Immunol.*, 1946, **54**, 9.

[6] Rosebury, T., Ellingson, H. V., and Meiklejohn, G., with assistance of Schabel, F., *J. Infect. Dis.*, 1947, **80**, 64.

[7] Wong, S. C., and Cox, H. R., *Ann. N.Y. Acad. Sc.*, 1948, **51**, 290.

TABLE II.
Results Obtained with Phenosulfazole in the Treatment of Mice Infected with Other Viruses.

Virus inoculum	Route of infection	Exp. No.	Wt of mice, g	Drug per day, mg	Mortality ratio* of mice infected with virus dilutions								LD_{50} titer
					10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	
Poliomyelitis MEF-1	I.C.	1	10-12	10	10/12								
				0†	6/6								
		2	10-12	16	10/10	8/10							
				16‡	10/10	10/10							
				0†	9/10	9/10							
		3	10-14	8‡	12/12								
				8§	12/12								
				0†	12/14								
Eastern Equine Encephalomyelitis	I.C.	1	14-16	20						6/6	2/5	3/6	$10^{-7.4}$
				0†						6/6	6/6	0/6	$10^{-7.5}$
	I.P.	2	14-16	20				3/6	4/6	1/6	0/6		$10^{-4.7}$
				0†				1/6	1/6	0/6	0/6		$<10^{-4.0}$
Venezuelan Equine Encephalomyelitis	S.C.	1	20-22	4				6/6	6/6	5/5	6/6	6/6	$>10^{-8.0}$
				0†						6/6	0/6	0/6	$10^{-6.5}$
		2	20-22	8				6/6	6/6	5/6	3/6	3/6	$10^{-7.3}$
				0†				6/6	6/6	4/6	1/6	1/6	$10^{-6.4}$
Russian Spring-Summer Encephalitis	S.C.	1	18-20	20			4/4						
				0†‖			4/4						
Influenza PR-8	I.N.	1	16-18	22					8/9				
				0†					8/9				
		2	16-18	22§					9/9				
				0†					8/9				

* Numerator = No. of mice dying; denominator = No. of mice in group.
† Untreated controls.
‡ Drug treatment started 6 hr after virus inoculation.
§ Drug treatment started 1½ hr after virus inoculation.
‖ The 10^{-3} dilution of Russian Spring-Summer encephalitis contained 25 to 100 mouse LD_{50}.

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.