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Sulfapyridine and Serum Therapy in Experimental Lobar Pneumonia of Rats.

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This group of experiments was planned to test the effect of sulfapyridine ("M. and B. 693", "Dagenan"*) upon the course and outcome of experimental pneumonia in rats. The report by Whitby[1] and several reports by American workers have indicated that this new compound is superior to sulfanilamide in combatting pneumococcal infections. The experiments so far reported have been confined mainly to infections of mice and rats by intraperitoneal and subcutaneous injection of various types of pneumococci. Cooper, Gross and Lewis[2] have recently reported upon Type II pneumococcal meningitis in rats.

In our first group of experiments we have used only one strain of pneumococcus, a Type I strain with high virulence for rats which we obtained from Dr. O. H. Robertson. It has been used for several years in producing experimental pneumonia. Young adult rats weighing 160 to 260 g were used for the experiments. The method used for production of the pulmonary infections has been previously described.[3, 4] Eighteen-hour dextrose-bouillon cultures were diluted with physiological saline, the final dilution in 5% commercial gastric mucin, and each animal received 0.1 cc of viscid mixture by intrabronchial insufflation. The sulfapyridine was suspended in 15% gum tragacanth or, more conveniently, in 5% mucin and administered by stomach tube, each animal receiving about 2 cc of mixture. Type I therapeutic serum (Felton)† was injected intraperitoneally diluted to 2 cc with saline.

In a preliminary orientation experiment, 18 rats were infected with cultures diluted to 10^{-4} and 12 of them treated with the sulfapyridine four hours later. Each of 6 animals received 125 mg for the first dose, followed by 60 mg at daily intervals for 5 days. Six

* "Dagenan" kindly furnished by Merck and Company, Inc., Rahway, N. J.

1 Whitby, L. E. H., *Lancet*, 1938, **1**, 1210.

2 Cooper, F. B., Gross, P., and Lewis, M., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 37.

3 Nungester, W. J., and Jourdonais, L. F., *J. Bact.*, 1935, **29**, 34.

4 Gunn, F. D., and Nungester, W. J., *Arch. Path.*, 1936, **21**, 813.

† Donated to us by Parke, Davis and Company, Detroit, Mich., and by E. R. Squibb and Sons, New York.

received 60 mg as the initial dose and 36 mg at daily intervals for 5 days. The 6 untreated controls died with lobar pneumonia and fibrino-purulent pleuritis within 48 hours. All 6 of those receiving the smaller dose of the drug were dead within 48 hours. Three of those receiving the larger dose were dead by the seventh day and the 3 surviving until the twenty-fourth day apparently in good condition were sacrificed and examined. Two showed only the India ink tracer in the left lung. The other had soft fibrous adhesions and encapsulated empyema of the left side.

In the second experiment, the same infecting dose was used in 30 animals. Of the 6 untreated controls, 5 were dead within 48 hours and the sixth was dead on the fifth day after injection. All showed pneumonia of the left lung. Of 6 animals receiving an initial dose of 60 mg of sulfapyridine, followed by 5 daily doses of 36 mg, 2 survived indefinitely. Of the 6 receiving 125 mg as an initial dose and 60 mg maintenance dose, 3 died as the result of infection and one was accidentally killed in attempting to pass a stomach tube (perforation of esophagus). Of 6 animals receiving Type I anti-pneumococcic serum in doses of 200 units each, 4 hours after infection and 100 units on each of the 5 succeeding days, 3 died. Finally, 6 animals were given both serum and sulfapyridine, the

TABLE I.
Efficacy of Sulfapyridine and Specific Serum in Type I Pneumonia.

No. rats	Avg wt g	Culture dilution	Therapy	Total dose	Survivals		Deaths	
					No.	%	Av. surviv.	Traumatic
Group 1. Treated 4 hours after infection.								
24	190	10^{-4}	None		0	0	2.1	
12	180	"	Sulfapyr.	240 mg	2	17	3.7	
6	180	"	"	425	3	50+	4.	1*
18	230	"	"	850	10	55+	4.5	3*
6	190	"	Serum	700 U	3	50	5.3	
6	190	"	Sulfapyr. + serum	425 mg 700 U	5	83+	3.	
Group 2. Treated 18 hours after infection.								
22	175	10^{-4}	None		0	0	1.6	
12	180	"	Sulfapyr.	425 mg	3	25	1.6	
11	180	"	"	850	3	27+	5.7	
10	180	"	Serum	1400 U	5	50	1.8	
12	200	"	Sulfapyr. + serum	850 mg 1400 U	1	8+	4.5	3*
11	190	10^{-5}	None		0	0	2.5	
12	190	"	Sulfapyr.	1250 mg	2	16+	2.2	2*
12	170	"	Serum	1250 U	3	25	1.7	
12	180	"	Sulfapyr. + serum	1250 mg 1250 U	4	33+	2.7	

* In computing percentage of survivals, traumatic deaths were included with deaths from infection but were not included in the average survival period of rats dying spontaneously. This method works to the disadvantage of groups receiving sulfapyridine.

latter in doses of 60 mg as initial dose and 36 mg daily for maintenance dose. Only one of these died and the necropsy revealed a minimal lesion in the left lung, insufficient to account for the fatal outcome. The others survived indefinitely. The results of these 2 experiments are combined as Group 1 in the Table I, all animals in the group receiving the same infecting dose.

In a third experiment, therapy was not begun until 18 hours after infection. Sixty-seven rats were infected with 0.1 cc of a 10^{-4} dilution of an 18-hour culture and 47 rats with a 10^{-5} dilution. Of the former, 12 animals received a total of 425 mg of sulfapyridine over a period of 6 days. The initial dose in every case was approximately twice the maintenance dose. Three of these survived. Of 11 rats in which the total quantity of drug administered was 850 mg, 3 survived indefinitely. While the mortality was about the same in the group receiving the larger dose as in that receiving 425 mg, the average period of survival in those dying of infection was longer in the former (5.7 days in rats receiving 850 mg and 1.6 days in those receiving 425 mg). Ten rats in this series were treated by intraperitoneal injection of 400 U. of Type I antipneumococcic serum on the day after infection, followed by 200 U. daily for 5 days. Five of these survived indefinitely and 5 died within 1 to 4 days (avg 1.8 days). Twelve animals were given both sulfapyridine (850 mg) and serum (1400 U.). The results are shown in Group 2 of the table. One animal survived, 3 died as the result of trauma and the survival time of those dying was prolonged (4.5 days). Of 22 untreated controls which received the same infecting dose of pneumococci, all died in 1 to 4 days (avg 1.6 days) after infection.

In a group of 47 rats the infecting dose was smaller (dilution 10^{-5}) than in the preceding experiments. Twelve received 250 mg of sulfapyridine daily for 5 days, a total of 1250 mg for each rat. Two survived, 2 died of trauma and the average survival time of those dying of infection was 2.2 days. Twelve were given 250 U. of serum on each of 5 days; 3 survived and the average survival time of those dying was 1.7 days. Of 12 rats receiving both serum and drug, 4 were alive at the end of 3 weeks and the average time of survival of the rest was 2.7 days.

Summary. A combination of type-specific serum, administered intraperitoneally, and sulfapyridine, administered by stomach tube, was more efficacious in the treatment of experimental lobar pneumonia of rats than either alone, when treatment was begun within 4 hours after infection. The optimal dose of sulfapyridine under the conditions of our experiments was 0.5 to 1 g per kilo of rat per

day. After the infection was well established, following intrabronchial insufflation of relatively large doses (0.1 cc of 10^{-4} and 10^{-5} of 18-hour bouillon cultures) of pneumococci, serum was more efficacious than sulfapyridine in preserving life, but a combination of the 2 did not show a significant reduction in mortality even with the smaller infecting dose.

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Factors Affecting the Vitamin B_1 Content of Evaporated Milk.

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The work of several investigators who have studied the vitamin B_1 content of evaporated milk has been recently reviewed by Daniels.[1] She reported two trends in results: certain workers had found about 50% destruction of vitamin B_1 when evaporated milk was compared with raw milk, whereas others had observed only 0-20% destruction. Using a modification of the 10-day rat growth technic of Schlutz and Knott,[2] Daniels assayed raw Guernsey milk and commercial evaporated milk. Her results indicated 60% less vitamin B_1 in the evaporated milk than was present in the raw milk. Since certain studies in our laboratory differed from the results presented by Daniels, and because she had used repeated assays on the same animals but had not employed an improvement we had reported for our basic ration,[3] we have thought it advisable to investigate further the effect of the process of evaporation upon vitamin B_1.

Fresh raw milk was immediately iced and delivered by special messenger together with evaporated milk prepared from the same lot. By careful refrigeration the raw milk was kept sweet for the duration of the test. The evaporated milk was stored at room temperature.

Sixty-gram rats were fed the following ration: 15.0% vitamin-free casein, 15.0% dried autoclaved liver,* 45.5% sucrose, 17.0% Crisco, 3.0% cod liver oil and 4.5% modified Wesson's salts. After

1 Daniels, A. L., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 212.

2 Schlutz, F. W., and Knott, E. M., *J. Nutr.*, 1936, **12**, 583.

3 Schlutz, F. W., and Knott, E. M., *J. Biol. Chem.*, 1937, **119**, lviii.

* Fresh hog liver was ground and autoclaved for 5 hrs at 120°.