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Effect of the Host in Action of Sulfonamides on Elementary-Body Agents of Murine and Feline Pneumonitis.*

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The effect of sulfanilamide and its derivatives on experimental infections with 4 agents of the lymphogranuloma-psittacosis group has been studied. Infections in mice with 2 of these agents, mouse pneumonitis and lymphogranuloma venereum, are inhibited with sulfonamides,[1,2] while infections with the agents of feline pneumonitis[3] and meningopneumonitis[4] are not.[2,5] Growth of the latter virus and psittacosis virus in chick embryos or mice is retarded by penicillin.[6,7] Clinical observations suggest that the sulfonamides have no effect in human infections with psittacosis and closely related agents.[8]

A close biological similarity of the agents from mice and cats and the formation of a complement-fixing antigen which cross-reacts with other members of the lymphogranuloma-psittacosis group have been noted.[9,10] Both agents produce pneumonia of almost identical character in mice inoculated intranasally, but neither agent will produce lethal infection in these animals by any other route except when large doses of yolk sac material are inoculated.[5] These properties distinguish the two agents from the other members of this group which produce fatal infections in mice inoculated intracerebrally.

Three similar strains forming elementary bodies, 2 from mice and one from hamsters,[11] were, like the original strains of mouse pneumonitis, inhibited by sulfonamides.[12] Since the biologically similar agent from cats was apparently exceptional in being drug resistant, it seemed possible that an investigation of the differences between resistant and susceptible strains might give some information about mechanisms of chemotherapy of certain virus diseases. One line of approach was to compare the cat and mouse viruses in several different hosts.

Materials and Methods. Sulfamerazine† and other relatively insoluble drugs were suspended in 10% gum acacia and injected intraperitoneally. Mice, rats, cotton rats (*Sigmodon hispidus eremicus*), hamsters (*Cricetus auratus*), and chick embryos were used in the experiments. Generally the animals were weighed and the dosage adjusted in relation to body weight. The strain of mouse pneumonitis virus was originally isolated by Dr. Clara Nigg who kindly sent it to us. The feline pneumonitis virus was obtained from Dr. James A. Baker of the Rockefeller Institute at Princeton, New Jersey. Both strains produced characteristic focal pulmonary lesions[3,11] when given intranasally in high dilutions, and the character of these lesions was the same in

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† 2-sulfanilyl aminomethylpyrimidine. Manufactured by Parke, Davis & Co.

TABLE I.
Effect of Sulfamerazine on Infections with the Agent of Mouse Pneumonitis.

Animal	Dilution of virus	Daily drug dosage mg/g	No. days	Day killed	Results with test animals: Lesions*	Results with test animals: Avg lung wt (g)	Results with controls: Lesions*	Results with controls: Avg lung wt (g)
Hamster	10^{-1}	0.3	3	4	3 3 2 2	2.30	5 5 4 3	2.6
"	10^{-3}	0.3	7	7	1 0 0 0 0	0.75	2 2 1	1.0
"	10^{-2}	0.1	3	4	1 1 0 0	0.90	3 3 2 2	2.05
"	10^{-3}	0.1	3	7	0 0 0	—	3 3 2	—
"	10^{-4}	0.1	3	7	1 0 0	—	1 1 1	—
"	10^{-2}	0.025	3	4	3 2 2 0	1.40	3 3 2 2	2.05
Cotton Rat	10^{-3}	0.3	6	6	1 0 0 0 0 0	0.90	3 2 2 1	1.70
Rat	10^{-2}	0.6	7	8	1 1 0 0	1.50	5 5 2 1	2.60
Mouse	10^{-1}	0.1	4	4	0 0 0 0 0 0	—	5 5 5 5 5 5	—
"	10^{-2}	0.1	4	4	0 0 0 0 0 0	0.166	3 3 3 3 3 3	0.300
"	10^{-3}	0.01	3	7	0 0 0 0 0 0	—	2 2 2 2 2 2	—
"	10^{-3}	0.005	3	7	1 0 0 0 0 0	—	same	—
"	10^{-3}	0.0025	3	7	1 1 f 0 0 0	—	"	—

* Pulmonary lesions are shown by numeral for each animal and were graded as follows: 5 = animal died with pulmonary consolidation; 4, 3, or 2 = 4/4, 3/4, or 2/4 of lung consolidated; 1 = ⅛ to ¼ of lung consolidated; f = 5 to 20 minute discrete foci; 0 = no lesion.

the various species of animals used in the experiments. Elementary bodies were demonstrable in impression smears stained by the method of Macchiavello. The viruses were inoculated intranasally in dilutions of mouse lung containing 10 to 1,000 times the minimal amount required to produce pulmonary lesions within a period of 7 days. In all experiments the virus was inoculated 2 to 4 hours after the first dose of drug had been given intraperitoneally and drug was administered daily thereafter. Pulmonary lesions were graded by gross examination and by weighing the lungs. The effect of variations in body weight was minimized by selecting the animals so that average weights of the test group and the control group were approximately equal.

Chick embryos 6 to 7 days old were inoculated into the yolk sac with egg-adapted agents of feline and murine pneumonitis, and a single dose of drug was given by the same route either before or after the virus. All embryos were cultured in glucose broth for bacterial contamination either at death or

TABLE II.
Effect of Sulfamerazine on Infections with the Agent of Feline Pneumonitis.

Animal	Dilution of virus	Daily drug dosage mg/g	No. days	Day killed	Results test animals: Lesions	Results test animals: Avg lung wt (g)	Results controls: Lesions	Results controls: Avg lung wt (g)
Hamster	10^{-3}	1.0	4	6	1 f f 0 0	—	3 3 2 2 2 2	—
"	"	0.6	6	6	f 0 0 0 0	0.95	2 2 1 1 1	1.19
"	10^{-4}	0.6	6	7	f f 0 0 0	0.76	f f f f 0 0	0.78
"	10^{-3}	0.3	5	5	3 1 1 1 1 f	1.30	3 3 3 3 2	1.60
"	10^{-4}	0.3	5	5	1 1 1 f f 0	0.85	1 1 1 1 1 1	0.97
"	10^{-3}	0.3	6	7	1 1 1 1	0.85	3 2 2 1	1.10
Cotton Rat	10^{-4}	0.6	6	7	1 1 1 1 f f	1.33	3 3 2 2 2 1	1.59
" "	10^{-4}	0.3	5	5	2 2 1 1 1 1	1.17	3 2 2 2 2 2	1.34
" "	10^{-4}	0.4	7	7	5 2 2 2 1 1	1.36	5 4 3 3 3 2	1.53
" "	10^{-3}	0.3	6	6	5 3 3 2	1.75	5 5 3 3	2.20
Rat	10^{-2}	0.6	7	8	1 1 1 f f f	1.50	5 5 3 2 2 1	3.10
"	10^{-2}	0.6	8	9	f f 0 0	1.10	1 f f 0	1.20
Mouse	10^{-3}	1.2	6	8	5 3 3 3 3 2	—	5 3 3 3 2 2	—
"	10^{-4}	1.2	6	8	2 2 1 1 1	—	2 2 2 2 1 1	—
"	10^{-3}	0.6	6	6	3 3 3 3 2 2	0.38	3 3 3 2 2 1	0.30
"	10^{-4}	0.6	6	6	3 3 1 1 1 1	0.23	3 3 2 1 1 1	0.25
"	10^{-5}	0.6	6	7	1 1 1 1 1 1	0.25	1 1 1 1 1 f	0.25

Lesions graded as in Table I.

TABLE III.
Blood Concentrations of Sulfamerazine After Single Daily Intraperitoneal Injections.

Animal	No. tested	Average sulfamerazine blood levels mg/100 cc 1st day dose = 0.9 mg/g		2nd day dose = 0.6 mg/g	
		Time after injection		Time after injection	
		4 hr	24 hr	4 hr	24 hr
Cotton rat	4	20.4	4 8	18.0	5.3
Hamster	4	17.8	6.3	15.8	3.5
Rat	7	39.8	21.6	43.2	21.2
Mouse	12	34.5	11.1	42.2	13.9

after the termination of the experiment.

Effect of Sulfonamides on the Mouse Pneumonitis Virus in Animals. Using the method of single daily intraperitoneal dosage over a period of 4 days, the minimal inhibiting dose of sulfanilamide and 4 of its derivatives against a constant amount (dilution 10^{-3}) of the mouse pneumonitis virus was determined in mice. Under these conditions the minimal inhibiting dose of sulfanilamide was found to be 1.0 mg per gram of body weight; that of sulfapyridine 0.30 mg/g, sulfathiazole 0.15 mg/g, sulfadiazine 0.005 mg/g, and sulfamerazine 0.0025 mg/g per day. Measurements of the blood levels at intervals after giving the drugs indicated that the rather marked differences in the effective amounts could be attributed, at least in part, to the rate of absorption and excretion of these compounds. After intraperitoneal injection of sulfamerazine appreciable blood levels remained after 24 hours, while drugs such as sulfanilamide and sulfathiazole were excreted more rapidly. Sulfamerazine was, therefore, chosen for use in the experiments to be described.

The results presented in Table I show that sulfamerazine inhibits infections with the mouse pneumonitis agent in mice, rats, hamsters and cotton rats. Small doses of drug (0.1 to 0.025 mg/g) were less effective in preventing the appearance of lung lesions in hamsters than in mice when the animals were inoculated intranasally with dilutions of 10^{-2}. Similarly, with virus dilutions of 10^{-1} a drug dosage of 0.1 mg/g gave complete inhibition in mice, while in hamsters 0.3 mg/g failed to produce significant reduction in the lesions. As the virus titered slightly higher in mice than in hamsters the greater effectiveness of the drug in mice probably cannot be attributed to a lower susceptibility of these animals.

Experiments with the Agent of Feline Pneumonitis in Animals. Tests similar to those with the agent of mouse pneumonitis were done with the virus of feline pneumonitis, with the results shown in Table II. In hamsters, sulfamerazine in amounts of 0.6 to 1.0 mg per gram of body weight daily produced a moderate inhibitory effect and 0.3 mg/g had a slight effect on the pulmonary lesions resulting from inoculation of virus at a dilution of 10^{-3} or 10^{-4}. This drug dosage was about 6 to 10 times as great as the amount required to produce a comparable effect on the agent of mouse pneumonitis in hamsters. In cotton rats a slight effect was noted, particularly with drug in amounts of 0.6 mg/g and a virus dilution of 10^{-4}. In 2 experiments with white rats an inhibitory effect was found, but the results were unsatisfactory because the control animals receiving a dilution of 10^{-2} in one experiment did not develop definite lung lesions. No inhibitory effect was found in mice even with doses of sulfamerazine of 1.2 mg/g, an amount between 200 and 500 times as great as that producing inhibition of the mouse pneumonitis virus in these animals. It should be noted that the agent of feline pneumonitis was not significantly more virulent for mice than for cotton rats or hamsters, as judged by titration.

Blood Levels with Sulfamerazine. The possibility that the species differences in the effect of sulfamerazine on the feline pneumonitis virus might be connected with the blood levels was considered. The results of determining sulfamerazine concentrations in the blood at 4 and 24 hours after a single daily intraperitoneal injection, as shown in Table III, indicated no relationship between the blood levels

TABLE IV
Effect of Sulfamerazine on Yolk-Sac Infection of Chick Embryos with the Agents of Murine and Feline Pneumonitis.

Virus	Dilution	Drug dosage, mg	Time	Day of death of chick embryos: Drug treated	Controls
Mouse Pn.	10^{-2}	20	3 hr after	6 S S S S S S*	4 4 4 4 5 5 5 6
Mouse Pn.	10^{-2}	4	3 hr after	8 8 9 S S S S S*	4 4 6 6 6 6 6 6
Cat Pn.	10^{-7}	20	3 hr after	6 6 6 6 6 6 6 6 10	6 6 6 6 6 6 6 6
Cat Pn.	10^{-7}	20	2 hr before	5 6 6 6 6 6 6 6	4 6 6 6 6 6 6 6

S—Survived 10 to 12 days.
*—Virus detected in surviving chick embryos by subinoculation of mice.

and the effect on the cat virus. In mice no inhibitory effect was observed, although the blood concentration was on the average almost twice as great as in hamsters or cotton rats. On the other hand, the differences in blood levels between mice and hamsters might account for the observation that relatively more drug was required in hamsters to inhibit the mouse pneumonitis virus.

Experiments in Chick Embryos. At a period of 10 days after inoculation of the embryos with a single dose of 20 mg of sulfamerazine into the yolk sac the drug was found in concentrations of 7 mg% in the amniotic fluid, 26 mg% in the allantoic fluid, and 16 mg% in the yolk and blood. This observation indicated that only one dose was required. Doses of 20 mg produced almost complete protection and doses of 4 mg partial protection against death of the embryos from a dilution of 10^{-2} (over 1,000 M.L.D.) of the mouse pneumonitis virus when given 3 hours after inoculation of the virus (Table IV). No inhibition of the infection with the virus of feline pneumonitis at a dilution of 10^{-7} (1 M.L.D.) was obtained with sulfamerazine given before or after inoculation of the virus. This drug also failed to inactivate the virus at a dilution of 10^{-3} in egg yolk when mixtures were allowed to stand for 4 hours *in vitro* at 37°C before inoculation of the chick embryos.

Discussion. The difference in sensitivity to sulfamerazine between the pneumonitis viruses from cats and mice appears to be quantitative in nature. In hamsters the inhibiting dose of drug for the cat agent was about 10 times that for the mouse virus. In mice the comparable ratio was found to be over 200 to 1 as determined by two factors: (1) maximum tolerated doses of drug failed to inhibit the cat virus, and (2) a smaller dose than required in hamsters would protect mice against the mouse virus. The differences between the two viruses are analogous to the phenomenon of drug resistance or sensitivity often observed with strains of bacteria in one species and might be due to similar factors. The results also suggest that unknown physiological mechanisms in the host may influence the effect of the sulfonamides (1) on the virus within the cells or (2) on extracellular virus either during the stage of initial infection or during spread of the virus particles from one cell to another. On the basis of the available data this effect does not seem to be related to the concentration of sulfamerazine in the blood or to the minimal infecting dose of the agent.

Summary. The effect of sulfamerazine on pulmonary infections in mice, hamsters, rats, and cotton rats, and on yolk sac infections in chick embryos with the agents of murine and feline pneumonitis was studied. The drug inhibited the action of the mouse virus in all the species tested and was effective in very small doses, 0.0025 mg per gram of body weight in mice and somewhat larger doses, 0.1 mg/g, in hamsters. With the agent of feline pneumonitis, large doses of sulfamerazine, near the toxic limits, produced moderate inhibition of the lung lesions in hamsters and white rats, slight inhibition in cotton rats, and no effect in mice or chick embryos.