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Comparison of Antistreptococcal Activities of p-Caproylaminobenzenesulfonhydroxamide and Sulfanilamide.

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The recent appearance of a number of papers[1-4] dealing with various aspects of the chemotherapeutic activity of p-caproylaminobenzenesulfonhydroxamide indicates an increasing interest in this compound. To facilitate further investigation, we report here the results of a comparative study of this sulfonhydroxamide and p-aminobenzenesulfonamide in the treatment of streptococcal infections in mice.

Methods and Materials. 1. *Chemotherapeutic Experiments.* Hemolytic streptococci (strain 1896) were grown for 6 hours in meat infusion broth containing 5% rabbit blood. The culture was diluted to permit the administration of 0.5 cc in each intraperitoneal injection. Dilutions of 10^{-3}, 10^{-4}, and 10^{-5}, approximately equal to 10,000, 1000, and 100 lethal doses respectively, were used. Virulence was maintained by frequent mouse passage.

The drugs were pulverized and suspended in 10% gum acacia. Each mouse received 0.5 cc of the appropriately diluted suspension by stomach tube at the intervals stated.

Mice weighing 18 to 20 g were inoculated intraperitoneally with 0.5 cc of the diluted streptococcus culture. Five or 10 mg doses of the drugs were fed to the mice immediately after infection, and then once daily for 5 additional days. Observations were made over a period of 10 days. The results are summarized in Table I. Under these conditions p-caproylaminobenzenesulfonhydroxamide was somewhat more effective than the sulfanilamide which was used for comparison.

A second series of infected mice were given 2 mg doses of the sulfonhydroxamide at 4-hour intervals over a period of 96 hours. Single daily doses were then given for 3 additional days. For compari-

[1] Cooper, F. B., Gross, P., and Lewis, M., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 491.

[2] Main, E. R., Shinn, L. E., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 593.

[3] Kohl, M. F. F., and Flynn, L. M., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 455.

[4] Shinn, L. E., Main, E. R., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 596.

TABLE I.

Materials—Six-hour culture *Streptococcus hemolyticus* 1896. Dilution of culture, 10^{-3}, 10^{-4}, 10^{-5} equivalent to 10,000, 1,000 and 100 lethal doses respectively.

Method—Mice were infected intraperitoneally with 0.5 cc of culture, and fed immediately after the injection and once daily for 5 additional days.

No. of mice	Compound	Dose per mouse in mg	Dilution of culture	% survival Time in days 1	2	3	4	5	6	7	8	9
45	Sulfanilamide	5	10^{-3}	73	33	24	24	24	24	24	24	24
45	The sulfonhydroxamide	5	10^{-3}	88	49	44	38	38	38	35	35	35
45	Sulfanilamide	10	10^{-3}	88	40	33	33	33	33	33	24	22
45	The sulfonhydroxamide	10	10^{-3}	91	67	51	49	47	47	47	44	44
45	Sulfanilamide	5	10^{-4}	93	69	60	58	56	51	49	47	47
45	The sulfonhydroxamide	5	10^{-4}	98	80	69	67	64	64	64	60	58
45	Sulfanilamide	10	10^{-4}	96	73	71	67	67	67	64	62	58
45	The sulfonhydroxamide	10	10^{-4}	93	84	80	80	78	78	76	73	71
45	Sulfanilamide	5	10^{-5}	93	71	69	67	64	64	64	62	62
45	The sulfonhydroxamide	5	10^{-5}	96	82	82	82	82	82	76	73	71
45	Sulfanilamide	10	10^{-5}	100	84	82	82	82	78	73	73	73
45	The sulfonhydroxamide	10	10^{-5}	100	98	98	98	93	91	89	89	89
15	Controls	—	10^{-4}	13	0	0	0	0	0	0	0	0
30	"	—	10^{-5}	24	0	0	0	0	0	0	0	0

son, similar experiments were performed using equal-weight doses of sulfanilamide as the chemotherapeutic agent. Observations were made each time the drugs were administered, but in the interests of space a limited number of observations are shown in Table II

At culture dilutions of 10^{-4} and 10^{-5} no significant differences between the two drugs were observed. At a dilution of 10^{-3} sulfanilamide gave a greater protection than the sulfonhydroxamide during the 4-day period in which the drugs were administered at 4-hourly intervals. This greater effectiveness probably results from the higher average blood level maintained with sulfanilamide (Fig. 2) rather than from a higher chemotherapeutic activity (at equal blood levels). When the surviving animals were given single daily doses of the drugs, the differences observed at this culture dilution disappeared.

2. *Colorimetric Study of Blood Concentrations.* Sulfanilamide determinations were performed by the method of Bratton and Marshall.[5] For the estimation of p-caproylaminobenzenesulfonhydroxamide and its deacylated analogue the following method was devised:

To 1 cc of blood add 19 cc of 95% alcohol, shake, allow to stand for 15 minutes and filter. To 5 cc of the filtrate add 1 cc of 4 N-H_2SO_4 and heat for 75 minutes on a water bath. Cool and

[5] Bratton, A. C., and Marshall, E. K., Jr., *J. Biol. Chem.*, 1939, **128**, 537.

TABLE II.

Materials—A 6-hour culture of streptococcus (1685) equivalent to 1,000,000, 100,000, and 10,000 lethal doses.

Method—Mice were infected intraperitoneally with 0.5 cc of diluted culture and treated orally with 2 mg of sulfanilamide or the sulfonhydroxamide immediately after infection and then at 4-hour intervals for 96 hours. Twelve mg doses were then given daily for 3 additional days.

No. of mice	Drug	Dilution of culture	No. of animals surviving Time in hr								
			24	36	48	60	96	120	144	168	192
15	Amide	10^{-5}	15	15	15	15	15	15	14	14	14
15	Hydroxamide	10^{-5}	15	15	15	15	15	15	14	12	12
15	Amide	10^{-4}	15	15	15	15	13	13	10	8	7
15	Hydroxamide	10^{-4}	15	15	15	14	14	14	11	7	7
15	Amide	10^{-3}	15	15	14	13	9	8	2	1	1
15	Hydroxamide	10^{-3}	15	13	12	10	5	5	3	0	0
5	Controls	10^{-3}	0	0	0	0	0	0	0	0	0
5	"	10^{-4}	0	0	0	0	0	0	0	0	0
5	"	10^{-5}	1	0	0	0	0	0	0	0	0
2	"	10^{-6}	1	0	0	0	0	0	0	0	0
2	"	10^{-7}	2	0	0	0	0	0	0	0	0
2	"	10^{-8}	2	1	0	0	0	0	0	0	0
2	"	10^{-9}	2	2	0	0	0	0	0	0	0
2	"	10^{-10}	2	2	1	1	1	1	1	1	1

Amide—Sulfanilamide.
Hyroxamide—p-Caproylaminobenzenesulfonhydroxamide.

adjust the volume to 15 cc and filter.* The diazotization and coupling are then carried out essentially as in Marshall's procedure. To 10 cc of the filtrate add 1 cc of 0.1% sodium nitrite and mix. After 3 minutes add 1 cc of 0.5% ammonium sulfamate and mix. After 3 minutes add 1 cc of 0.1% N-(1-naphthyl)ethylene diamine dihydrochloride and mix. After 5 minutes add 5 cc of alcohol† and read in the Evelyn colorimeter using filter No. 540. This gives the "total" p-caproylaminobenzenesulfonhydroxamide. The "free" deacylated form is determined by evaporating 5 cc (or 10 cc if the concentration is low) of the original alcoholic filtrate to dryness. Cool, and add 1 cc of 4 N-H_2SO_4 and water to 15 cc. Filter and diazotize 10 cc of this filtrate as described above.

Several series of recovery experiments were performed in which the drug, its deacylated free base, and a mixture of both substances were added to whole blood. An average recovery of 96% (±4)

* Diazotization and coupling cannot be satisfactorily performed in the presence of alcohol due to an alteration of reaction rates which permits nitrosation of the beta component. This is prevented by evaporating off the alcohol.

† This dissolves lipoid material to give an optically clear solution.

was obtained in 30 such experiments. Final values are, therefore, divided by 0.96.

Results of the blood determinations in mice are shown in Figs. 1 and 2. Each point was established by analyzing the pooled blood of 5 mice. Two or more groups of 5 were used for each determination. Following the oral administration of single doses of the drug, blood levels of the free and total sulfonhydroxamide were remarkably constant indicating uniform absorption. Only slight differences in the blood levels were obtained following 5 or 10 mg doses, probably indicating a limited absorption. In equal doses, sulfanilamide affords much higher blood concentrations.

When the drugs were fed at 4-hour intervals, the blood levels of the sulfonhydroxamide were much lower than those obtained with sulfanilamide. However, as shown in Fig. 2, the level was main-

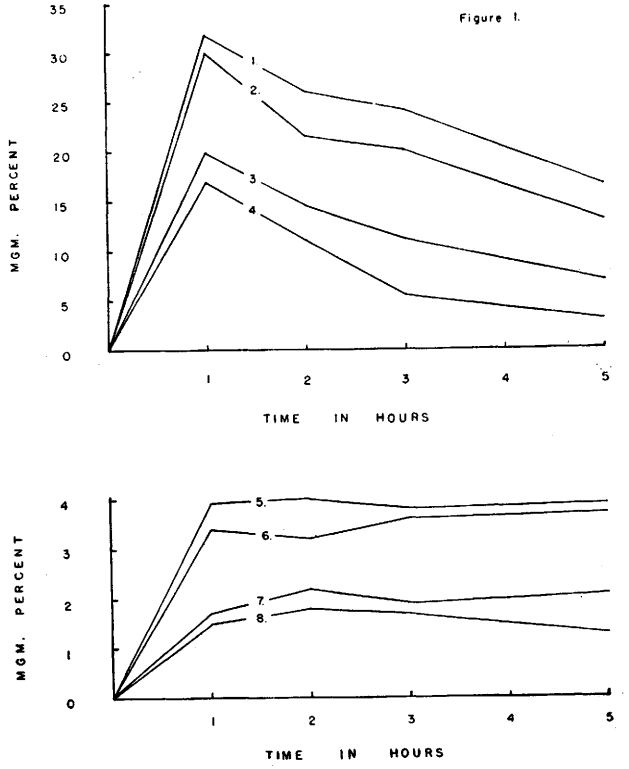

FIG. 1.

Blood Concentrations Following a Single Oral Dose of the Drugs.
Curves 1 and 2: Total and free sulfanilamide following 10 mg dose.
Curves 3 and 4: Total and free sulfanilamide following 5 mg dose.
Curves 5 and 7: Total and free sulfonhydroxamide following 10 mg dose.
Curves 6 and 8: Total and free sulfonhydroxamide following 5 mg dose.

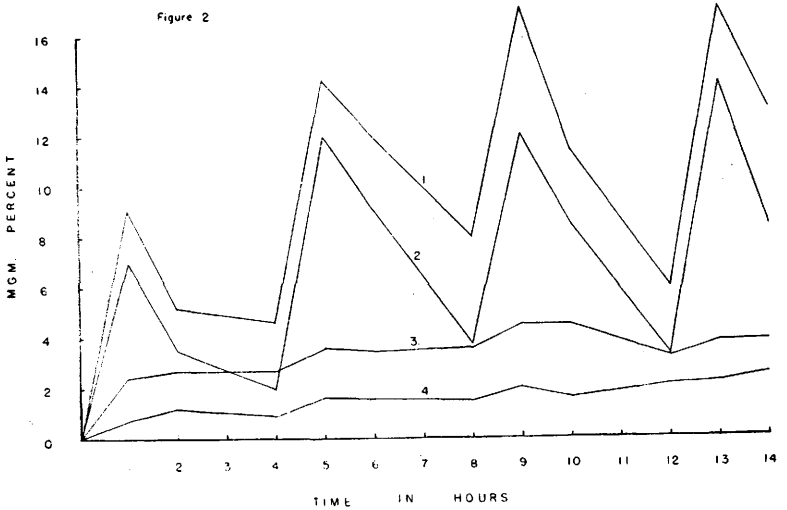

FIG. 2.

Blood concentrations following 2 mg doses of the drugs administered every 4 hours. Curves 1 and 2 represent the total and free sulfanilamide. Curves 3 and 4 represent the total and free sulfonhydroxamide, respectively.

tained much more steadily with the sulfonhydroxamide than with sulfanilamide.

Discussion. Recently Shinn, Main, and Mellon[4] demonstrated an immediate, although transient, bacteriostasis due to the sulfonhydroxamide group of p-caproylaminobenzenesulfonhydroxamide which was followed by a secondary effect due, presumably, to hydrolysis of the caproyl group. Such a mechanism suggests that the sulfonhydroxamide might differ from sulfanilamide in antistreptococcal activity. In agreement with the data of Cooper, Gross, and Lewis[1] we have found no striking differences in the activity of sulfanilamide and the sulfonhydroxamide when the drugs were given every 4 hours by mouth. However, the sulfonhydroxamide produced this effectiveness at lower blood concentrations than sulfanilamide. This difference is even further accentuated by comparison of the blood levels on a molecular basis, since one molecule of the sulfonhydroxamide is equivalent to 1.6 molecule of sulfanilamide.

Since for an optimal therapeutic effect the maintenance of an effective blood concentration is essential,[6] a drug which, because of solubility and other properties, is maintained at constant levels in the blood is desirable. Fig. 2 shows that more constant levels are maintained with the sulfonhydroxamide than with sulfanilamide. This may explain why sulfanilamide and the sulfonhydroxamide

[6] Marshall, E. K., Jr., Litchfield, J. T., Jr., and White, H. J., *J. Pharm. and Exp. Therap.*, 1940, **69**, 89.

are approximately equally effective when fed at intervals of 4 hours, whereas the sulfonhydroxamide is the more effective when the drugs are fed once daily.

Summary. 1. A colorimetric method for the determination of p-caproylaminobenzenesulfonhydroxamide has been described. 2. This drug is approximately equal, weight for weight, to sulfanilamide in antistreptococcal activity when fed at intervals of 4 hours, but it is somewhat more effective than sulfanilamide when fed only once daily. 3. When the sulfonhydroxamide and sulfanilamide were given in equal-weight oral dosage, the former gives the lower and more constant blood levels.

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Inhibition by Sulfapyridine of the Curative Action of Nicotinic Acid in Dogs.

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When *Staphylococcus aureus* is inoculated in a medium deficient in nicotinic acid, the addition of nicotinic acid will support growth, but the addition of nicotinic acid and sulfapyridine will not do so. The addition of coenzymes and sulfapyridine, however, gives excellent growth.[1] Dorfman[2] has shown that sulfapyridine inhibits the increased respiration of dysentery bacilli caused by nicotinic acid amide. The present investigation was undertaken to see if a similar relationship could be demonstrated in dogs on a diet deficient in nicotinic acid.

Young dogs were fed such a diet[3] until weight loss and diarrhea developed, although typical tongue lesions did not appear. Drugs and vitamins were administered orally in gelatin-coated capsules. Sodium sulfapyridine was given at 9 a. m. and 5 p. m. in doses sufficient to maintain a blood level of 3 to 10 mg per 100 cc just before the morning dose. Nicotinic acid was given in doses of

1 *a.* West, R., and Coburn, A. F., *J. Exp. Med.*, 1940, **72**, 91; *b.* West, R., and Coburn, A. F., *Tr. A. Am. Physicians*, 1940, 173.

2 Dorfman, A., Rice, L., Koser, S. A., and Saunders, F., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 750.

3 Koehn, C. J., Jr., and Elvehjem, C. A., *J. Biol. Chem.*, 1937, **118**, 693.