Chemotherapeutic Activity of 6-Sulfanilamido-2,4-Dimethylpyrimidine (Elkosin) (19667)

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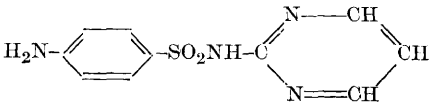
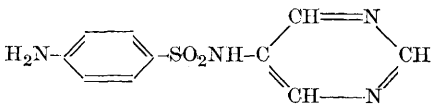
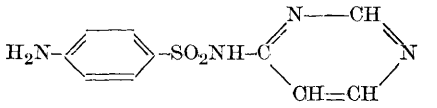
Among the heterocyclic substitutes of sulfanilamide, the sulfanilamidopyrimidines occupy an important place as chemotherapeutic agents because they include several of the most effective and best tolerated sulfonamides known to date. A comparison of the properties of members of this group of compounds clearly demonstrates the marked differences in tolerance and therapeutic activity which take place by slight variations in the substitutions and the attachment of the pyrimidine ring to the sulfanilamide nucleus. 2-Sulfanilamidopyrimidine (sulfadiazine) possesses excellent clinical activity(1) and according to Roblin and coworkers(2,3) it exerts very good protection in experimental mouse infections. On the other hand, the 6-isomer is completely ineffective in the animal body although it exhibits bacteriostatic activity *in vitro* equal to that of sulfadiazine. The 5-isomer was found to have less *in vivo* activity than the 2-isomer.



2-Sulfanilamidopyrimidine (Sulfadiazine)

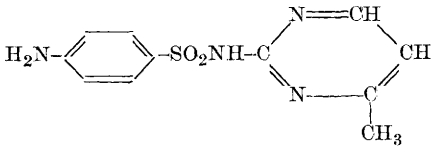


5-Sulfanilamidopyrimidine

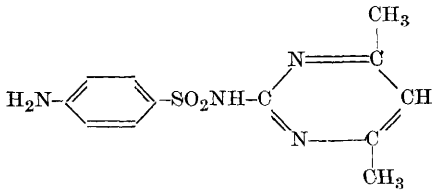


6-Sulfanilamidopyrimidine

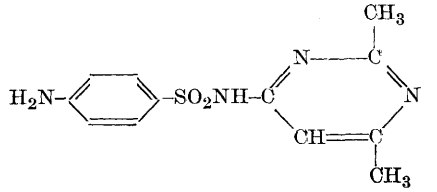
Mono- and di- substitutions on the pyrimidine ring, especially those with one or two methyl groups, also bring about considerable changes with regard to therapeutic and pharmacologic behavior when compared with the parent, unsubstituted sulfanilamidopyrimidines. Of these substituted compounds, 2-sulfanilamido-4-methylpyrimidine (sulfamerazine)(2), 2-sulfanilamido-4,6-dimethylpyrimidine (sulfamethazine)(3,4) and 6-sulfanilamido-2,4-dimethylpyrimidine (Elkosin)(5-7) are of chemotherapeutic importance.



2-Sulfanilamido-4-methylpyrimidine (sulfamerazine)



2-Sulfanilamido-4,6-dimethylpyrimidine (sulfamethazine)



6-Sulfanilamido-2,4-dimethylpyrimidine (Elkosin)

Among the methylated derivatives, the 2,4-dimethyl compound, Elkosin, merits special consideration. According to Meier, Allemann and Meyenburg(5), it is appreciably more soluble in urine over a wide pH range when compared with sulfamethazine and sulfadiazine and has as well, a very low order of acetylation. These workers reported on the broad therapeutic effectiveness of Elkosin in streptococcal and pneumococcal infections in mice, since doses ranging from 0.5 to 24 g/kg administered daily for 6 consecutive days were highly effective.

The results of an experimental study carried out in our laboratory, of the chemotherapeutic properties of Elkosin in a variety of mouse infections are presented in this paper.

TABLE I. Therapeutic Activities of Elkosin, Sulfadiazine and Sulfisoxazole as Indicated by Average Survival Time.

Test organism	Avg survival time (days) of infected mice treated with: Elkosin, g/kg				Sulfadiazine, g/kg				Sulfisoxazole, g/kg				Untreated controls
	5	2.5	1	.5	5	2.5	1	.5	5	2.5	1	.5	
a. Bacilli													
S. pyogenes	21	17.7	15.3	10.2	17.1	17.8	16.2	21	15.6	17.2	16.6	14.9	.4
D. pneumoniae	13	15.2	13.8	6.1	13.1	8.2	16.1	11.5	1.3	10.3	9.3	6	1.4
Cl. tetani	1	.6	.5	1	.5	.7	.6	.7	.6	.8	.8	.7	.6
Kl. pneumoniae	17.3	21	21	21	15.1	13.9	19.5	21	21	20.9	20	17.3	.5
S. typhosa	3.9	7.7	4.2	4.6	3	.8	4.4	5	.5	4.9	6.1	8.2	.5
S. typhi-murium	8	6.1	4	9.1	7.7	8.3	8.9	7.6	8.1	4.3	8.6	4.7	.6
S. schottmuelleri	21	21	18.7	16.7	14.8	21	21	21	18.3	19.3	19.5	19.2	2.2
E. coli	21	20.2	19.5	19.4	13.9	16.4	14.9	18.9	9.6	14.3	21	21	1.6
b. Fungi													
N. asteroides	17.8	18.1	18.1	19.9	9.1	15.9	20	20	6.6	17.7	17.2	13.1	6.2
C. albicans	21	21	—	—	.9	.8	1.2	.9	.6	.8	.8	.7	.8

Methods. Chemotherapeutic tests with CFW mice, each weighing approximately 18 g were performed with the following organisms. *Strep. pyogenes,* strain C203; *Diplococcus pneumoniae,* type 1; *Clostridium tetani; Klebsiella pneumoniae; Salmonella typhosa; Salmonella typhi-murium; Salmonella schottmuelleri; Escherichia coli; Nocardia asteroides* and *Candida albicans.* All animals were *infected intraperitoneally* with 100 to 5000 lethal doses of the various organisms studied. The infecting doses of *Salm. typhosa, Salm. typhi-murium, Salm. schottmuelleri, E. coli, Noc. asteroides* and *Candida albicans* were administered in 4% gastric mucin, prepared according to the method of Runyon(8). For purposes of comparison, sulfadiazine and sulfisoxazole(9) were included in these tests. The latter substance, although not a sulfanilamidopyrimidine, was selected because of its recent introduction for use in urinary tract infections. Suspensions of the test substances were given *per os* one hour before infection. Treatment was continued daily in 2 equally divided doses for 5 days and mice were observed for a total of 21 days. Random subculture of organs from succumbing mice were made in order to verify that death was due to the infecting organisms employed.

Results. A. *Toxicity for mice.* Groups of 10 uninfected mice were given Elkosin, sulfadiazine and sulfisoxazole at daily dose levels of 1.0 and 5.0 g/kg for 5 consecutive days. The drugs were administered *per os* daily in 2 equally divided doses. No deaths occurred as a result of this treatment nor did the mice show any indication of toxic effects. A repetition of this experiment yielded similar results.

B. *Protection tests.* The therapeutic effects of the sulfonamides, shown in Table I, are expressed as "average survival time (days)" per mouse.

Summary. 1. 6-Sulfanilamido-2,4-dimethylpyrimidine (Elkosin) possesses a high degree of effectiveness in experimental mouse infections induced by *Strep. pyogenes, Diplo. pneumoniae* and representative species of the gram negative bacteria. No evidence of protection was observed with *Clos. tetani* and only slight activity was obtained in *Salm. typhosa* infection. 2. Equally favorable results were obtained in infections with *N. asteroides* and *C. albicans.* In the case of the latter infection, Elkosin proved to be highly effective.

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Propagation of Mumps Virus in Suckling Mice and in Mouse Embryo Tissue Cultures. (19668)

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Adaptation of mumps to mice, undertaken to facilitate investigation of this disease, has been accomplished in 2 different ways. In one series of experiments 20 preliminary passages in mouse embryo tissue culture were carried out before virus grew consistently in brains of suckling mice. Later it was found that mumps virus could be more readily adapted to suckling mice by prior passage in suckling hamsters(1). Illness and death occurred only in the latter series of mice, infections being inapparent in those originally inoculated with tissue culture material. It is felt that these investigations have practical interest, as they may pave the way for use of mice in mumps neutralization tests. In addition, mumps infections of mice give some indication of the varied potentialities of this virus as an agent of disease.

Materials and methods. Tissure culture. With modifications, methods employed in tissue culture were ones developed by Earle and associates(2-6). The nutrient fluid consisted of horse serum 40%, balanced saline (Earle's) 40%, and 1:1 extract from 9-day chick embryos, 20%. Antibiotics added to the saline gave it a concentration of 5 mg of streptomycin and 500 units of penicillin per ml. Supporting tissue consisted of mouse embryos in an advanced stage of development. These were comminuted by forcing them through an 18-8 stainless steel sieve of 24 mesh, .014 inch wire diameter(6). Virus containing inocula were mixed directly with tissue pulp and the mixture pipetted into T-60 flasks,* prior to addition of 8 ml of nutrient fluid. Once the cell suspension was evenly distributed over the floor of the flask a sheet of perforated cellophane was dropped on it. Supernatant fluids were drained off and fresh fluid added so as to give a total of 3 fluid changes a week. In later passages, mouse embryo juice was used in the last 2 of these changes. At weekly intervals, the cellular material was scraped from the cellophane, ground, and frozen before using as a virus inoculum for the next tissue culture passage. Roughly 0.5 ml of cellular debris plus fluid were carried over from one passage to another. Precise determination of total dilutions of virus included in the mixture of cells and fluid, was not attempted. Serum ultrafiltrates were assayed in a few alternative passages, but multiplication of virus did not appear to be sustained in this medium. *Virus.* The strain of mumps virus employed for both animal and tissue culture experiments was one originally isolated from human milk(7) and carried through 6 to 7 passages in the amniotic cavity of embryonated eggs. *Mouse passages.* Almost all passages were carried out with A strain mice, although a few regular Swiss mice were employed initially. Suckling mice were inoculated intracerebrally at 1 or 2 days of age and brains were harvested a week later, being ground without abrasive and made up as extracts diluted 1:10 in meat infusion broth.

Identification of virus. Supernatant fluid from each weekly tissue culture passage was inoculated into 7-day embryonated eggs by the amniotic route. Amniotic fluids, harvested separately a week later, were used in hemagglutination tests to determine the presence of

* The design of this flask will be published later.