

Effects of Sulfanilamide and Sulfamethylthiazol* on Experimental *Brucella (var. melitensis)*† Infection in Mice.

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Sulfanilamide and related compounds have been used in the treatment of brucellosis since 1936. Their value, however, is subject to controversy. We have attempted to determine the effect of sulfanilamide and sulfamethylthiazol on the experimental infection in mice.

Experiment I. The organisms were grown for 24 hours on tryptose agar slants and the growth was suspended in saline to give a turbidity comparable to McFarland's barium sulphate standard No. 1. Mice were inoculated intraperitoneally with varying amounts of this suspension and 0.1 cc was found to kill the majority of animals in from 4 to 5 days. This dose was employed in Experiment I.

The Drugs. Sulfanilamide and sulfamethylthiazol were used in a one percent solution and a one percent suspension in water respectively. The powder was moistened with a small amount of distilled water and made in a mortar into a fine paste which was suspended in the required amount of water. An even suspension was secured by thorough agitation just prior to the administration of the drug, which was given by mouth with a 1 cc tuberculin syringe and a 1½-inch 20-gauge needle with a blunt end. A dose of 0.5 cc (corresponding to 5 mg of the drug) was given twice daily for 2 days; from there on, once daily until the fifth day when the treatment was suspended.

The animals were kept under careful observation on a diet of Purina dog chow and water during 15 days, at the end of which the survivors were killed and autopsied.

Sixty mice were used. The animals weighed 18 to 20 g and were from 5 to 6 weeks old. They were divided into 5 groups of 12 mice each, as follows:

Group I: *Brucella melitensis* alone.

Group II: Organisms plus sulfamethylthiazol.

Group III: Organisms plus sulfanilamide.

* Kindly supplied to us by Mr. Rassow, local agent of the Winthrop Chemical Company.

† The strain (No. 2456) utilized in these experiments was obtained through the kindness of Dr. F. I. Huddleson. This strain was isolated from human blood, February 14, 1939.

Group IV: Sulfamethylthiazol alone.

Group V: Sulfanilamide alone.

Of the 12 mice belonging to Group I, 9 died before the fifteenth day with an average survival period of 5.8 days. Three animals survived the experimental period. Of these, 2 gave a negative, and one a positive blood culture. *Brucella* organisms were cultured from the spleen of all 3 and from cheesy material found in the peritoneum, in the region of the genital organs, and from the lymph nodes in one male animal. From the 9 mice which died during the experimental period, organisms were recovered from the blood in 6 cases. The other 3 were decomposed when found and were not autopsied.

Of the 12 mice treated with sulfamethylthiazol (Group II) 2 died immediately after the administration of the drug during the course of the experiment. Five died during the experimental period with an average survival period of 14.8 days, and 5 were still alive after 15 days. Four of the 5 mice which died within the experimental period were autopsied, and 3 gave a positive blood culture. *Brucella* organisms were obtained in pure culture from the spleens of all 4. From the spleens of the 5 survivors, the *Brucella* organisms were recovered in all cases, but from the blood the organism could only be cultured once.

In Group III (with sulfanilamide), one died immediately after the administration of the drug during the course of the experiment. Six died during the experimental period with an average survival period of 11 days, and 5 were still alive after 15 days. Blood cultures were not attempted, except in one case, from the animals which died during the experimental period because they were decomposed when found. In the case autopsied, organisms were recovered in pure culture from the blood and from the spleen. The 5 survivors were autopsied and none of them showed the organism in blood culture. However, the organism was isolated from the spleen in every case.

In Groups IV and V, where the animals received the drug alone, all survived the experimental period in apparent good health, except one, which died immediately after the administration of the drug.

Comments. Sulfanilamide and sulfamethylthiazol, administered by mouth during 5 consecutive days, markedly extended the survival period of mice infected intraperitoneally with *Brucella melitensis*. Sulfamethylthiazol was more effective than sulfanilamide. Under the conditions described, these drugs inhibited the infection, but the organisms were not killed in the tissues of the host, although in some instances the number of colonies was small, indicating a marked

inhibition of the proliferation of the organisms in the tissue.

In order to corroborate these results, a second experiment was made.

Experiment II. A stock culture of the same strain employed in Experiment I was inoculated into a mouse and the organisms recovered from the heart's blood were used in the preparation of a saline suspension which was adjusted to the same turbidity as before.

Sixty mice were utilized, divided into 3 groups as follows:

Group A: Sulfanilamide plus organisms.

Group B: Sulfamethylthiazol plus organisms.

Group C: Organisms alone.

The mice were given a single dose (0.5 cc) of the drug by mouth and immediately inoculated intraperitoneally with 0.1 cc of the *Brucella melitensis* suspension. Groups A and B were kept thereafter on a diet containing one percent of the drug.‡ Group C was kept on a diet of Purina dog chow and water.

Two weeks after initiating the experiment, 9 animals in Group A, 7 in Group B, and 18 in Group C had died. The great majority of animals could not be autopsied due to advanced decomposition. Three animals in Group B (2, 4 days, and one, 7 days after inoculation) were the only cases autopsied, and in all 3 the specific organism was recovered from the blood. Thirty-six days after inoculation, the animals still alive (11 in Group A, 13 in Group B, and 2 in Group C) were killed and autopsied and cultures made from the heart's blood and splenic tissue in every instance. The amount of splenic pulp used for culture was approximately the same in all cases. Material was smeared on the surface of freshly prepared tryptose

TABLE I.
Results of Cultures of Blood and Splenic Tissue of the Mice Which Survived the Experimental Period of 36 Days.

Treatment	No. of animals	Spleen		Blood	
		Positive	Negative	Positive	Negative
Sulfanilamide	11	10*	1	0	11
Sulfamethylthiazol	13	8†	5	0	13
None	2	2‡	0	0	2

*In one case growth was abundant. In the rest, only few colonies on slant.

†Few colonies in all.

‡Abundant growth in both instances contrasting markedly with the comparatively poor growth obtaining from the treated animals.

‡ Purina dog chow was ground to a fine powder. The sulfanilamide was also ground to a fine powder and intimately mixed with the food. The same was done with the sulfamethylthiazol which was supplied to us in powder form.

agar slants and incubated aerobically during 4 days at 37°C. The results are summarized in Table I.

Comments. Sulfamethylthiazol is more effective than sulfanilamide in the treatment of experimental *Brucella* infection in mice. The results suggest that the proliferation of the organisms is only partially inhibited by treatment with a tendency towards the establishment of a chronic infection. These drugs may be of use in the study of chronic infection in the experimental animal.