

Use of Sulfonamides in Treatment of Experimental *Clostridium welchii* Infection.

LUCILE R. HAC* AND AGNES C. HUBERT.† (Introduced by H. Close Hesseltine.)

From the Department of Obstetrics and Gynecology, The University of Chicago and The Chicago Lying-In Hospital.

The efficacy of oral sulfonamide therapy in experimental *Cl. welchii* infection in mice has been discussed previously.¹ In that study, it seemed desirable to produce a concentration of drug in the blood of 10-20 mg %. This required that the drug be administered in the food 3 to 4 days before inoculation with *Cl. welchii*, and consequently the results obtained were viewed more from the standpoint of prophylaxis than treatment, although all the animals showed a generalized infection 6 to 18 hours after inoculation, and had large lesions at the site of inoculation.

The present study was undertaken in order to evaluate more accurately the treatment of this infection, and to determine the effect of high local concentrations of the drugs on the lesion which resulted at the site of inoculation.

Infection was produced as previously described¹ by intramuscular inoculation into the inner aspect of the thigh of a whole, unwashed, 18-hour culture. No other necrotizing agent was used. The amount of inoculum used was sufficient to kill 90-100% of the untreated animals. This amount had been determined by previous standardization (usually 0.25 ml of a 1:4 dilution of culture was used), and as a further check 10 control animals were inoculated with each group of animals studied.

Various suspensions of sulfadiazine, sulfathiazole, and sulfanilamide were used; 0.25 ml or 0.1 ml of a 20%, 10%, 5%, or 1% suspension was injected subcutaneously into

the inguinal region of the infected leg, before, at the same time, and at 1-, 2-, and 3-hour intervals after inoculation with *Cl. welchii*.

The suspensions of the drug were prepared in honey and water. For a 20% suspension: 2 g of drug was finely pulverized in a mortar and 2.4 ml of honey added gradually with continued grinding. When a smooth paste had been prepared 7.6 ml of water was added drop-wise with stirring. Smoother suspensions could be obtained with sulfadiazine than with the other drugs. All the suspensions tended to precipitate slowly, but if shaken frequently, they proved entirely satisfactory for injection with a 26-gauge needle.

At first it was thought that with this technic of injecting the drug near the site of inoculation it would be possible to study the local effect of the drug. Actually, it proved to be a means of producing high sustained concentrations of the drugs in the blood. Charts 1 and 2 give the curves of the concentrations of the drugs (free-sulfonamide) in the blood when 20% and 5% suspensions of the drugs were injected. Each point on the curve represents the average of 10-12 determinations. Since equipment for micro-determinations was not available, it was necessary to sacrifice an animal for each determination.

The trends of the curves are not altered appreciably by the amounts of drug conjugated as the acetyl-sulfonamide, nor by varying the concentration of honey used. The initial rise, therefore, seems to be dependent upon the water content. The tremendous concentrations of drug in the blood obtained with the 20% suspension of sulfanilamide proved so toxic that most of the animals died within 6-10 hours.

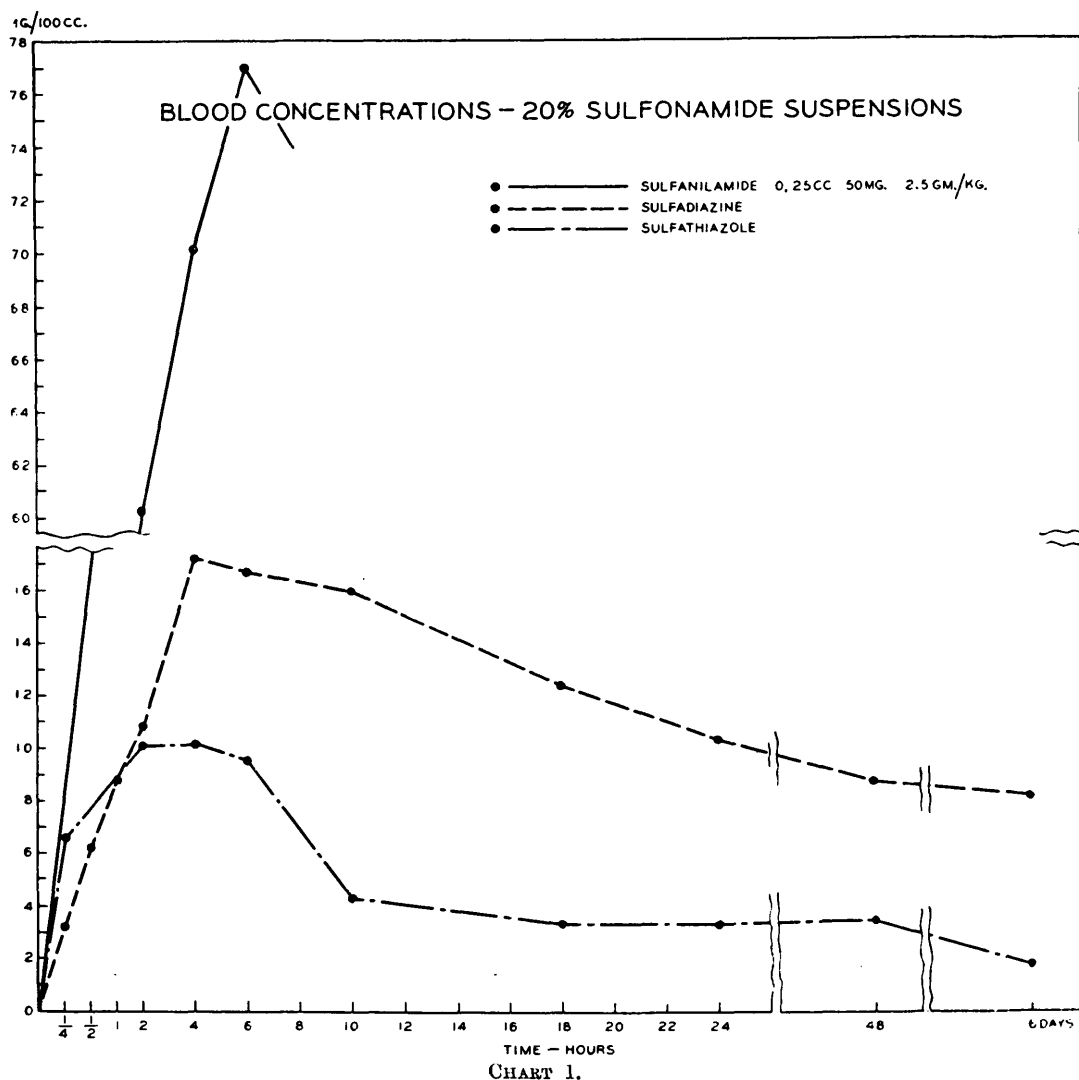
It was of interest to know which drug, what dosage, and what height of blood concentration was necessary to produce the best survival rates in infected animals. The results

* A. B. Kuppenheimer Foundation.

† Joseph B. DeLee Fellow 1942-43.

The sulfonamides used in this study were furnished through the courtesy of Lederle Laboratories, Inc. (sulfadiazine), and the Department of Medical Research, Winthrop Chemical Co. (sulfathiazole and sulfanilamide).

¹ Hac, L. R., Eilert, M. L., and Adair, F. L., *Proc. Soc. Exp. Biol. and Med.*, 1942, **51**, 108.



(averages of repeated experiments) obtained with various doses of the drugs, when a single injection of the drug was made at the same time as, and at 1-, 2-, and 3-hour intervals after the inoculation with *Cl. welchii* are given in Table I.

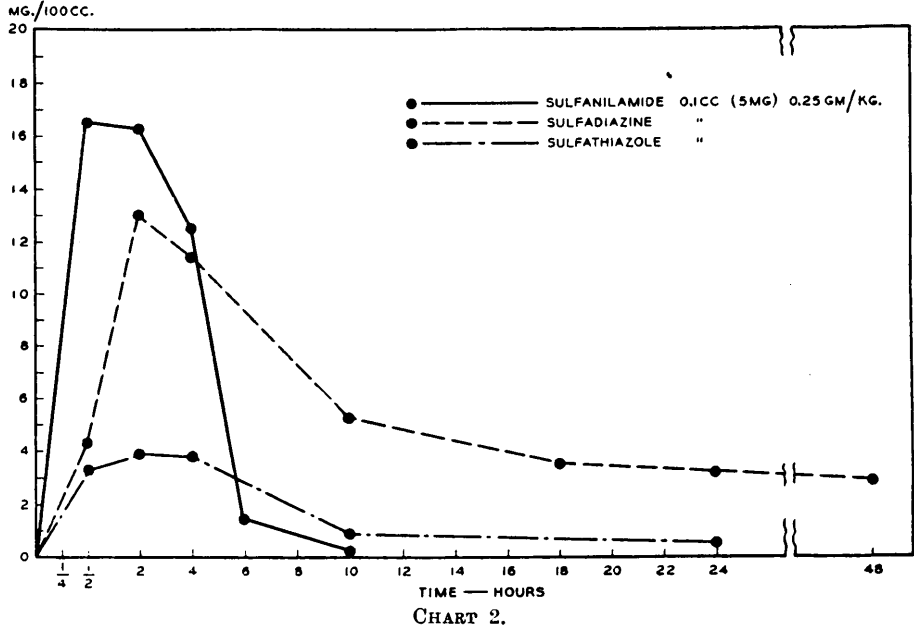
Sulfadiazine seems to be only very slightly, if at all, more efficacious than sulfathiazole, but both are considerably more effective than sulfanilamide. Repeated injections of the drug in order to maintain the initial high concentration of drug in the blood proved no more efficacious than a single dose, and, surprisingly, a 1 mg dose protected as effectively as a 50 mg dose.

No better survival was obtained when the drug was injected into the inguinal region of the infected leg than when similarly injected into the non-infected (opposite) leg, which would seem to indicate that protection was due largely to the concentration of drug in the blood rather than to the local concentration.

Protection was markedly decreased by a delay of one hour, and there was very little protection after 2 hours.

Each animal had a large lesion at the site of inoculation, similar to that described when oral therapy was used. Most of the animals were killed at the end of one week, but ob-

CONCENTRATIONS OF DRUGS IN THE BLOOD. 5% SULFONAMIDE SUSPENSIONS



servations of the lesion were made in some for as long as 6 weeks when healing had usually become complete.

Summary. 1. A suspension of sulfonamides injected subcutaneously into the inguinal region of mice produced high sustained concentrations of the drug in the blood. 2. A single dose of a suspension of sulfathiazole or

sulfadiazine injected immediately following inoculation with *Cl. welchii* protected 45-53% of the mice. A 1 mg dose gave as much protection as a 50 mg dose. A delay in therapy of 1 or 2 hours markedly reduced the protective action. Sulfanilamide gave very little protection.

TABLE I.
Effect of Drug, Dosage, and Time of Therapy on *Cl. welchii* Infection.

Time	Dose, mg	No. animals	% survival		
			Sulfathiazole	Sulfadiazine	Sulfanilamide
3 hrs before <i>welchii</i>	50	30		97	
Same time as "	50	100	45	53	
" " " "	5	100	40	48	30*
" " " "	1	100	50	51	10*
1 hr after "	50	50	20	38	0*
2 " " "	50	50	14	16	
3 " " "	50	25	12	8	

* 30 animals.