ions through the membrane is indicated by the intensity of the color (red) in the tubes Although frog skins show a wide range of individual variation a careful comparison of symmetrical skin-areas seemed justified. Atropine 10^{-5} resulted in a decrease of permeability of the membrane for thiocyanate as indicated by the color test, while higher concentrations (10^{-3}) showed no definite effect. The same was true of the stain taken up by the membranes after 24 hours. These results suggest that prolongation of procaine anesthesia by atropine may be due to changes in permeability.

Results obtained with other drugs of the atropine group, acetylcholine, as well as clinical trials will be reported elsewhere; it may be stated here that syntropan seems to be superior to atropine.

Summary. Prolongation of procaine HCl anesthesia by addition of atropine sulfate in low concentration 10^{-5} is reported; tests were made on rabbit's cornea after subconjunctival injection. The possibilities of an effect of low amounts of atropine sulfate on permeability are discussed.

Acknowledgment is due to Prof. A. J. Clark (University of Edinburgh) for suggestions and interest in the first stage of these experiments and to the Earl Moray Fund of the University of Edinburgh for financial aid.

11597

Sulfanilamide, Sulfapyridine and Sulfathiazole and Experimental Infections in Mice Due to *Shigella paradysenteriae* Flexner.

MERLIN L. COOPER AND HELEN M. KELLER. (Introduced by Glenn E. Cullen.)

From the Children's Hospital Research Foundation, and the Department of Pediatrics, College of Medicine, University of Cincinnati.

Our earlier studies[1] of acute diarrhea in 207 infants and young children revealed that 50% of the patients who were admitted to the Children's Hospital during the summer of 1938 with diarrhea had dysentery bacilli *(Shigella paradysenteriae)* in their stools. The majority of these patients were infected with the Flexner type, a few with the Sonne type. The mortality was 17%. With the advent of sulfanilamide and allied compounds and a search of the

[1] Cooper, M. L., Furcolow, M. L., Mitchell, A. G., and Cullen, G. E., *J. Pediat.*, 1939, **15**, 172.

literature failing to find that such drugs had been used in experimental infections with Flexner type of dysentery bacilli, animal experiments were planned to determine the effectiveness of sulfanilamide, sulfapyridine and sulfathiazole.

The culture used had been isolated a year previously from the stool of an infant acutely ill with dysentery. Colony studies on MacConkey's medium revealed "rough" and "smooth" colonies. The "smooth" were avirulent in broth and in 3% mucin. The "rough" were slightly virulent in broth and quite virulent in 3% mucin. The virulence in mucin varied some, but a 20-hour Difco Brain Heart Infusion Broth culture diluted 1-100,000 in freshly prepared 3% autoclaved suspension of mucin killed the majority of white mice

TABLE I.

Therapeutic Effectiveness of Sulfanilamide, Sulfapyridine, and Sulfathiazole Administered in Diet. ½ cc of 1:100,000 culture in 3% mucin given intraperitoneally.

Mice	Diet plus 1% sulfathiazole ad lib. before culture	Diet plus 1% drug ad lib. after culture	Results
6	2 days	Sulfathiazole	All survived
6	3 "	"	" "
6	4 "	"	" "
6		Sulfanilamide	2 died
6		Sulfapyridine	6 "
6		Sulfathiazole	3 "
18			14 "

TABLE II.

Therapeutic Effectiveness of Sulfanilamide, Sulfapyridine and Sulfathiazole When Administered by Stomach Tube and in the Diet. ½ cc of 1:100,000 culture in 3% mucin given intraperitoneally.

Mice	Drug by stomach tube, 3, 6, 17, 27 hours after culture 4 doses*	mg	Results Died
6	Sulfanilamide	2	0
6	"	1	1
6	"	½	5
6	"	1/10	3
6	Sulfapyridine	2	0
6	"	1	0
6	"	½	0
6	"	1/10	5
6	Sulfathiazole	2	0
6	"	1	0
6	"	½	0
6	"	1/10	1
18			16

*Immediately after the last dose of drug by stomach tube the mice were placed on a diet, *ad libidum*, for 7 days containing 1% of the respective drug administered previously by stomach tube.

when injected intraäbdominally in ½ cc doses. The white mice weighed 17 to 20 g each.

Early in our experiments we found, as seen in Table I, that all mice were protected if given, *ad libidum,* a complete diet, to which had been added 1% of sulfathiazole, for 2 or more days before and continued for 7 days after injecting the organisms. The animals remained apparently normal. Table I shows also that sulfanilamide, sulfapyridine, and sulfathiazole were only partially effective therapeutically when administered in the diet *ad libidum after* injection of the culture. All the mice became sick within 5 hours after injecting the bacteria and since they ate very little food there was a low intake of drug.

In Table II is seen the therapeutic efficiency of surprisingly small doses of sulfanilamide, sulfapyridine, and sulfathiazole when given by stomach tube. All the mice survived in previous experiments in which 10 mg to 5 mg of these drugs were administered by stomach tube. All of 18 mice survived when treated with 2 mg of these drugs. Only one of 18 receiving 1 mg died, while 5 of 18 receiving 0.5 mg died, and 9 of 18 receiving 0.1 mg died. These mice had 4 doses of the drugs and were then given, *ad libidum,* the diet containing 1% of the respective drugs. Administration of the drugs by stomach tube apparently carried the majority of the mice through the early hours of acute infection, after which they ate sufficient food to insure adequate drug intake to obtain a therapeutic effect. All the mice were observed for a month and remained normal.

In the experiment set forth in Table III, one quantity, 2 mg of sulfanilamide, sulfapyridine, and sulfathiazole, was the standard dose throughout. The time elapsing between the injection of the organ-

TABLE III.

Effect of Delaying the First Dose of Drug on the Therapeutic Efficiency of Sulfanilamide, Sulfapyridine, and Sulfathiazole.

Mice	Culture 1:100,000 in 3% mucin	2 mg drug by stomach tube at indicated hours after the culture*	hr	Results Died
6	½ cc intraperit.	Sulfanilamide	*2*, 8, 22, 27	0
6	,, ,,	,,	*3*, 9, 22, 27	0
6	,, ,,	,,	*4*, 10, 22, 27	0
6	,, ,,	,,	*5*, 11, 23, 28	0
24	,, ,,	Sulfapyridine	,, ,, ,, ,,	0
24	,, ,,	Sulfathiazole	,, ,, ,, ,,	0
12	,, ,,			9

* Immediately after the last dose of drug by stomach tube the mice were placed on a diet, *ad libidum,* containing 1% of the respective drug administered previously by stomach tube.

ism and the administration of the first dose of drug by stomach tube varied between 2 and 5 hours. This interval was without significance, however, since all the mice survived. All the mice were very sick within 5 hours after injecting the culture and the rapid favorable response to the drugs was marked. All the mice were observed for a month and remained well.

Blood level determinations on pooled blood from mice which had been on each of the 1% drug diets for 7 days gave the following data:

Sulfanilamide-free	5.8	mg	%	and	conjugated	2.9	mg	%
Sulfapyridine	7.3	"	"	"	"	1.1	"	"
Sulfathiazole	2.9	"	"	"	"	0.5	"	"

After this paper had been submitted for publication our attention was called to the work of Buttle,[2] who cured mice infected with the Sonne type of dysentery bacilli by administering 25 mg of sulfanilamide twice daily for 7 days. This worker used only Sonne cultures because he had " . . . not yet obtained Shiga or Flexner strains of sufficient virulence to enable us to test the effect of the drug in these infections."

Summary. All mice survived when fed, *ad libidum,* a complete diet containing 1% of sulfathiazole for 2 or more days before and 7 days after injecting *Shigella paradysenteriae* Flexner. Seven of 18 mice survived when offered, *ad libidum,* 1% drug diets of sulfanilamide, sulfathiazole, and sulfapyridine immediately after injecting the organisms. Fifty-seven of 72 mice survived when given 4 feedings by stomach tube of these 3 drugs in doses varying between 2 mg and 1/10 mg. All of 72 mice survived when given 4 feedings of 2 mg of these 3 drugs by stomach tube, regardless of whether the first dose of drug was administered 2, 3, 4, or 5 hours after the injection of the organisms. Nine of 48 control mice survived.

[2] Buttle, G. A. H., *Proc. Roy. Soc. Med.*, 1937, **31**, 154.