wise susceptible, but in somewhat lesser degree, to similar injections of rabbit-*avirulent,* and hitherto supposedly harmless, strains of *C. diphtheriæ;* about 85% of the mice showing highly characteristic reactions within 10 days. (c) Mice receiving minute doses of toxin intracerebrally exhibit the same definitive and fatal results observed in the mice receiving the rabbit-virulent and rabbit-avirulent strains. (d) A number of cultures of diphtheroids *(C. xerosis* and *C. pseudodiphthericum)* were harmless for mice when injected as indicated above. Several other organisms have failed to produce any reactions in any way resembling those produced by *C. diphtheriæ* and diphtheria toxin.

11617

Comparative *in vitro* Study of Various Sulfanilamide Derivatives on Typhoid-Dysentery Organisms.

CHARLES H. RAMMELKAMP AND MARJORIE L. JEWELL. (Introduced by Chester S. Keefer.)

From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston, Mass.

Following the introduction of sulfathiazole by Fosbinder and Walter[1] it soon became evident that the thiazole derivatives exhibited bacteriostatic action against a number of organisms *in vitro.*[2-5] Previous experience in investigating infections of the urinary tract[6] due to *B. coli, B. lactis aerogenes* and *B. proteus* suggested that sulfathiazole might be an effective bacteriostatic agent against other Gram-negative organisms. The present study was undertaken to determine the comparative action *in vitro* of sulfanilamide, sulfapyridine, sulfamethylthiazole and sulfathiazole on representative organisms of the typhoid-dysentery group. The organisms used in this study were *Eberthella typhosa, Salmonella paratyphi, Salmonella schottmuelleri, Shigella dysenteriæ, Shigella paradysenteriæ*

1 Fosbinder, R. J., and Walter, L. A., *J. Am. Chem. Soc.,* 1939, **61**, 2032.

2 Long, P. H., and Bliss, E. H., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 324.

3 Rammelkamp, C. H., and Keefer, C. S., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 664.

4 Lawrence, C. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 92.

5 Hill, J. H., *J. Urol.,* 1940, **43**, 491.

6 Rammelkamp, C. H., and Stoneburner, L. T., *New Eng. J. Med.,* in press.

(Flexner) and *Shigella sonnei*. Broth, urine and blood were used as media to study the effect of the various drugs.

Effect in Veal Infusion Broth. Methods. The chemical-broth media was prepared by adding powdered crystals to veal infusion broth so that the final concentration was 10 mg per 100 cc. The media was then sterilized by heating at 56° C for 2 hours. Sixteen-hour broth cultures of all organisms were used. To 5 cc of the control and broth containing the various drugs 0.5 cc broth dilution of the 16-hour culture was added. Incubation was carried out at 37°C for 24 hours. At specific intervals 0.5 cc of the culture was removed, proper dilutions made, and agar plates poured. These agar plates were then incubated for 24-48 hours and the colonies counted.

Results. Chart 1 shows in graphic form the results of these experiments in veal infusion peptone broth. The growth curves of the organisms using both a large and a small inoculum are recorded.* In general little bacteriostatic effect resulted when the original culture

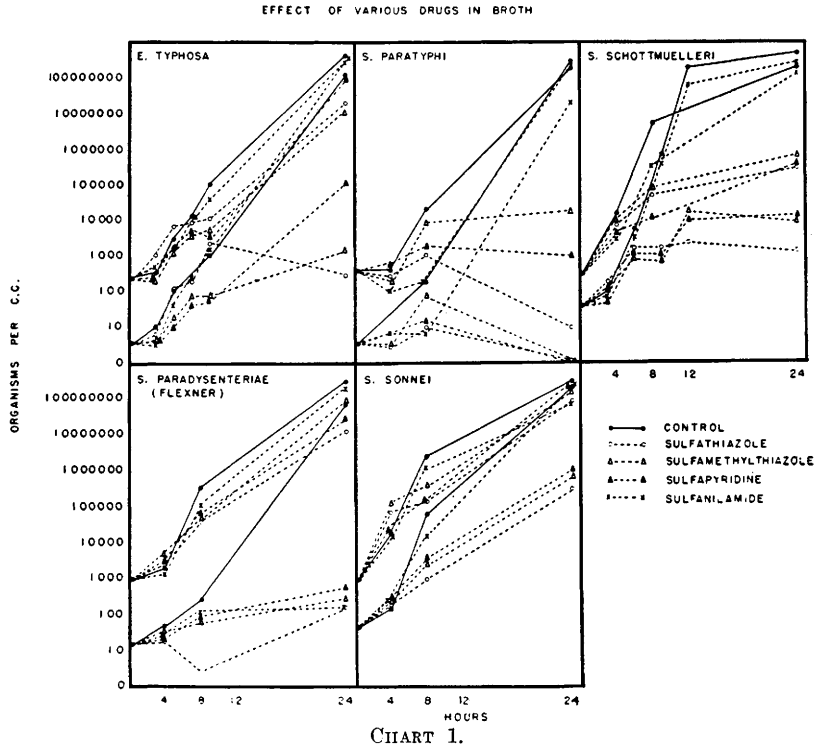

CHART 1.
The concentration of each drug is 10 mg per 100 cc.

* Growth curves using *Shigella dysenteriæ* (Shiga) are not reported because of failure of this organism to grow well in plain broth.

contained over 100 organisms per cubic centimeter. With the use of a large inoculum, sulfanilamide failed to inhibit the growth in any instance, whereas sulfathiazole, sulfamethylthiazole, and sulfapyridine showed some bacteriostatic action. In the experiments using *S. paratyphi* and *S. schottmuelleri* this inhibiting effect was striking.

The bacteriostatic effect becomes more apparent when small inocula are used; however, even then sulfanilamide showed no effect except in the case of *Shigella paradysenteriæ* (Flexner). Sulfathiazole usually caused more bacteriostasis than either sulfamethylthiazole or sulfapyridine, but the difference is not marked. In all curves there is an initial growth period of at least 4 hours before inhibition by the various drugs was noted.

Effect in Urine. Methods. The urine used in these experiments was obtained from pooled samples of urine from normal individuals

TABLE I.
Action of Various Drugs in Urine.

Organisms	Inoculum organisms per cc	Colonies per cc—24 hours				
		Control	Sulfanilamide	Sulfapyridine	Sulfamethylthiazole	Sulfathiazole
E. typhosa	75,000	112,000,000	1,000	8	24	28
	1,400	312,000,000	300	48	56	72
	120	263,000,000	4	8	0	0
S. paratyphi	6,300	115,000,000	1,510,000	4	2,400	0
	1,780	458,000,000	29,600	12	0	8
	100	6,640,000	418	9	2	0
	4	25,000	10	0	0	0
S. schottmuelleri	42,000	117,000,000	4,520,000	580	4	280
	1,500	87,000,000	2,750,000	104	40	14
	300	296,000,000	600,000	528	328	404
	50	112,000,000	4,000	12	4	0
	30	68,000,000	100,000	0	0	0
S. paradysenteriæ (Flexner)	15,000	98,000,000	20	8	0	8
	5,400	68,000,000	40	4	8	0
	830	125,000,000	68,000	0	0	0
	66	2,000,000	4	4	0	0
S. sonnei	21,000	71,000,000	20,000	200	12	0
	5,100	169,000,000	100,000	340	604	900
	540	260,000	20	4	8	0
	60	180,000,000	40	4	24	4
S. dysenteriæ	42,000	1,200,000	1,000	28	200	12
	5,200	128,000,000	78,000,000	14,400	1,200	72
	820	134,000,000	72,000,000	500	160	400
	34	330,000,000	520,000,000	320	32	0

The final concentration of each drug was 10 mg per 100 cc.

and adjusted to pH 7.0. The various drugs were added so that the final concentration was 10 mg per 100 cc. The urine was then passed through a Berkefeld filter. All dilutions of the cultures were made in 0.85% sodium chloride. 0.5 cc of the proper dilution was then added to 5 cc of the control and the urine containing the various drugs. Growth curves were then done following the technic used above.

Results. In Table I the results of the *in vitro* studies using urine as the media are recorded. Since the growth curves are similar in character to those in Chart 1, only the number of organisms present after 24 hours' incubation are recorded. Again, experiments using large and small inocula are included for each organism. In the control cultures, growth was maximal in most instances. Sulfanilamide caused some inhibition of growth in the majority of experiments, however, the urine was never sterilized after 24 hours of incubation. The other drugs caused not only inhibition, but also some bactericidal action. Sulfathiazole and sulfamethylthiazole were only slightly superior to sulfapyridine.

Effect in Blood. Methods. Because relatively large numbers of typhoid-dysentery organisms are killed in normal defibrinated blood, the media was prepared as follows. Venous blood was obtained from normal individuals, defibrinated, and the serum separated by centrifugalization. The serum was then placed in a water bath at 57°C for 60 minutes. The red cells were washed in saline and added to the treated

TABLE II.
Action of Various Drugs in Blood.

Organism	Inoculum organisms per cc	Colonies per cc—24 hours				
		Control	Sulfanilamide	Sulfapyridine	Sulfamethylthiazole	Sulfathiazole
E. typhosa	50,000	268,000,000	55,000,000	340,000	1,520,000	290,000
	1,290	50,000,000	12,000	4	24	24
S. paratyphi	7,000	180,000,000	3,000,000	164	120	40
S. schottmuelleri	6,100	85,000,000	104,000,000	1,040	600	264
	400	50,000,000	72,000,000	36	4	48
S. para dysenteriæ (Flexner)	41,500	23,000,000	12	28	4	4
	300	19,000,000	15,000	48	148	100
S. sonnei	31,700	73,000,000	44,000,000	130	124	84
	2,300	122,000,000	30,000	900	36	24
S. dysenteriæ	6,400	44,000,000	80	8	8	8
	200	4,000,000	12,000,000	60	2	0

The final concentration of each drug was 10 mg per 100 cc.

serum in a concentration of about 10%. To 3 cc of this complement free blood, 0.6 cc of a 70 mg/100 cc saline solution of the various drugs and 0.6 cc of the proper saline dilution of the 16-hour broth cultures were added. The final concentration of the drug in the culture was 10 mg/100 cc. Growth curves were then followed, using a technic similar to that outlined above.

Results. Again, only the number of organisms present after 24 hours' incubation is recorded in Table II, however, the growth curves showed that the inhibiting effect, when present, did not become manifest until after 4 hours' incubation. Sulfanilamide usually caused some inhibition of growth. Sulfapyridine, sulfamethylthiazole and sulfathiazole all caused bacteriostasis in every experiment and, indeed, some killing of the organisms was noted.

Discussion. The results of the *in vitro* studies in broth confirm in part the experiments reported by Lawrence.[7] He found that sulfathiazole and sulfamethylthiazole were somewhat more effective than sulfanilamide and sulfapyridine in dextrose broth against *E. typhosa, Shigella dysenteriæ, S. paratyphi* and *S. schottmuelleri.* In his experiments, the largest inoculum used was 185 organisms per cc. Although he reported some degree of bacteriostasis, in only one instance was there bactericidal action as well.

When urine or blood was used as the culture media, bactericidal as well as bacteriostatic effects were demonstrable. The difference between sulfathiazole, sulfamethylthiazole and sulfapyridine on the test organisms used in blood and urine is not marked, however, all 3 drugs showed a greater effect than sulfanilamide.

Conclusions. The effect of sulfathiazole, sulfamethylthiazole, sulfapyridine and sulfanilamide in concentrations of 10 mg per 100 cc on large and small inocula of *E. typhosa, S. paratyphi, S. schottmuelleri, Shigella dysenteriæ, Shigella paradysenteriæ* and *Shigella sonnei* in veal infusion peptone broth, urine and blood was studied. It was found that sulfanilamide had little bacteriostatic effect in broth cultures, whereas, if a small inoculum is used, sulfathiazole, sulfamethylthiazole and sulfapyridine showed a bacteriostatic action. In urine and blood, sulfanilamide usually caused some inhibition of growth. The action of sulfathiazole, sulfamethylthiazole and sulfapyridine in urine and blood was not only bacteriostatic but was often bactericidal. Sulfathiazole was only slightly superior to sulfamethylthiazole and sulfapyridine when urine and blood were used as media.

Of the 6 organisms studied, *S. paradysenteriæ* (Flexner) appears

[7] Lawrence, C. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 162.

to be most susceptible to the action of the sulfonamide drugs. No further conclusions as to the comparative susceptibility of the strains used in this study seems justified.

We are indebted to Squibb Institute for Medical Research for the sulfathiazole and to Winthrop Chemical Company for the sulfamethylthiazole used in these experiments.

We wish to thank Miss Eleanor M. Fleming for her technical assistance.

11618

Effect of Ether-Soluble Fraction of Bile on Hepatic Glycogen Storage.

R. W. VANCE, E. SNAPP AND A. C. IVY.

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

Mizuta and Ikegami[1] have reported the presence in cattle bile of an ether-soluble substance which promotes glycogen formation in the liver. We decided to repeat their experiments because the data presented are not convincing and the concept may possess significance.

Methods. We have conformed as closely as possible to the general experimental plan of the Japanese observers. Young adult rabbits were fed our standard stock diet for 2 weeks. They were then placed in a stock in a wire cage to prevent coprophagia and fasted for 72 hours. The animals were then given 2 or 4 g of glucose per kilo body weight slowly intravenously (10 minutes) and 30 minutes later were given the bile extract similarly. 2.5 hours later, the animals were given pentobarbital intravenously, which induces anesthesia immediately. The liver was frozen *in situ* with CO_2 within a minute. The frozen liver was crushed to powder and a weighed portion placed in 30% KOH, digested and analyzed for glycogen by Somogyi's method. The results were calculated and expressed as the percent of glycogen in the powder.

The extracts were made from freshly frozen cattle bile. One extract, A, was made by rendering the thawed bile slightly alkaline (pH 8.0), evaporating to dryness *in vacuo* at a low temperature for 24 hours, and extracting the powder with ether and removing the ether *in vacuo*. A second extract, B, was made by slightly acidifying the thawed bile (pH 4.0) with phosphoric acid, steam distilling for

[1] Mizuta, N., and Ikegami, Y., *Jap. J. Gastroenterol.*, 1937, **9**, 258.