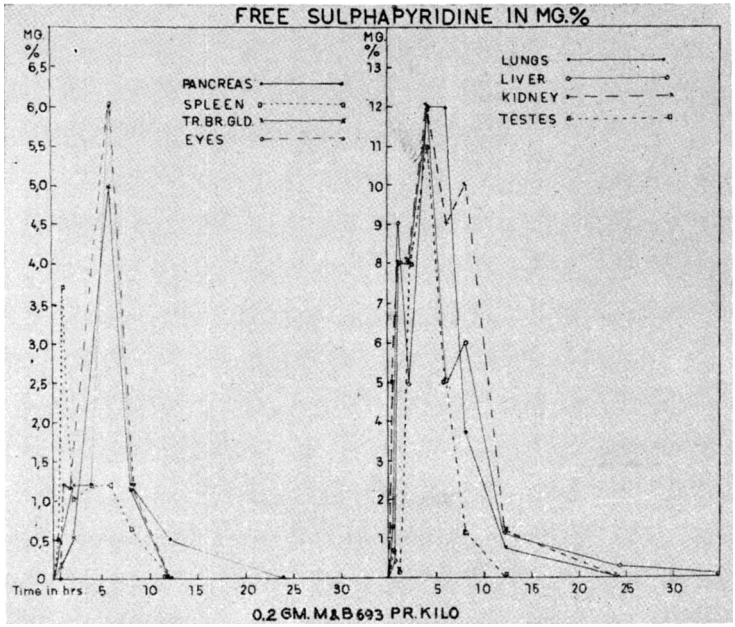

FIG. 4.

0.2 g of the drug per kg, and this is nearly identical with the maternal blood concentrations of free and conjugated sulfapyridine.

Conclusion. The orally administered sulfapyridine is nearly completely absorbed from the stomach after 4 hours, and is distributed to every liquid, tissue and organ in the guinea-pig body, mostly in the free form. About 70% of the ingested drug is excreted in the urine after 36 hours and resorption from the larger gut mucosa may account for the discharge of the balance with the feces.

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Bacteriostatic Effect of Sulfapyridine, Sulfanilamide and Prontosil Rubrum *in vitro* on Mycobacteria.

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In view of the inhibitory action of certain sulfanilamide compounds on the development of an infection of guinea pigs with human and bovine tubercle bacilli,[1] the present study was undertaken to

[1] Birkhaug, K. E., *Brit. Med. J.*, 1939, **2**, 54 (Review of literature).

secure more exact information on the *in vitro* effect of sulfapyridine, sulfanilamide, and prontosil rubrum on mammalian, avian and certain paratubercular strains of mycobacteria. Carpenter and Gibbons[2] have reported that sulfanilamide and disulon in 1 :2,500 to 1 :10,000 concentrations either completely or partially inhibited the growth of chromogenic mycobacteria while no such bacteriostatic effect was noted on bovine and human tubercle bacilli. Rist[3] found that sulfanilamide (1162 F) in 200 mg % concentration completely inhibited the film formation of bovine and human strains of tubercle bacilli while 20 mg % concentration only partially delayed growth of these strains. An avian strain which grew in the depth of the tube was completely inhibited in a 20 mg % concentration of sulfanilamide (1162 F). However, the compound 1358 F (4-4'-diaminophenylsulphone) exerted the same bacteriostatic effect in 10 times weaker concentrations than the 1162 F compound. Rist, Bloch and Hamon[4] extended the study *in vivo* and found that both these sulphonamide compounds inhibited the multiplication of virulent avian tubercle bacilli in rabbits and guinea pigs. Birkhaug[1] reported a similar *in vivo* bacteriostatic effect of prontosil soluble on virulent bovine tubercle bacilli in the guinea pig.

In our study 14 strains of mycobacteria were employed, of which 4 were bovine, 3 of each were human and avian tubercle bacilli, all of considerable virulence, and 4 paratubercular cultures: *M. johnei, M. piscium, M. Boquet and M. Vallée.* Concentrations of 10, 50 and 90 mg % of sulfapyridine, sulfanilamide and prontosil rubrum (4-sulphamido-2, 4-diaminoazo benzol) were made in Sauton's synthetic medium adjusted to pH 7.4. The solutions were made at 100°C and held at 38°C to avoid recrystallization. Ten cc of each concentration was placed in 2.5x20 cm cotton-plugged sterile tubes. Similar amounts of Sauton's medium were used for controls. The 14 strains of mycobacteria were previously grown on Sauton's medium for 3 weeks and all had formed surface films. A 3 mm loopful of film (approximately one mg dry weight) from each strain was floated on the surface of the liquid in each tube. A rubber cap was placed above the cotton plug to prevent evaporation. These cultures were left in a slanting position in the incubator for 7 weeks at 37.5°C. At that time they were autoclaved and filtered through previously weighed small filter papers. After being completely dried at 90°C until the weight became constant, the filter papers were again weighed and the difference between the first and second weights was recorded as

2 Carpenter, C. M., and Gibbons, L. L., *J. Bacteriol.*, 1939, **37**, 226.

3 Rist, N., *C. R. Soc. de Biol.*, 1939, **130**, 972.

4 Rist, N., Bloch, F., and Hamon, V., *Ibid.*, 1939, **130**, 976.

bacterial growth. The experiment was repeated twice and the averages of these 3 trials are shown in Table I.

TABLE I.

Bacteriostatic Action of sulfapyridine, Sulfanilamide, and Prontosil rubrum on Mammalian, Avian, and Paratubercular Acid-fast Bacilli.

Dry weight of bacterial growth (mg) in 10 cc of Sauton's liquid medium containing 10, 50, and 90 mg% conc. of the sulphonamide compounds, after 7 weeks' incubation at 37.5°C.

	Sulfapyridine			Sulfanilamide			Prontosil rubrum			Control
				Mg% concentrations						
Strain	10	50	90	10	50	90	10	50	90	Control
Human R-51	56	29	16	96	60	51	72	49	14	107
" H-37	67	30	19	101	71	61	61	42	12	103
" S-46	38	12	0	128	67	30	21	0	0	130
Bovine Ep.-2	86	48	23	154	93	45	89	13	10	152
" VR-12	39	17	11	112	77	46	132	15	11	130
" T5080	84	62	11	121	72	51	66	0	0	118
" T3396	51	0	0	104	82	47	55	0	0	109
Avian B-44	50	0	0	111	104	102	19	0	0	115
" NR-61	62	0	0	117	101	98	21	0	0	119
" NR-62	18	0	0	111	78	70	57	0	0	108
Myc. johnei I	71	64	34	163	155	142	117	0	0	165
Myc. piscium	50	32	0	104	63	42	83	55	28	109
Myc. paratub. Boquet	18	10	0	134	87	49	0	0	0	142
Myc. paratub. Vallée	63	10	0	104	64	41	45	0	0	104
Avg wt mg	54	23	8	119	84	63	60	13	5	122
Statistics: t	9.0	13	19	0.45	4.6	6.1	5.5	15	21	
vs. Control P	0.01	0.01	0.01	>0.9	0.01	0.01	0.01	0.01	0.01	

By summing up the average weights for each concentration of the 3 sulphonamide compounds and by analyzing the difference between the mean weights of the control and each sulphonamide concentration by the statistical method suggested by Fisher,[5] it is quite apparent that both sulfapyridine and prontosil rubrum in 10 mg % concentration are capable of producing a significant inhibition of mycobacterial growth which sulfanilamide is not able to do before the concentration is elevated to 50 mg %. But even at that concentration the inhibition of growth is only 32% less than the control weight. Not before the concentration of sulfanilamide reaches 90 mg % is the inhibition of mycobacterial growth comparable with that produced in 10 mg % concentrations of sulfapyridine and prontosil rubrum.

Conclusion. Sulfapyridine and prontosil rubrum possess more potent anti-mycobacterial properties *in vitro* than sulfanilamide.

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[5] Fisher, A. R., *Statistical Methods for Research Workers*, Oliver and Boyd, London, 1936, pp. 128-166.