TABLE III.
Jaundice Following Sulfanilamide.

Days	1	3	4	5	6	8	10	12
Total bilirubin	5.9	4.6	2.9	1.6	1.1	0.8	1.0	0.4
Direct bilirubin (30 min)	.5	.5	.7	.8	.5	.5	.5	.2
Direct van den Bergh Reaction	—	—	+	+	+	+	+	—

the total is illustrated also by data obtained in a patient with jaundice following administration of sulfanilamide.

Conclusions. The chief clinical value of the quantitative determination of direct serum bilirubin appears to lie in the detection of hepatic functional impairment in the presence of a normal serum bilirubin concentration. Values constituting more than 50% of the total with readings at 5 minutes or more than 70-75% with readings at 30 minutes are probably abnormal. There is no apparent significant relationship between the amount or proportion of direct bilirubin and the production of a positive direct van den Bergh reaction in sera with normal total bilirubin content.

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Response of *Sulfapyridine-fast* Pneumococci to Sulfathiazole and Sulfamethylthiazole.

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(Introduced by S. Tashiro.)

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Our experimental studies,[1] as well as those of MacLeod,[2] have shown that inadequate treatment of pneumococcal infections with sulfapyridine leads to development of organisms which are resistant or *fast* to that drug. Whether these *sulfapyridine-fast* pneumococci are equally *fast* to other sulfanilamide derivatives, such as sulfathiazole and sulfamethylthiazole, is a matter of theoretical and practical interest. Consequently, we have studied the reactions of 2 of our *sulfapyridine-fast* strains to these latter drugs.

Essentially identical experiments have been performed with each *fast* strain and with the parent strains from which these *fast* organ-

1 Schmidt, L. H., and Dettwiler, H. A., in press.

2 MacLeod, C. M., *J. A. M. A.*, 1939, **113**, 1405.

TABLE I.

Response of *Sulfapyridine-fast* and Parent Strains of Pneumococcus to Sulfathiazole and Sulfamethylthiazole.

Type and strain	No. organisms in infecting dose	No. mice infected	Treatment	No. of deaths Days after infection								Avg hr survival of mice that died	30-day survivors	
				1	2	3	4	5	6	7-10	11-30		No.	%
I	560	30	Sulfapyridine	0	29	1	0	0	0	0	0	41	0	0
McGovern		30	Sulfathiazole	1	26	3	0	0	0	0	0	40	0	0
Fast		30	Sulfamethylthiazole	0	28	1	1	0	0	0	0	38	0	0
		10	None—Controls	1	9	0	0	0	0	0	0	27	0	0
I	520	30	Sulfapyridine	0	0	0	0	1	1	1	0	144	27	90
McGovern		30	Sulfathiazole	0	0	1	3	1	0	3	0	131	22	73
Parent		30	Sulfamethylthiazole	0	0	0	1	4	0	2	0	141	23	77
		10	None—Controls	3	7	0	0	0	0	0	0	26	0	0
III	230	30	Sulfapyridine	0	30	0	0	0	0	0	0	39	0	0
Wistuba		30	Sulfathiazole	1	28	1	0	0	0	0	0	38	0	0
Fast		30	Sulfamethylthiazole	0	28	2	0	0	0	0	0	39	0	0
		10	None—Controls	6	4	0	0	0	0	0	0	27	0	0
III	430	30	Sulfapyridine	0	0	0	2	0	0	20	0	167	8	27
Wistuba		30	Sulfathiazole	0	0	0	3	1	1	18	0	160	7	23
Parent		30	Sulfamethylthiazole	0	0	1	1	0	3	20	2	171	3	10
		10	None—Controls	9	1	0	0	0	0	0	0	23	0	0

isms were derived.* Mice in groups of 100 were infected intraperitoneally, each mouse receiving 10^{-6} cc of a 12 hour blood broth culture. Ten mice from each group were untreated. The remaining 90 were divided into 3 equal groups which were treated with sulfapyridine, sulfathiazole or sulfamethylthiazole†—20 mg doses of the respective drug being administered at 2, 8, 14 and 22 hours after infection and at 8 hour intervals thereafter for either 5 additional days (in the experiments with the parent strains) or as long as the mice survived (in the experiments with the *fast* strains). The drugs were administered as 10% suspensions in 10% acacia.

The results in the accompanying table show conclusively that *sulfapyridine-fast* pneumococci were also equally resistant to sulfathiazole and sulfamethylthiazole. Thus the mice infected with strain McGovern lived an average of 41 hours under sulfapyridine treatment and 40 and 38 hours under sulfathiazole and sulfamethylthiazole treatments. In the experiment with strain Wistuba, the average survival times were 39, 38 and 39 hours under therapy with the respective drugs.

The table shows also that these drugs were essentially equal in effectiveness against infections with the parent strains. This finding, consistent with those of other workers,[3-7] precludes the possibility that either of the organisms was naturally resistant to sulfathiazole or sulfamethylthiazole. It should be noted, however, that each of the 3 drugs had a greater curative action against infections with the Type I strain (McGovern) than against infections with the Type III strain (Wistuba). This difference in type response is in agreement with our earlier observations.[8, 9]

The finding that pneumococci which are *sulfapyridine-fast* are equally *fast* to sulfathiazole and sulfamethylthiazole offers additional

* The preparation of these *sulfapyridine-fast* strains has been described elsewhere.[1]

†We are indebted to Merck and Company, Inc., for the sulfathiazole and sulfamethylthiazole used in this study.

[3] McKee, C. M., Rake, G., Greep, R. O., and Van Dyke, H. B., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 417.

[4] Cooper, F. B., Gross, P., and Lewis, M., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 421.

[5] Barlow, O. W., and Homburger, E., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 317.

[6] Long, P. H., and Bliss, E. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 324.

[7] Litchfield, J. T., Jr., White, H. J., and Marshall, E. K., Jr., *J. Pharm. and Exp. Therap.*, 1940, **69**, 166.

[8] Schmidt, L. H., and Hilles, C., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 611.

[9] Schmidt, L. H., Hilles, C., Dettwiler, H. A., and Starks, E., in press.

proof that the mode of action of these drugs in pneumococcal infections is fundamentally the same. Moreover, it suggests the futility of attempting therapy with a second of these compounds when the pneumococcal infection becomes resistant to treatment with any one of these drugs.

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A New Material (Lygranum) for Performance of the Frei Test for Lymphogranuloma Venereum.

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No completely satisfactory product for the performance of the Frei intradermal test for the diagnosis of lymphogranuloma venereum has yet been obtained. The materials commonly employed for the purpose are a suspension, in sterile physiological saline, of human pus or mouse brain containing the heat-inactivated virus of the disease. Many critical reports upon the value of these materials, which are termed Frei antigens, have already appeared, *e. g.*[1] It was felt that a suspension of elementary bodies of the virus of lymphogranuloma venereum might be the ideal agent for the performance of the Frei test. Accordingly, this virus was grown in the yolk sac of the developing chicken embryo[2] according to the method of Cox[3] and a considerable yield of elementary bodies obtained. One cc amounts of suspensions of virus-infected yolk sac showing many elementary bodies were inoculated into the yolk sac of eggs of 6 days' previous incubation. As soon as the embryos were moribund or dead the yolk sacs were collected, washed lightly to remove excess yolk and then weighed. They were ground thoroughly in a mortar with sterile quartz fragments and sufficient sterile physiological saline added to make a 10% suspension. This suspension was centrifuged for 1 hour at 2500 RPM in the cold and the sediment discarded. The super-

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1 Grace, A. W., *Arch. Dermat. and Syph.*, 1939, **39**, 347.

2 Rake, G., McKee, C. M., and Shaffer, M. F., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 332.

3 Cox, H. R., *Pub. Health Rep.*, 1938, **53**, 2241.