ment-fixing antibodies in the serum of a laboratory worker recovered from infection with the virus of lymphocytic choriomeningitis.

Summary. A soluble specific antigen, which occurs in tissues of animals infected with the virus of lymphocytic choriomeningitis, fixes complement in the presence of immune serum; the virus freed from the soluble antigen fixes complement poorly or not at all. Patients recovered from lymphocytic choriomeningitis develop in their serum antibodies that react with the soluble antigen to fix complement.

10310

Sulfanilamidopyridine (2-Para-aminobenzenesulfonamidopyridine) in Experimental Infections with Type XXII Pneumococcus.*†

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Our recent observations[1] indicated that sulfanilamide had definite therapeutic properties in experimental pneumococcus infections in mice, and that the effectiveness of the drug varied with the type of pneumococcus used as the infecting agent. A marked curative action was obtained in infections with 19 of the 30 types of pneumococci (I, V, VII, IX, X, XII, XIII, XIV, XV, XVI, XVII, XVIII, XIX, XXI, XXIII, XXV, XXVIII, XXXI, and XXXII); in infections with the remaining 11 types (II, III, IV, VI A and B, VIII, XI, XX, XXII, XXIV, XXVII, and XXIX) the drug prolonged life but did not lead to recovery.

Recently, Whitby[2] reported that sulfanilamidopyridine (2-para-aminobenzenesulfonamidopyridine) had curative properties when administered to mice infected with Types I, VII and VIII pneumococci and prolonged life in infections with types II, III and V. In experiments on Type I, he found that sulfanilamidopyridine was a more effective therapeutic agent than sulfanilamide. This observa-

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1 Schmidt, L. H., and Hilles, C., in press.

2 Whitby, L. E. H., *Lancet*, 1938, **1**, 1210.

tion suggested that sulfanilamidopyridine might have a favorable therapeutic action in infections due to those types of pneumococci which were refractory to sulfanilamide therapy. This has been investigated; the results in Type XXII infections are reported here.

Therapeutic Properties of Sulfanilamidopyridine. In the initial experiment 2 groups of mice were infected intraperitoneally with 10^{-6} cc of a 12-hour blood broth culture of a Type XXII pneumococcus; this quantity of culture contained approximately 200 pneumococci and was equivalent to 100 minimum lethal doses.‡ The first group (10 mice) served as untreated controls. The second group (20 mice) was treated with sulfanilamidopyridine in a manner similar to that described by Whitby; 20 mg of the drug were administered, orally, 2 hours after infection and 6, 12 and 18 hours thereafter, then at 24-hour intervals for 5 successive days. Cultures of tail blood were made at the conclusion of treatment; the mice were observed closely for 24 additional days; survivors were sacrificed then, and cultures of heart blood were made. The results are summarized in Fig. 1 (Groups 1 and 2).

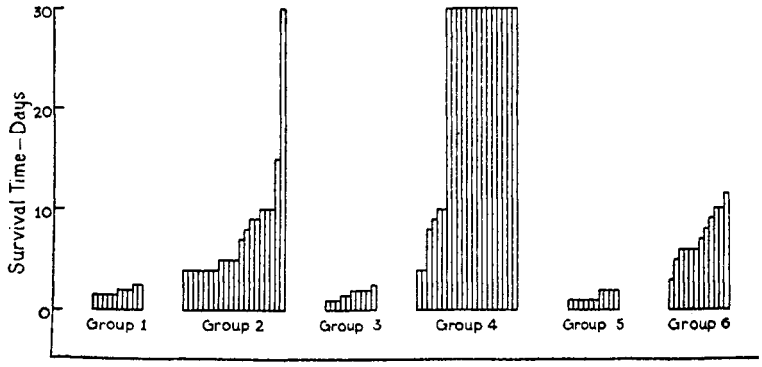

FIG. 1.

Therapeutic Properties of Sulfanilamidopyridine and Sulfanilamide in Experimental Infections with Type XXII Pneumococcus.

Each column represents one mouse.

Groups 1, 3 and 5: Untreated controls.

Group 2: Sulfanilamidopyridine administered orally; 20 mg 2 hours after infection and 6, 12 and 18 hours thereafter, then at 24-hour intervals for 5 succeeding days.

Group 4: Sulfanilamidopyridine administered orally; 20 mg 2 hours after infection and every 6 hours thereafter for 4 days, then at 12-hour intervals for 2 days.

Group 6: Sulfanilamide injected subcutaneously; 10 mg 2 hours after infection and every 6 hours thereafter for 8 days.

‡ The stock culture of this pneumococcus was obtained from Miss Annabel Walter, Bureau of Laboratories, Department of Health, New York City. The procedure for increasing the virulence of organisms and determining the minimum lethal dose has been described elsewhere.[1]

Treatment with sulfanilamidopyridine, in the above manner, prolonged life but led to recovery in only one of the 20 mice. All of the treated animals survived 4 days, and 9 lived 6 days or longer, whereas all the untreated controls died within 60 hours after infection. Seven of the 9 mice that survived 6 days or more had positive blood cultures at the time therapy was discontinued, and yet 6 of these 7 mice lived an additional 3 to 9 days. These results were not significantly better than the results obtained with sulfanilamide (Fig. 1, Groups 5 and 6).

In a subsequent experiment, sulfanilamidopyridine was administered at more frequent intervals. Mice were infected with 100 lethal doses of Type XXII pneumococcus as in the preceding experiment; 20 mg of sulfanilamidopyridine were administered 2 hours after infection and every 6 hours thereafter for 4 days, then at 12-hour intervals for 2 additional days. Fourteen of the 20 mice so treated recovered (Fig. 1, Groups 3 and 4). Of the 6 mice that did not recover, 2 lived 4 days; of the remaining 4, one died on the 8th, one on the 9th and 2 on the 10th day after infection—all died after therapy had been discontinued.

This experiment shows that sulfanilamidopyridine has definite curative properties in infections with type XXII pneumococcus. The fact that more intensive therapy was required in Type XXII infections than in Types I and VII is of interest, for in our previous experiments[1] it was noted that the dosage of sulfanilamide required for effective treatment varied in infections with different types of pneumococci.

Mode of Action of Sulfanilamidopyridine. Four mice in the last experiment described—those that died after therapy had been discontinued—had positive blood cultures on the 6th day after infection. Cultures from 2 of these mice contained typical Type XXII pneumococci. The culture from the third animal contained Gram positive diplococci which were bile soluble and gave a negative Neufeld reaction when mixed with antipneumococcus sera Types I to XXXII. The culture from the 4th mouse contained both the typical Type XXII pneumococci and the diplococci giving a negative Neufeld reaction. This last culture has been studied in some detail.

Pure cultures of the "Neufeld positive" and "Neufeld negative" organisms were obtained—both fermented inulin and were bile soluble. There were marked differences in the growth of these organisms on blood agar. The colonies of the "Neufeld positive" organisms were smooth—as were those of the stock culture; the colonies of the "Neufeld negative" organisms were rough.

The "Neufeld negative" organisms were passed through mice; after 2 successive passages they gave a positive Neufeld reaction. The "Quellung" at this stage was not as marked as that of the stock Type XXII pneumococcus; after 5 successive passages, the "Quellung" reaction of the originally "Neufeld negative" organisms was identical with that of the stock XXII.

Observations by Whitby,[2] and Telling and Oliver[3] have suggested that sulfanilamidopyridine exerts a definite action on the capsules of Types I and III pneumococci. Our observations on Type XXII pneumococcus offer further evidence that sulfanilamidopyridine therapy leads to production of decapsulated pneumococci. Neither our own observations nor those of Whitby and Telling indicate whether this is the result of capsular degeneration or inhibition of capsule formation.

The effect of sulfanilamidopyridine on the pneumococcus capsule may partially explain the therapeutic properties of the drug. It is generally agreed that decapsulated pneumococci are readily attacked by the phagocytes of the host and for that reason are less virulent than capsulated forms. That the "Neufeld negative" organisms were relatively avirulent is indicated by the following experiment.

A group of 30 mice was infected intraperitoneally with 100 M.L.D. of the stock Type XXII pneumococcus (160 organisms by plate count); a second group was infected with 150 "Neufeld positive" organisms isolated from the 4th mouse mentioned above; the third group received 150 "Neufeld negative" organisms isolated from the same mouse. Mice from each of these groups were sacrificed at 6, 12 and 24 hours after infection; in addition, mice from the third group were killed at 48, 96 and 144 hours. Cultures and smears of peritoneal washings were made; heart blood samples were taken and the number of organisms therein determined by means of pour plates.

TABLE I.

Comparison of *In Vivo* Growth of Stock Type XXII Pneumococcus with That of "Neufeld Positive" and "Neufeld Negative" Type XXII Pneumococci Isolated from a Mouse Treated with Sulfanilamidopyridine.

Infecting Organisms	No. of pneumococci per cc of blood Hr after infection			Cultures from peritoneal washings Hr after infection		
	6	12	24	6	12	24
"Stock"	23×10^3	13×10^4	32×10^6	+	+	+
"Neufeld Positive"	28×10^3	31×10^4	140×10^6	+	+	+
"Neufeld Negative"	0	0	0	—	—	—

[3] Telling, M., and Oliver, W. A., *Lancet*, 1938, **1**, 1391.

The data in Table I show that the stock XXII pneumococci and the "Neufeld positive" organisms (those obtained from the sulfanilamidopyridine treated mouse) invaded the blood stream quickly and multiplied equally well. "Neufeld negative" organisms could not be cultured from the blood or peritoneal washings at any time.

Summary. Experimental infections with Type XXII pneumococcus, which are refractory to sulfanilamide therapy, can be treated satisfactorily with sulfanilamidopyridine. The amount of drug required for effective treatment is larger than that necessary in Types I, VII, and VIII infections (Whitby). Avirulent decapsulated Type XXII pneumococci have been isolated from the blood of mice treated with sulfanilamidopyridine. This supports the suggestion of Whitby, and Telling and Oliver that the drug exerts a definite action on the pneumococcus capsule.

10311

Sulfanilamide in the Treatment of Experimental Trypanosomiasis of Rats.

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The reputed effectiveness of sulfanilamide in the treatment of malaria induced us to determine whether or not the compound was therapeutically effective in the treatment of trypanosomiasis of rats. Buttle, Gray and Stephenson[1] were unsuccessful with sulfanilamide therapy against malaria in canaries and *Trypanosoma equiperdum* infections of mice.

Sixteen white rats, weighing from 150 to 170 g, were inoculated intraperitoneally with approximately 500,000 *Trypanosoma equiperdum;* 24 hours later some showed a few trypanosomes in the tail blood. Treatment was started at this time, 4 receiving 0.08 g per kilo by intravenous injection 24, 30, 48, 54, 72, and 78 hours after inoculation; 4 were given 0.160 g and 4 given 0.200 g per kilo at the same intervals. Four were kept as untreated controls and all died of trypanosomiasis 4 to 5 days after inoculation; 3 additional animals were kept as drug controls and survived 8 days when the experiment was terminated.

[1] Buttle, G. A. H., Gray, W. H., and Stephenson, D., *Lancet*, 1936, **1**, 1286.