that *p*-aminobenzoic acid inhibits the action of other sulfonamide drugs on a wide variety of bacteria.

Conclusions. *P*-aminobenzoic acid inhibits the antibacterial action of sulfathiazole but has no effect on pneumococcidal antibodies in fresh defibrinated blood.

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Effect of P-Aminobenzoic Acid on Therapeutic and Toxic Action of Sulfapyridine.*

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In an attempt to determine the chemical identity of the factor responsible for the inhibition of sulfanilamide by certain substances, (*e.g.*, yeast extract, bacterial extracts), Woods[1] reported experiments which demonstrated that *p*-aminobenzoic acid in very high dilution was capable of inhibiting the effect of sulfanilamide on the growth of the *Streptococcus hemolyticus in vitro*. The antibacterial effect of sulfapyridine was similarly impaired, but 5 times as much *p*-aminobenzoic acid was required for inhibiting sulfapyridine as was found necessary to nullify sulfanilamide. The difference was explained by the observation that sulfapyridine was 5 times as potent as sulfanilamide against the strain of *Streptococcus hemolyticus* employed in the experiments. In an accompanying paper from the same laboratories, Selbie[2] demonstrated that *p*-aminobenzoic acid inhibited the therapeutic action of sulfanilamide in mice infected with hemolytic streptococci. Selbie recorded that the survival rate of mice receiving 25 mg doses of sulfanilamide was greatly reduced by administering to the mice as little as 2.5 mg of *p*-aminobenzoic acid. The two drugs were mixed *in vitro* and introduced simultaneously by esophageal cannula.

The present report concerns the inhibiting effect of *p*-aminobenzoic acid on the therapeutic action of sulfapyridine in pneumococcal infection in mice, and also deals with the effect of one drug upon the toxic action of the other.

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1 Woods, D. D., *British J. Exp. Path.*, 1940, **21**, 74.

2 Selbie, E. R., *Ibid.*, 1940, **21**, 90.

Swiss mice weighing 16-20 g were used throughout. Sulfapyridine, suspended in tragacanth mucilage, was introduced by stomach tube in a uniform constant dose of 24 mg to all mice. It was given twice on the day of inoculation and once daily thereafter. *P*-aminobenzoic acid was given subcutaneously in 3 daily doses of equal size, but as may be noted in Table I, for purposes of titration the amount of *p*-aminobenzoic acid differed in individual experiments. Aqueous solution of *p*-aminobenzoic acid was effected by the addition of an equi-molecular quantity of sodium hydroxide. An 18-hour broth culture of a strain of pneumococcus Type I, which regularly induced fatal infection in mice in doses of 10^{-7}, was employed. The dosage of culture, injected intraperitoneally, was kept constant at a dilution of 10^{-4}. The culture was injected immediately after the first dose of sulfapyridine.

In the technical procedures, therefore, it may be noted that the sulfapyridine was given by mouth, *p*-aminobenzoic acid subcutaneously, and pneumococci intraperitoneally. The procedure obviated the possibility of chemical action between the drugs *in vitro*. Treatment was continued for 6 days.

Table I records the results of a series of these experiments. With doses of *p*-aminobenzoic acid, slightly greater or less than the size of the dose of sulfapyridine, the animals died within the same time as the virulence controls. As the dose of *p*-aminobenzoic acid was decreased, the time of death became definitely delayed and more scat-

TABLE I.
Inhibiting Effect of P-aminobenzoic Acid on the Curative Action of Sulfapyridine for Type I Pneumococcus Infection in Mice.

Sulfapyridine (daily dose), mg	P-aminobenzoic acid (total daily dose), mg	No. mice	Deaths Day after infection with pneumococci 1	2	3	4	5	6	7	8	9	10	11	Survivals	% survivals
—	—	10	8	2										0	0
24	30	5	1	4										0	0
24	22.5	14	5	8	1									0	0
24	15.0	4	3	1										0	0
24	7.5	14	—	10	2	2								0	0
24	6.0	4	—	—	2	1	—	1						0	0
24	3.0	14	—	2	2	4	1	2	1	—	—	—	1	1	7
24	1.5	14	—	—	4	2	2	2	2	—	—	—	—	2	14
24	0.75	10	—	—	—	1	1	—	—	1	—	—	1	6	60
24	—	24	—	—	—	*1	—	—	†2	—	—	—	—	21	91

*Died during administration of sulfapyridine; postmortem cultures plus for pneumo. I.

†One of these had sterile postmortem cultures.

tered. When the amount of *p*-aminobenzoic acid was reduced to 7.5 mg per day (2.5 mg t.i.d.), there were no deaths on the first day after inoculation, but 10 of the 14 mice succumbed on the second day. At a dosage of 3.0 mg per day the times of death were definitely prolonged and were scattered over a period of several days; one of the mice survived. With further decrease in the amount of *p*-aminobenzoic acid administered, there was a sharp increase in the number of mice surviving the infection. However, even with the smallest dose used (0.75 mg per day) there was some depression of sulfapyridine effect as evidenced by the delayed death of 4 of the 10 mice.

In toxicity experiments, in which no organisms were injected, *p*-aminobenzoic acid caused no untoward symptoms in the doses and by route of administration used in the present study. Furthermore, the combined therapeutic dosage of sulfapyridine plus *p*-aminobenzoic acid, without pneumococcal infection, likewise had no apparent effect on normal mice. Selbie[2] indicated that the toxicity of the combined drugs was somewhat less than that of sulfanilamide alone. In order to determine whether or not the nullifying effect of *p*-aminobenzoic acid on the therapeutic efficacy of sulfapyridine might be accompanied by an alteration in the toxic action of sulfapyridine, the drugs were introduced into uninfected mice according to the titrations indicated in Table II.

The subcutaneous injection of 25 mg of the sodium salt of sulfapyridine (0.5 cc of 5% sol.) in 16-20 g mice caused convulsions proceeding to death in less than 2 hours. *P*-aminobenzoic acid was given by oral dosage of 50 mg a day (in 2 equal doses) for 2 days prior to the injection of the toxic dose of sulfapyridine. There is evidence that *p*-aminobenzoic acid is readily adsorbed from the gastro-intestinal tract in the results of Selbie[2] who demonstrated the effectiveness of small oral doses in nullifying sulfanilamide effect

TABLE II.
Effect of P-aminobenzoic Acid on Acute Toxicity of Sulfapyridine.

P-aminobenzoic acid	Sodium sulfapyridine monohydrate, mg subcut.	No. mice	No. died	No. survived
—	25	9	9	0
50 mg/day *per os* for 2 days	25	6	5	1
25 mg subcut. 30 min. preceding sulfapyridine	25	4	4	0
—	20	3	1	2
50 mg/day *per os* for 2 days	20	3	1	2
—	15	2	0	2
50 mg/day *per os* for 2 days	15	2	0	2

in mice and the studies of Harrow, *et al.*,[3] on the mechanism of detoxification of orally administered *p*-aminobenzoic acid by the formation of the acetylated derivative. However, as a further control in the present experiments, some of the animals were given a single subcutaneous dose of 25 mg of *p*-aminobenzoic acid 30 minutes prior to the injection of sulfapyridine.

The toxic effects of the 25 mg dose of sodium sulfapyridine on normal animals and animals receiving *p*-aminobenzoic acid were compared, and the results of this type of experiment are illustrated in Table II. It will be seen that *p*-aminobenzoic acid exerts no appreciable effect on the acute toxicity of sulfapyridine when administered in this manner. The time of onset and the duration of convulsions in the group receiving *p*-aminobenzoic acid did not differ significantly from that in the control group. The single animal that survived the 25 mg dose of sulfapyridine did so only after several hours of severe convulsions.

Summary. 1. *P*-aminobenzoic acid (given subcutaneously) was found capable of nullifying the curative effect of sulfapyridine (given *per os*) for type I pneumococcus infection in mice. 2. *P*-aminobenzoic acid had no observable effect upon the immediate fatal toxicity of sulfapyridine for mice.

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Effect of Intramuscular Injection of Sodium Citrate on the Prothrombin Time of the Blood.*†

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Neuhof and Hirshfeld[1] observed that intramuscular injections of sodium citrate caused an increase in the coagulability of the blood. A fall in the coagulation time occurred within 40 minutes of an intragluteal injection of 30 cc of 30% citrate and was maintained

[3] Harrow, Benj., Power, F. W., and Sherwin, C. P., PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 422.

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[1] Neuhof, H., and Hirshfeld, L., *Ann. Surg.*, 1922, **76**, 1.