mitting minimal multiplication. No absorption of penicillin onto the organisms could be demonstrated. Incubation of small numbers of organisms with penicillin under conditions suitable for active growth, however, resulted in the disappearance of the organisms. The failure to demonstrate these killed organisms by the usual staining technics suggested that they had undergone a subsequent lysis. This is in accord with the observations of other workers.[3, 4]

Summary. Conditions which increase the rate of growth of bacteria increase the rate at which penicillin acts. Conditions which decrease the rate of growth decrease also the rate at which penicillin acts. Penicillin is most effective when active multiplication takes place.

3 Fleming, A., *Brit. J. Exp. Path.*, 1929, **10**, 226.
4 Chain, E., *et al.*, *Lancet*, 1940, **2**, 226.

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Relationship of Penicillin to Sulfonamide Action.

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Ungar[1] reported on the synergistic action of both para-aminobenzoic acid and sulfapyridine on penicillin. In a previous communication from this laboratory,[2] it was stated that para-aminobenzoic acid increased the rate of action of penicillin whereas sulfadiazine decreased the rate of its action.

Experimental. Unless otherwise specified the Oxford strain of *Staphylococcus aureus* (strain H) grown in Knight's synthetic medium was used throughout the present study. Before use the culture was transferred 3 times in Knight's basic medium to which had been added M/100 nicotinic acid. A solution of penicillin was diluted serially in Knight s medium containing 100 μg of para-aminobenzoic acid per cc, in Knight's medium containing 20 μg of sulfadiazine per cc, and in Knight's medium containing 20 μg of sulfapyridine per cc, respectively. *Staphylococcus aureus* was inoculated into each tube in an amount sufficient to give a final dilution of 10^{-2} or approximately 10,000,000 organisms per cc. Incubation was carried out at 37°C for 24 hours. The last tube in which no visible growth occurred was accepted as the titer of the penicillin solution.

The titer of the penicillin in Knight's medium was 0.035 Oxford units per cc. With sulfapyridine the titer increased twofold to 0.018 units per cc. With para-aminobenzoic acid or sulfadiazine no increase was observed. (Table I.)

A similar experiment was carried out using hemolytic streptococci (strain C203MV) grown in beef infusion broth. No increase in the titer of the penicillin was observed with sulfadiazine, sulfapyridine, or para-aminobenzoic acid. (Table I.)

It was apparent that, although para-aminobenzoic acid and the sulfonamides may affect the rate of action of penicillin, they do not significantly affect the titer of penicillin.

Further preliminary experiments were carried out to determine any possible relationship between the action of penicillin and that of sulfadiazine or sulfapyridine.

West and Coburn[3] showed that sulfapyridine is capable of exerting a bacteriostatic

1 Ungar, J., *Nature*, 1943, **152**, 245.
2 Hobby, G. L., and Dawson, M. H., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 178.
3 West, R., and Coburn, A. F., *J. Exp. Med.*, 1940, **72**, 91.

TABLE I.
Effect of Sulfonamides and Para-aminobenzoic Acid on Titer of Penicillin *in Vitro*.

Organism	Medium	Sulfapyridine, γ/cc	Sulfadiazine, γ/cc	Para-aminobenzoic acid, γ/cc	Titer of penicillin, Oxford units/cc
Staphylococcus aureus (strain H) 10-6	Knight's basal medium containing M/100 nicotinic acid and penicillin	0	0	0	0.035
		20	0	0	0.018
		0	20	0	0.035
		0	0	100	0.035
Hemo. streptococcus (strain C203MV) 10-6	Beef infusion broth containing penicillin	0	0	0	0.018
		20	0	0	0.035
		0	20	0	0.035
		0	0	100	0.035

TABLE II.
The Effect of Sulfonamides, Para-aminobenzoic Acid and Penicillin on Growth of *Staphylococcus aureus* in Synthetic Medium Containing Nicotinic Acid.

Organism	Knight's basal medium, cc	Nicotinic acid M/1000 cc	Sulfadiazine M/1000 cc	Sulfapyridine M/1000 cc	Para-aminobenzoic acid cc	Penicillin 10 Oxford units/cc cc	Growth 96 hr
Staphylococcus aureus (strain H), 10-2	1.8	0	0	0	0	0	—
		.1	0	0	0	0	+
	1.8	.1	0	0.15	0	0	—
		.1	0.15	0	0	0	—
		.1	0	0	0.15 M/10,000	0	++++
	1.8	.1	0	0.15	0.15 M/50,000	0	+
	1.8	.1	0.15	0	0.15 M/10,000	0	+
Staphylococcus aureus (strain H), 10-2	1.8	.1	0	0	0	.05	—
		.1	0.15	0	0	.05	—
		.1	0	0.15	0	.05	—
		.1	0.15	0	0.15 M/10,000	.05	—
		.1	0	0.15	0.15 M/50,000	.05	—

action in the presence of nicotinic acid but is inhibited by the presence of coenzymes. Experiments similar to those of West and Coburn were carried out using penicillin as well as sulfapyridine and sulfadiazine. As shown in Table II sulfapyridine and sulfadiazine were both active in the presence of nicotinic acid. Likewise penicillin was fully effective in the presence of nicotinic acid. Para-aminobenzoic acid greatly enhanced growth of the organisms in the basal medium containing nicotinic acid; it inhibited both sulfadiazine and sulfapyridine but did not inhibit penicillin activity.

Extracts of red blood cells were prepared according to the method of West and Coburn. As shown in Table III growth in Knight's basal medium was greatly enhanced by the addition of these extracts. Their presence inhibited the bacteriostatic action of sulfapyridine but did not inhibit the action of either sulfadiazine or penicillin.

It was apparent that penicillin, like sulfadiazine, was not affected by the presence of coenzymes.

Defibrinated rabbits' blood was shown to increase the rate of action of penicillin. There was no evidence, however, that extracts of red blood cells made from human blood produced any such effect. (Table IV.)

It has been shown by numerous investigators that organisms multiply at a normal rate for 5 to 7 hours in the presence of sulfadiazine

TABLE III.
Effect of Red Blood Cell Extracts on Action of Sulfapyridine, Sulfadiazine, and Penicillin on *Staphylococcus aureus in Vitro.*

Organism	Knight's basal medium cc	Red blood cell extract M/1000 cc	Sulfadiazine M/1000 cc	Sulfapyridine M/1000 cc	Penicillin 10 Oxford units/cc cc	Amt of growth 96 hr
Staphylococcus aureus (strain H), 10-2	1.8	0	0	0	0	—
		.15	0	0	0	++++
		.15	0	0.15	0	++++
		.15	0.15	0	0	—
		.15	0	0	0.05	—

TABLE IV.
Effect of Red Blood Cell Extract on Action of Penicillin *in Vitro.*

Temperature	Medium	Red blood cell extract cc	Penicillin 5.9 units/cc cc	No. organisms (× 1000) per cc 0 hr	No. organisms (× 1000) per cc 24 hr
37°C	Knight's basal medium 6 cc + nicotinic acid 0.1cc—M/100	0	0	228,950	264,000
		0.03	0	228,950	262,000
		0	0.07	228,950	257,000
		0.03	0.07	228,950	240,000
	,,	0	0	2,289	121,000
		0.03	0	2,289	255,000
		0	0.07	2,289	1.3
		0.03	0.07	2,289	1.2
4 °C	,,	0	0	228,950	490,000
		0.03	0	228,950	330,000
		0	0.07	228,950	280,000
		0.03	0.07	228,950	500,000
	,,	0	0	2,289	2,250
		0.03	0	2,289	1,575
		0	0.07	2,289	2,560
		0.03	0.07	2,289	1,445

or sulfapyridine and that bacteriostasis occurs only after this relatively long lag period. Repeated experiments have shown that penicillin is most effective during active multiplication but that the lag period before killing commences is short and the concentration of organisms unimportant so long as multiplication occurs and sufficient penicillin is present.[4]

In further experiments studies were carried out to determine the effect of concentration of organisms on the action of sulfadiazine and sulfapyridine.

Hemolytic streptococci were used throughout this experiment. Sulfadiazine and sulfapyridine were added to undiluted broth cultures containing 292,500,000 organisms per cc and to cultures diluted so as to contain 2,925,000 organisms and 29 organisms per cc respectively. The final concentration of each, sulfadiazine and sulfapyridine, was 99 μg per cc. Broth controls containing no drug were tested simultaneously. Incubation was carried out at 37°C.

In the presence of an initial concentration of 292,500,000 organisms per cc, a slight decrease in the number of organisms occurred in plain broth after 24 to 48 hours. The tubes containing sulfadiazine and sulfapyridine showed a similar change.

In the presence of an initial concentration of 2,925,000 organisms per cc, rapid multiplication took place in plain broth. Equally

[4] Hobby, G. L., and Dawson, M. H., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 178.

TABLE V.
Effect of Sulfonamides on Varying Concentrations of Hemolytic Streptococci (Strain C203MV) at 37°C.

Hr. of incubation	No. of organisms (× 1000) per cc		
	Sulfadiazine 99 μg/cc	Sulfapyridine 99 μg/cc	Broth
0	292,000	292,000	292,000
24	190,000	240,000	260,000
0	2,920	2,920	2,920
24	208,000	252,000	285,000
0	29	29	29
24	5,400	8,500	122,500

rapid multiplication occurred in the presence of either sulfadiazine or sulfapyridine.

In the presence of a small initial number of organisms per cc rapid multiplication occurred in plain broth whereas much slower multiplication occurred in the presence of sulfadiazine or sulfapyridine.

It is apparent that the sulfonamides were effective only after a lag period during which the organisms were in contact with the drug. On the addition of sulfadiazine or sulfapyridine after the organisms had passed into the phase of growth acceleration, no bacteriostasis took place. Neither sulfadiazine nor sulfapyridine acted on initially large numbers of organisms in spite of active multiplication. This is in contrast to the action of penicillin.

Summary. Under the experimental conditions used there was no evidence of a synergistic action between sulfadiazine or sulfapyridine and penicillin. Para-aminobenzoic acid did not increase the titer of penicillin although it does increase its rate of action.

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Inhibitory Effect of Certain Amino Acids on Growth of Young Male Rats.

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Previous attempts to supplement gelatin with known amino acids so as to provide optimum growth have been unsuccessful (Jackson *et al.*[1] Kruse[2]). Threonine was not recognized at the time and we attempted to determine whether this was the necessary factor.

Arginine, histidine, lysine, valine, leucine, isoleucine, methionine, tryptophane, phenylalanine, and threonine were fed at levels in excess of Rose's minimum levels[3] with 20% gelatin (Group 2). A second group was fed the amino acids alone (Group 3). A third group was fed gelatin alone (Group 1).

The animals fed the supplemented gelatin made slight gains whereas loss of weight occurred in the same period on gelatin alone. The animals fed the amino acids alone grew better than those on the gelatin-amino acid diet, despite the fact that gelatin supplied "non-essential" amino acids. However, the rate of growth on the amino acids alone was not optimal.

It seemed to us that gelatin might be in-

[1] Jackson, R. W., Sommer, B. E., and Rose, W. C., *J. Biol. Chem.*, 1928, **80**, 167.

[2] Kruse, H. D., Day, H. G., and McCollum, E. V., *Am. J. Hygiene*, 1934, **19**, 260.

[3] Rose, W. C., *Physiol. Rev.*, 1938, **18**, 109.