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## Use of Para-Aminobenzoic Acid to Inhibit Sulfonamide Action in Bactericidal Tests.

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(Introduced by W. T. Salter.)

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The use of sulfonamide drugs in the treatment of bacterial infections has raised new problems in the study of immunological changes occurring during the course of disease. Blood samples obtained from patients under treatment with sulfonamides frequently contain concentrations of these drugs which in themselves are bactericidal, thus masking immune reactions. Addition of appropriate amounts of *p*-aminobenzoic acid to such blood samples inhibits sulfonamide action.<sup>1</sup> Although antibody activity, in the case of the pneumococcus, may be differentiated from drug action, if bactericidal tests are terminated at 3 hours,<sup>2</sup> this procedure is not always desirable, nor is it feasible with certain other bacteria. It seemed necessary to determine whether or not *p*-aminobenzoic acid inhibits the sulfonamide effect without interfering with the bactericidal action of antibody. The following studies were therefore carried out.

Bactericidal tests were done on fresh defibrinated blood, using stock strains of pneumococci as in previous studies from this laboratory.<sup>3</sup> Sulfathiazole and *p*-aminobenzoic acid were added to the blood separately and in combination. In Experiment I, blood was obtained from a normal subject with a high titer of "natural" pneumococcal antibodies. In Experiment II the subject was convalescent from Type I lobar pneumonia; in Experiment III a subject whose blood lacked bactericidal action for Type V pneumococcus was chosen and the appropriate antibody added *in vitro*.

The results are shown in Table I. Neither the natural bactericidal action of defibrinated blood nor the bactericidal action associated with recovery from pneumococcus infection was interfered with by the addition of *p*-aminobenzoic acid. Blood which had no

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<sup>1</sup> Strauss, E., Lowell, F. C., and Finland, M., *J. Clin. Invest.*, in press.

<sup>2</sup> Finland, M., Lowell, F. C., and Spring, W. C., Jr., *New Eng. J. Med.*, 1940, **222**, 739.

<sup>3</sup> Spring, W. C., Jr., Lowell, F. C., and Finland, M., *J. Clin. Invest.*, 1940, **19**, 163.

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TABLE I.  
Effect of Antibody, Sulfathiazole and Para-aminobenzoic Acid, Alone or in Combination, on Pneumococcal Action of Fresh Defibrinated Human Blood.

Exp. No.	Material added to blood	Concentration, mg%	Growth inhibition		Pneumococcal action at 48 hr		Remarks	
			24 h.	48 h.	Inoculum	Growth		
I	Control		10 <sup>6</sup>	10 <sup>5</sup>	10 <sup>5</sup>	0	Normal subject with "natural" bactericidal antibody. Stock Type I pneumococcus used.	
	PABA	1	10 <sup>6</sup>	10 <sup>5</sup>	10 <sup>5</sup>	0		
	ST	10	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>6</sup>	1		
	PABA	} 1	10	166	10 <sup>5</sup>	10 <sup>5</sup>		0
	ST							
II	Control		10 <sup>7</sup>	10 <sup>7</sup>	10 <sup>7</sup>	0	Convalescent from Type I pneumococcus pneumonia; blood taken 7 days after crisis and 2 days after cessation of sulfonamide therapy. No serum treatment given. Stock Type I pneumococcus used.	
	PABA	1	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>6</sup>	0		
	ST	10	10 <sup>7</sup>	10 <sup>7</sup>	10 <sup>7</sup>	0		
	PABA	} 1	10	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>6</sup>		0
	ST							
III	Control		0	0	—	—	Normal subject having no natural bactericidal activity. Stock Type V pneumococcus used.	
	Pn V Antibody	} 1 unit per ml	10 <sup>6</sup>	10 <sup>4</sup>	10 <sup>4</sup>	0		
	Pn V Antibody							
	PABA	} 1	10	10 <sup>6</sup>	10 <sup>5</sup>	10 <sup>5</sup>		0
	ST							
	PABA	} 1	10	0	0	—		—
	ST							
	Pn V Antibody	} 1 unit per ml	10	10 <sup>6</sup>	10 <sup>4</sup>	10 <sup>4</sup>		0
	ST							
	PABA	} 1	10	10 <sup>6</sup>	10 <sup>4</sup>	10 <sup>4</sup>		0
PABA								

*Growth Inhibition.* Numbers represent the largest inoculum which showed no color change in the blood.

*Pneumococcal Action.* Listed only when inhibition was noted.

*Growth* = number of colonies grown in agar pour plates.

*Inoculum* refers to original number added to the blood. Larger inocula yielded too many colonies to count; smaller ones yielded no growth.

*Abbreviations.* PABA = para-aminobenzoic acid.

ST = sulfathiazole.

Pn V antibody = concentrated Type V antipneumococcus rabbit serum (Lederle).

h. = hours.

killing power against pneumococci acquired that property on the addition of either sulfathiazole or homologous antipneumococcus rabbit serum. The action of sulfathiazole was completely inhibited by *p*-aminobenzoic acid, whereas the action of the antibody was unaffected by it.

General applicability of this principle seems indicated by the fact

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 pneumococcal 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<sup>1</sup> 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<sup>2</sup> 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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<sup>1</sup> Woods, D. D., *British J. Exp. Path.*, 1940, **21**, 74.

<sup>2</sup> Selbie, E. R., *Ibid.*, 1940, **21**, 90.