

Antagonism Between Effects of P-Aminosalicylic Acid and Salicylic Acid on Growth of *M. tuberculosis*.

G. IVÁNOVICS. (Introduced by G. Gomori.)

From the Institute of General Pathology and Bacteriology, University of Szeged, Hungary.

It has been reported^{1,2} that the bacteriostatic action of PAS is not antagonized by small amounts of salicylic acid. Lehmann³ found in some of his experiments that a combination of PAS and salicylic acid gave a moderate inhibition of oxygen uptake of tubercle bacilli, although each of these acids, added separately, increased the rate of oxygen uptake. It was expected that an antagonism between salicylic acid and P-aminosalicylic acid (PAS) can also be demonstrated in cultures of tubercle bacilli if the experimental conditions are chosen correctly.

Methods. Cultures were grown in the liquid medium described by Dubos. It contained 0.05% of Tween 80 and 0.5% of bovine albumin fraction. A freshly prepared solution of the Na salt of PAS was added to the medium and serial dilutions by a factor of 2 were made. The tubes were inoculated with 0.0001 mg (dry weight) of tubercle bacilli grown in Dubos medium, and the total volume of the cultures was made up to 5 ml with dist. water. After 16 days of incubation at 37°C, 0.5 ml of phosphate buffer pH 7.0 containing 2% formaldehyde and 0.1% Tween 80 was added to each tube and the amount of tubercle bacilli was determined by turbidimetric readings in a Leitz universal colorimeter. Using a control culture as reference, the growth rate of bacilli in the presence of antiseptics was expressed in percentages of the full growth. Each dilution of drug or combination of drugs was tested in duplicates, and turbidimetric readings were averaged.

Four strains of *M. tuberculosis var. hominis* were used in these experiments. Two of them were isolated from sputa recently (K3 and K5); the others (Nos. 7800 and 5100) have been maintained for several years in our collection.

Results. Using strain 7800, the effect of PAS alone and in combination with sodium salicylate was studied simultaneously.

The striking antagonism between Na salicylate and PAS is shown in Table I. Although a concentration of 200 μ M of Na salicylate alone markedly inhibited the growth of tubercle bacilli, this amount antagonized the effect of about 30 minimal inhibiting concentrations of PAS. The tuberculostatic action of PAS is antagonized only by a considerable concentration of Na salicylate, and the molar ratio of PAS to Na salicylate in the range of minimal inhibition varied between 0.08 to 0.25, *i.e.*, one mole of PAS was antagonized by 4 to 12 moles of Na salicylate in this experiment.

In the presence of small concentrations of Na salicylate antagonism gradually diminishes, and at the highest dilution of drug tested (20 μ M) only a slight effect was seen.

As the tuberculostatic action of PAS is antagonized by p-aminobenzoic acid (PAB),⁴ we determined the molar ratio of PAS to this metabolite under similar experimental conditions. It was found that one mole of PAB abolishes the effect of about 4 moles of PAS. On comparing these values it is seen that the antagonistic effect of PAB is 16 to 50 times greater than that of salicylate.

In the presence of salicylate at a concentration of 620 μ M the growth of strain 7800 was found to be zero on turbidimetric read-

¹ Youmans, G. P., Raleigh, G. W., and Youmans, A. S., *J. Bact.*, 1947, **54**, 409.

² Fitzgerald, R. J., and Bernheim, F., *Am. Rev. Tuberc.*, 1948, **58**, 210.

³ Lehmann, J., *Svenska Läkartidn.*, 1946, **43**, 2029.

⁴ Goodacre, C. L., Mitchell, B. W., and Seymour, D. E., *Quart. J. Pharm. and Pharmacol.*, 1948, **21**, 301.

TABLE I.
Growth Rate of *M. tuberculosis* var. *hominis* (Strain 7800) in Percentages in the Presence of PAS and Salicylate. (Uninhibited growth = 100%.)

Salicylate, μ M	PAS μ M								
	50	25	12.5	6.25	3.12	1.56	0.76	0.38	0.00
200	0	18	29	32	40	40	46	57	65
80	0	0	0	9	46	61	65	68	94
40	0	0	0	0	8	35	50	70	96
20	0	0	0	0	0	11	53	72	100
0	0	0	0	0	0	0	26	67	100

ing. This complete inhibition was not antagonized by either PAB or pantothenic acid. A slight growth (about 8 to 12%) was, however, found if a certain concentration (about 2 μ M of PAS), which in itself alone is fully bacteriostatic, was added to the tube.

Similar observations were made with strains 5110 and K3. The effect of salicylate on strain K5, however, was found to be very slight.

The tuberculostatic effect of PAS was not antagonized by compounds related to salicylic acid. No antagonism was found when the Na salts of benzoic, phthalic, o-aminobenzoic, o-phenolsulfonic, o-chlorobenzoic, o-nitrobenzoic, dithiosalicylic, o-sulfobenzoic, p- and m-oxybenzoic, 2, 4-dioxybenzoic and 2-oxy-4-aminosulfobenzoic acids were tested in the range of concentrations between 40 and 200 μ M. m-Aminophenol and pantothenic acid were also found to be ineffective.

Discussion. The action of salicylate on bacteria is a variable one. The growth of some non-acid fast bacteria is prevented by the drug at high concentrations by inhibiting the synthesis of pantothenic acid in the cells.⁵ The non-pathogenic acid fast bacteria are capable of oxidizing salicylic acid,⁶ and the oxygen uptake of resting virulent tubercle bacilli is increased in the presence of this drug;⁷ however, it is not metabolized⁸ by the

cells. Fitzgerald and Bernheim⁹ concluded from their experiments that acid fast bacteria produce an adaptive enzyme in the presence of salicylate, and that the formation of this enzyme is inhibited by streptomycin. On the basis of these observations it can be assumed that both non-acid fast bacteria requiring no exogenous pantothenic acid for growth and various acid fast bacteria either contain or form adaptively an enzyme with specific affinity to salicylic acid. However, the synthesis of pantothenic acid in tubercle bacilli is not inhibited by this drug. The nature of this enzyme (or enzymes) occurring in a wide variety of bacteria is entirely obscure at present.

Salicylate antagonizes the tuberculostatic effect of PAS at concentrations so high as to be toxic by themselves. As found previously, salicylate has a twofold action on bacteria: in addition to a specific effect at high dilutions, the drug will damage the cells in high concentrations by its protein-denaturing effect.^{5,10} This latter action of the drug renders the study of antagonism of the two drugs investigated more difficult; however, it seems that it is a competitive one.

Summary. The bacteriostatic effect of p-aminosalicylic acid is antagonized by high concentrations of salicylic acid. This effect of salicylate is highly specific and not shared by related compounds. Pantothenic acid does not inhibit the tuberculostatic effect of salicylate.

⁵ Ivánovics, G., *Z. f. physiol. Chem.*, 1942, **276**, 32.

⁶ Bernheim, F., *J. Biol. Chem.*, 1943, **143**, 383.

⁷ Bernheim, F., *Science*, 1940, **92**, 204.

⁸ Fitzgerald, R. J., and Bernheim, F., *J. Bact.*, 1947, **54**, 671.

⁹ Fitzgerald, R. J., and Bernheim, F., *J. Bact.*, 1948, **55**, 765.

¹⁰ Ivánovics, G., Csábi, J., and Diezfalusy, E., *Hungarica Acta Physiol.*, 1948, **1**, 171.