Ind. Jour. Med. Res., **49**, 2, March, 1961.

EFFECT OF P-AMINOSALYCYLIC ACID (PAS) ON THE LOSS OF ACID-FASTNESS PRODUCED IN TUBERCLE BACILLI BY ISONIAZID.

K. G. VARMA.

(*From the Tuberculosis Chemotherapy Centre, Madras.**)

[Received for publication, October 10, 1960.]

The microbiological assay method for estimating free isoniazid in serum described by Mandel *et al.* (1956), used as the assay end-point the dilution pro-ducing loss of acid-fastness in 50 per cent of bacilli. The advantage of this end point is that loss of acid-fastness is produced specifically by isoniazid, and not by either PAS (Mandel *et al., loc. cit.)* or streptomycin (Middlebrook, 1952). Mandel *et al. (loc.* cit.) claimed that the addition of as much as 1,000 µg./c.c. of PAS to undiluted human sera did not interfere with the assay of isoniazid in the serum using loss of acid-fastness as the end-point. Since Mandel *et al. (loc. cit.)* did not however, describe their results fully, it remained a possibility that PAS in con-centrations near that necessary to inhibit the growth of tubercle bacilli c ould act synergestically or antagonistically with isoniazid in producing loss of acid-fastness. This was investigated in the following experiments.

METHOD.

The method used for investigating the effect of PAS on the loss of acid-fast- ness produced by isoniazid was similar to that used for the microbiological assay of isoniazid (Mandel *et al., loc. cit.).* A series of twofold increasing concentrations of PAS from 0.25 to 2 µg./c.c. of sodium PAS without isoniazid or with 0.02, 0.03, 0.04 or 0.5 µg./c.c. isoniazid were prepared in 2 c.c. volumes of 7H-10 liquid medium (Cohn *et al.,* 1954 ; Gangadharam, Selkon and Bhatia, 1961), but without Tween 80 or glycerol. Each tube was inoculated with 0.1 c.c. of an 8-day old culture of H37Rv grown in 7H-10 medium contain-ing 0-05 per cent Tween 80. After incubation at 37° C. for five days, smears were prepared from the deposits of growth and stained by the Ziehl-Neelsen method.

RESULTS.

The percentage of acid-fast bacilli was first determined by exaining six oil-immersion fields and estimating approximately the percentage of acid-fast bacilli among those seen (Table I). The loss of acid-fastness in the series of PAS con-centrations with the isoniazid concentration which produced approximately 50 per cent loss of acid-fastness, namely 0.03 µg./c.c., was then more accurately determined by actually counting the acid-fast, non-acid-fast and doubtfully acid-fast bacilli. Each estimation was based on a count of at least 100 bacilli. The experiment was carried out in duplicate and the average of the results are given in Table II.

* Under the joint auspices of the Indian Council of Medical research, the Madras State Govern-ment, the World Health Organisation and the British Medical Research Council.

TABLE I.

Percentage of acid-fats bacilli in different concentrations of isoniazid and PAS.

Concentration of INAH in µg./c.c	CONCENTRATIONS OF SODIUM PAS DIHYDRATE IN µg./c.c.				
	0.00	0.25	0.5	1.0	2.0
0.00	100	100	100	100	100
0.02	80	80	70	70	70
0.03	40	40	35	35	30
0.04	*	5	5	5	5
0.05	0	0	0	0	0

* Contamined.

TABLE II

The effect of different PAS concentrations on the loss of acid-fastness produced by 0.03 µg./c.c. *of isoniazid.*

Staining character.	CONCENTRATIONS OF SODIUM PAS DIHYDRATE IN µg./c.c.				
	0.00	0.25	0.5	1.0	2.0
Acid-fast	39.8	27.2	34.8	26.8	41.6
Non-acid-fast	51.7	70.2	57.5	69.3	55.8
Doubtful	8.5	2.6	7.7	3.9	3.6

* Percentage of acid-fast, non-acid fast and doubtfully acid-fast bacilli as determined by actual count.

From the above results, it can be conclude that PAS in twofold increasing concentration from 0.25 µg./c.c to 2.0 µg./c.c of the sodium salt had no effect on the loss of acid-fastness produced by isoniazid.

REFERENCES.

COHN, M.L., ODA, U., KOVITZ, C., and MIDDLEBROOK, G. (1954) Studies on isoniazid and tibercle bacilli. I. The isolation of isoniazid-resistant mutants *in vitro. Amer. Rev. Tuberc.*, **70,** 465.

GANGADHARAM, P.R.J., BHATIA, A.L., RADHAKRISHNA, S., and SELKON, J.B. The rate of inactivation of isoniazid in South Indian patients with pulmonary tuberculosis. I. the microbiological assay of isoniazid in serum following a standard intramuscular dose (to be published).

MANDEL, W., COHN, M.L., RUSSELL, W.F, and MIDDLEBROOK, G. (1956) Effect of para-amino salicylic acid on serum isoniazid levels in men. *Proc. Soc. Exper. Biol. Med.*, **91,** 409.

MIDDLEBROOK, G. (1952) ... ... Sterilization of tubercle bacilli by isonicotinic acid hydrazide and the incidence of variants resistant to the drug *in vitro. Amer. Rev. Tuberc.*, **65,** 765.