

## PARA-AMINOSALICYLIC ACID—PART IV

### ATTEMPTS TO INDUCE RESISTANCE TO PARA-AMINOSALICYLIC ACID, IN STRAINS OF *MYCOBACTERIUM TUBERCULOSIS*

BY C. L. GOODACRE AND D. E. SEYMOUR

*From the Research Department, Herts Pharmaceuticals Ltd.*

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THE unfortunate limitation of streptomycin in the treatment of tuberculosis, due to the development of drug resistance, suggested to us the necessity for an investigation as to whether similar phenomena might occur with *p*-aminosalicylic acid.

The investigation was concerned with attempts to induce drug-resistance *in vitro*, and with a study of strains of *M. tuberculosis* isolated from patients before and during *p*-aminosalicylic acid treatment. The H37RV strain was cultivated in Dubos medium containing decreasing amounts of the acid. After a suitable incubation period, the bacilli in the tubes containing the highest concentration of *p*-aminosalicylic acid which was still allowing growth were freed from it by washing, and used to inoculate a similar series of tubes. No increased resistance was demonstrated by this method, in fact, no growth at all occurred at the dilutions used after two or more passages. These results are confirmed by Hurni<sup>1</sup> who reported on a similar study while this work was in progress. In view of the unsatisfactory results obtained with the above method, the following procedure was adopted in an attempt to induce drug resistance. A large inoculum (0.5 mg./ml. of dry bacterial substance) of *M. tuberculosis* H37RV was introduced into Dubos medium containing *p*-aminosalicylic acid in a concentration of 100 mg./ml. After 14 days' incubation, a similar concentration of *p*-aminosalicylic acid in Dubos medium was inoculated from this culture. Inhibition concentration tests were made at monthly intervals, and after 10 months (20 passages) the organisms showed a similar *p*-aminosalicylic acid sensitivity to that at the beginning of the experiment. A duplicate experiment was carried out using a medium containing no tween 80 with similar results.

These results suggest that under the above conditions the H37RV strain does not become resistant to the tuberculostatic action of *p*-aminosalicylic acid. In an attempt to obtain further and possibly more significant data we obtained cultures of *M. tuberculosis* isolated before and during treatment from patients suffering from pulmonary tuberculosis who received 20 g./day for 6/day week of sodium *p*-aminosalicylate given orally in divided doses. The strains, after cultivation in Dubos medium, were subjected to sensitivity tests by the method described in our previous paper<sup>4</sup> using a standard inoculum (0.001 mg./ml. of dry bacterial substance). The results of these experiments are shown in Table I and it will be seen that in only one instance (case 9) was there any indication of development of resistance to *p*-aminosalicylic acid. It is of interest to note, however, that there does exist a slight difference in sensitivity between different strains. These results together with those of

PARA-AMINOSALICYLIC ACID. PART IV

Lehmann<sup>2</sup>, Hurni<sup>1</sup>, and Seivers<sup>3</sup> suggest that "drug-fastness" is not a significant problem in the treatment of tuberculosis with this drug.

Whilst this paper was in course of preparation, Graessle and Pietrowski<sup>5</sup> reported that repeated exposure of *M. tuberculosis* H37RV to *p*-aminosalicylic acid for 120 days failed to produce an increase in the resistance of the strain.

TABLE I

Case No.	Before treatment	<i>p</i> -Aminosalicylic Acid treatment		
		2 months	3 months	4 months
1 ... ..	0·0243—0·0121			0·0975—0·0487
2 ... ..	0·0487—0·0243	0·0243—0·0121		
3 ... ..	0·0243—0·0121			0·0487—0·0243
4 ... ..	0·0243—0·0121			0·0487—0·0243
5 ... ..	0·0243—0·0121			0·0487—0·0243
6 ... ..	0·0243—0·0121			0·0243—0·0121
7 ... ..	0·0487—0·0243	0·0487—0·0243		0·0487—0·0243
8 ... ..	0·0487—0·0243	0·0487—0·0243		0·0487—0·0243
9 ... ..	0·0243—0·0121			3·125 —1·56
10 ... ..	0·0243—0·0121	0·0243—0·0121		0·0121—0·006
11 ... ..	0·0487—0·0243	0·0487—0·0243		0·0487—0·0243
12 ... ..	0·0487—0·0243	0·0243—0·0121		0·0487—0·0243
13 ... ..	0·39 —0·195	0·39 —0·195		0·0975—0·0487
14 ... ..	0·0487—0·0243	0·0487—0·0243		0·0487—0·0243
15 ... ..	0·0243—0·0121	0·0243—0·0121		0·0243—0·0121
16 ... ..	0·0243—0·0121			0·0243—0·0121
17 ... ..	0·0487—0·0243		0·0487—0·0243	
18 ... ..	0·0487—0·0243		0·0487—0·0243	
19 ... ..	0·0243—0·0121	0·0243—0·0121		
20 ... ..	0·0243—0·0121	0·0243—0·0121		
21 ... ..	0·0487—0·0243			0·0487—0·0243
22 ... ..	0·0243—0·0121			0·0243—0·0121
23 ... ..	0·0243—0·0121	0·0243—0·0121		
24 ... ..	0·0243—0·0121	0·0243—0·0121		
25 ... ..	0·0487—0·0243		0·0487—0·0243	

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SUMMARY

1. After repeated exposure of *M. tuberculosis* H37RV to *p*-aminosalicylic acid for 10 months, no increase in resistance developed.
2. Out of a total of 25 strains of *M. tuberculosis* isolated from patients receiving *p*-aminosalicylic acid, only one developed any increase in resistance after four months' treatment.

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