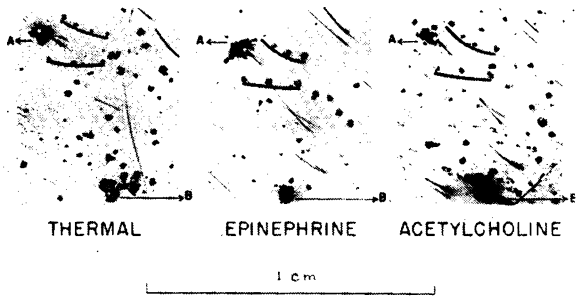



Fig. 1. Sweat responses to the three stimuli in one subject. *A* and *B* are the reference spots (moles), marked with India ink before photographing. The added lines are beneath two groups of sweat spots that appear in all three instances. Other coinciding spots may be seen.

were made so that the spots of sweat appeared black upon a pale transparent background. It was thus possible to attempt superimposition of the photographs in front of a bright light, using the two moles and the clipped hairs as reference points. This simple technique revealed the following: (i) Some of the individual sweat glands were stimulated on one occasion and not on another, despite the reapplication of the same stimulus and the reappearance of an approximately equal number of black spots in the same area. This "responsiveness" of the glands appeared to be capricious. (ii) It was nevertheless clear that, despite this individual variation, a very similar pattern of sweat glands was produced in each case by heat, epinephrine, and acetylcholine (Fig. 1) The similarity in pattern was so obvious that prints from any two stimuli could be superimposed in such a way that a large percentage of the spots coincided.

This visual impression was tested statistically as follows: Some of the positive transparencies were placed in a photographic enlarger, and the shadows were projected upon white paper at a standard distance and over a standard area. The black spots from a given print were then traced in a given color, and upon the same area of paper the spots of another print were traced in another color. Spots touching each other were considered coincident. In this manner the coincidence of spots derived from each subject was tabulated for the following combinations: (i) thermal patterns, (ii) epinephrine patterns, (iii) acetylcholine patterns, (iv) thermal and epinephrine, (v) thermal and acetylcholine, (vi) epinephrine and acetylcholine, (vii) a print produced by any of the three stimuli and the same print turned 180°, and (viii) a print produced by any of the three stimuli and a print produced by another stimulus rotated 180°. Random coincidence of spots would be directly proportional to the product of the number of spots in any two prints if all the spots were of equal size. Since both the differences in size of the spots and the average size of spots were negligible, as compared with the total area, for each pair of prints plotted a value ("J") was computed, representing the ratio of the number of coincident spots to the product of the total number of spots in each print that fell within the tabulated area. The average J-value for one stimulus with another at 180° and one print with another at 180°, or the two combined, was about 0.0027, whereas all other J-values were in the neighborhood of 4 times as great, about 0.01 or more.

No significant difference occurred between average J for the prints compared with themselves at 180° of rotation and average J for different prints so compared ($p > 0.2$). This suggests a random distribution of the sweat glands, and hence all prints compared at 180° were pooled to determine J for chance coincidence. There was no significant difference between J for the prints produced by identical stimuli and J for prints produced by different stimuli ($p > 0.5$). The difference, however, between J for the prints superimposed properly and J for the prints superimposed at 180° was highly significant ($p < 0.001$). In other words, coincidence among sweat patterns evoked by *different* stimuli was as good as that produced by the *same* stimulus on different occasions. The extent of this coincidence was far greater than that demonstrated to occur at random.

This degree of similarity among the patterns evoked by the three different stimuli would not be found if each stimulus involved a different set of sweat glands. The findings are consistent with the view that the same individual sweat glands are stimulated by epinephrine and acetylcholine, at the concentrations used, and reflexly by heat. This demonstration does not imply a dual innervation of the sweat glands.

#### References and Notes

1. E. E. Kisin, *Vestnik Vernerol i. Dermatol.*, No. 5, 27 (1948) [abstracted in *Chem. Abstr.* **48**, 2323 (1948)]; R. R. Sonnenschein, *Proc. Soc. Exptl. Biol. Med.* **71**, 654 (1949); R. R. Sonnenschein *et al.*, *J. Appl. Physiol.* **3**, 573 (1951); H. Haimovici, *ibid.* **2**, 512 (1950); T. M. Chalmers and C. A. Keele, *J. Physiol.* **114**, 510 (1951).
2. M. Wada, *Science* **111**, 376 (1950).
3. Supported by a Lilly Research grant. We wish to thank Donald Jenden for valuable advice and Frank Wilbar and Carmelita Salazar for technical assistance.

23 August 1954.

## Resistance of *M. tuberculosis* to the PAS Salt of INH

**H. Brodhage**

*Institute of Pathology and Bacteriology, Cantonal General Hospital, Lucerne, Switzerland*

Preliminary tests have shown that the p-aminosalicylate (PAS) salt of isonicotinylhydrazine (INH) (*1*) is unexpectedly highly active in *in vitro* tests against *M. tuberculosis* (H 37 Rv). This tuberculostatic effect was confirmed in therapeutic tests carried out on the guinea pig. Further *in vitro* tests on the activity of the PAS salt of INH (*2*) against *M. tuberculosis* (H 37 Rv) (*3*) and certain resistant strains are herewith reported.

In our *in vitro* experiments (serial dilution tests in

liquid Dubos-culture medium, each tube containing 5 ml and an inoculation with 1 drop of a 10-day culture of H 37 Rv-Dubos-Tween culture) the PAS salt of INH shows a tuberculostatic effect which seems often to exceed that of physical mixtures of PAS and INH. Whether this difference is statistically significant is still not clear (*4*).

On the basis of these experiments it appeared that it would be of interest to determine the tuberculostatic effect of the PAS salt of INH on resistant strains in routine testing. The primary cultures were inoculated into liquid Dubos-Tween culture medium. When growth had proceeded sufficiently, the culture was standardized to a predetermined degree of turbidity and then inoculated into liquid Dubos culture medium containing various concentrations of the tuberculostatics to be tested. These last-mentioned Dubos cultures contained no Tween and thus differed from the primary cultures. In this manner unreliable results owing to the addition of Tween are avoided (*5, 6*). This method simplifies the reading of results, since the bacilli show a flaky type of growth under these conditions.

The experiments with 7 of the 32 strains tested are summarized in Table 1. The results obtained during these resistance tests are unexpected in that bacilli resistant to both PAS and INH are sensitive to the PAS salt of INH.

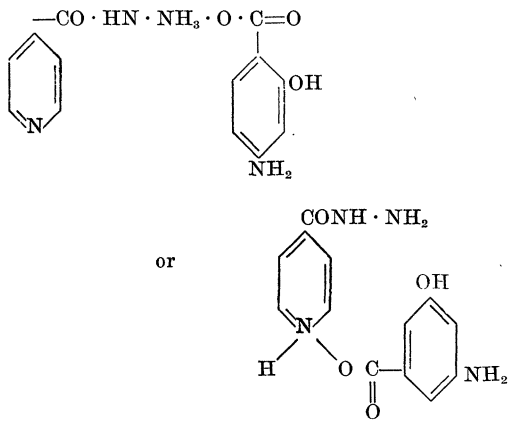
Table 1. Resistance determinations.

| Strain: | | 0.1 | 1.0 | 5.0 | 10 | 50 | 100 | Control |
|---|---|---|---|---|---|---|---|---|
| | | Drug concentration (μg/ml) | | | | | | |
| 147/54 | SM | +++ | ++ | ++ | – | – | – | +++ |
| | PAS | +++ | +++ | +++ | ++ | (+) | – | |
| | INH | +++ | – | – | – | – | – | |
| | Salt | ++ | – | – | – | – | – | |
| 790/54 | SM | +++ | – | – | – | – | – | +++ |
| | PAS | +++ | +++ | +++ | +++ | +++ | ++ | |
| | INH | ++ | – | – | – | – | – | |
| | Salt | +++ | – | – | – | – | – | |
| 2725/53 | SM | – | – | – | – | – | – | +++ |
| | PAS | +++ | – | – | – | – | – | |
| | INH | +++ | +++ | ++ | ++ | + | – | |
| | Salt | +++ | ++ | + | – | – | – | |
| 3071/53 | SM | – | – | – | – | – | – | +++ |
| | PAS | +++ | (+) | – | – | – | – | |
| | INH | +++ | +++ | +++ | +++ | ++ | + | |
| | Salt | +++ | (+) | – | – | – | – | |
| 2757/53 | SM | (+) | – | – | – | – | – | +++ |
| | PAS | +++ | +++ | +++ | ++ | ++ | ++ | |
| | INH | +++ | +++ | ++ | + | – | – | |
| | Salt | +++ | ++ | + | – | – | – | |
| 3102/53 | SM | – | – | – | – | – | – | +++ |
| | PAS | +++ | +++ | +++ | +++ | +++ | ++ | |
| | INH | +++ | +++ | +++ | ++ | ++ | + | |
| | Salt | +++ | +++ | ++ | – | – | – | |
| 2802/53 | SM | ++ | – | – | – | – | – | +++ |
| | PAS | +++ | +++ | +++ | ++ | +++ | ++ | |
| | INH | +++ | +++ | +++ | +++ | +++ | + | |
| | Salt | +++ | +++ | ++ | – | – | – | |

Identification of symbols: +++, ++, +, various degrees of growth; (+), doubtful growth; –, no growth; SM, Streptomycin; Salt, PAS salt of INH.

Resistance tests have shown that the PAS salt of INH possesses a superior tuberculostatic effect even where strains resistant to both PAS and INH are concerned. The effect is scarcely to be explained on the basis of the small amount of PAS present in the salt (*7*). It seems more probable that the PAS salt of INH possesses in itself a tuberculostatic effect of its own.

The PAS salt of INH behaves in many respects as a new chemical compound. Being a salt, it may, of course be split into its components in the same way as other organic salts. Its structure may be represented as




It may be crystallized from aqueous ethanol or methanol as fine, yellow needles and shows the following elementary analysis in percentages: found, C = 53.81, H = 4.95, N = 19.54; calculated for $C_{13}H_{14}N_4O_4$, C = 53.79, H = 4.86, N = 19.30. It melts between 140° and 142°C with decomposition and is soluble to the extent of 1 to 2 percent in water.

That the PAS salt in INH possesses its own specific tuberculostatic properties is borne out by the clinical results obtained by Hueck (*8*) and Krug (*9*), using this substance. Patients who did not respond to INH, PAS, or streptomycin therapy responded to treatment with the PAS salt of INH.

### References and Notes


1. M. Aufdermaur and H. Brodhage, *Praxis* **42**, 885 (1953).
2. Obtainable under the name of "Dipasic" from Ed. Geistlich Sons Ltd., Wolhusen, Switzerland. Thanks are expressed to them for supplying the required quantities of this substance.
3. The strain H 37 Rv was kindly supplied by the Centre de Collection de Types Microbiens, Lausanne (P. Hauduroy).
4. A. E. Wilder Smith and F. X. Wiederkehr, *Praxis* **42**, 884 (1953).
5. E. Wiesmann, *Schweiz. Z. Tuberk.* **10**, 277 (1953).
6. G. Canetti and G. Rocher, *Progr. Evplor. Tuberc.* (S. Karger, Basel–New York, 1950), vol. 3, p. 30.
7. E. Wiesmann, *Schweiz. Z. Tuberk.* **10**, 351 (1953).
8. O. Hueck, *Tuberk. Arzt. Jahrg.* **8**, No. 7, 423 (1954).
9. F. Krug, *Münch. med. Wochschr.*, **96**, 1126 (1954).


16 August 1954.