CHART I. Non-Inhibitors.

Compound	Conc./flask
Penicillin G	0–400 μ
Tetracycline	0–400 μg
Erythromycin	0– 1 mg
Gantrisin	0– 16 mg
Hydrocortisone	0–500 μg

might be expected in an infant, particularly when high doses are given. Chloromycetin shows slight inhibition within the accepted range and streptomycin just beyond the accepted range of serum levels.

1. Waters, W. J., *J. Ped.*, 1958, v52, 559.
2. Lacson, P. S., Waters, W. J., *Am. J. Dis. Child. Soc. Trans.*, 1957, v94, 510.
3. Crosse, M. V., Meyer, T. C., *ibid.*, 1957, v90, 602.
4. Allison, A. C., *Lancet*, 1955, v1, 669.
5. Zinkham, W. H., Childs, B., *Am. J. Dis. Child. Soc. Trans.*, 1957, v94, 420.
6. Silverman, W. A., Andersen, D. H., Blanc, W. A., Crozier, D. N., *Pediatrics*, 1956, v18, 614.
7. Dutton, I. D. E., Storey, G. J., *Biochem. J.*, 1954, v57, 275.

Received July 18, 1958. P.S.E.B.M., 1958, v99.

Inhibition of Diaminopimelic Acid Decarboxylase Activity in *Mycobacterium tuberculosis* by Isonicotinic Acid Hydrazide.* (24286)

HILDA POPE WILLETT (Introduced by D. T. Smith)
Department of Bacteriology, Duke University Medical Center, Durham, N. C.

In an attempt to determine the mechanism of action of isonicotinic acid hydrazide (INH) on *Mycobacterium tuberculosis* attention has been directed in r any studies toward an interference with the operation of essential enzymes. Inhibition of growth by INH can be neutralized competitively by pyridoxal, which the drug structurally resembles(1,2). A number of enzyme systems requiring pyridoxal phosphate as coenzyme have also been shown to react with INH(3-5). Although these studies suggest an antagonism, there is as yet no direct evidence that INH exerts its antituberculous activity by inhibiting an enzyme activated by pyridoxal phosphate.

In a recent study in our laboratory it was found that growth of tubercle bacilli in the presence of INH leads to a marked decrease in amount of lysine produced, and that lysine synthesis by the INH-resistant strain is also lower than that by the INH sensitive organism. Since one of the major pathways of lysine synthesis in microorganisms is by way of the pyridoxal phosphate-requiring enzyme, diaminopimelic acid (DAP) decarboxylase, we have investigated the possibility that this enzyme is operative in tubercle bacilli and that it is inhibited in the presence of INH.

Methods. The RlRv strain of *Myco. tuberculosis,* kindly furnished by Dr. William Steenken, was used as the source of enzyme in all of our studies. Cells were harvested after a 3-week growth period on the surface of Proskauer-Beck synthetic liquid medium. They were washed twice in a refrigerated centrifuge with sterile distilled water, resuspended in a small amount of 0.1 M phosphate buffer and ground in a Ten Broeck tissue grinder. Additional buffer was added, the cells recentrifuged, and suspended in a known quantity of buffer. The cells were disrupted in a Raytheon 9KC sonic oscillator followed by centrifugation at 16,000 x G for 30 min. The straw-colored supernatant contained the active enzyme and was used immediately. All operations were carried out in the cold and because of the long reaction time employed, sterile conditions were maintained throughout all the experiments.

The reaction mixture for detecting DAP decarboxylase activity contained 12.5 μM synthetic DAP, 10 μg pyridoxal phosphate,

* This study was aided by a grant from the Committee on Medical Research of the American Trudeau Soc., medical section of the National Tuberculosis Assn.

TABLE I. Inhibition of DAP Decarboxylase by INH.

INH conc. (μg)	A μg lysine	A % inhibition	B μg lysine	B % inhibition
100	4.3	98.3	34.4	86.4
10	121.7	51.8	175.2	30.7
1	187.9	25.7	227.2	10.2
.1	196.8	22.2	234.4	7.5
Control	252.9			

A = 1 hr preincubation with INH before addition of substrate.
B = No preincubation with INH.

1.0 ml of cell-free extract in a 0.1 M phosphate buffer, pH 7.0. Total reaction volume was 2.0 ml. Suitable controls were included in all experiments. Varying concentrations of INH were employed and in some vessels INH was preincubated with the enzyme preparation before the substrate was added. The reaction time was 18 hours, and the reaction stopped by heating at 100°C for 5 min. The precipitated protein was removed by centrifugation and the clear supernatant solutions used for detection of enzymic reaction products. Presence of lysine was detected by one-dimensional paper chromatography using phenol-water and butanol-acetic as solvents. Final identification of lysine was by use of the microbiological assay method using *Leuconostoc mesenteroides* as the test organism.

Results. Examination of the reaction mixture showed that DAP was converted to lysine by an enzyme present in cell-free extracts of tubercle bacilli. Identification of lysine as the end-product was made by comparison of the unknown ninhydrin-reacting spot with a lysine control in different solvents, and by demonstration of its utilization by the lysine-requiring bacterium, *L. mesenteroides*. No lysine was produced in the absence of DAP. No spot comparable to that of cadaverine was detected by chromatography.

INH was found to cause a marked reduction in activity of DAP decarboxylase. This inhibition was most pronounced when the drug was preincubated with the enzyme-containing extract before addition of substrate. Table I shows the effect of preincubation of the enzyme for 1 hour with INH before addition of the substrate, in a system in which extraneous pyridoxal phosphate was omitted.

When pyridoxal phosphate is added to the reaction mixture, degree of inhibition at the various drug levels is reduced. This protection against INH is greatest when the enzyme is preincubated with pyridoxal phosphate for 1 hour before addition of the substrate and INH (Table II). It is also more pronounced at the lower levels of INH. At the higher concentrations of the drug, the amount of pyridoxal phosphate employed was probably insufficient to neutralize effectively the large dose.

Discussion. Cell-free extracts of *Myco. tuberculosis* strain RIRv have been found to contain an enzyme which decarboxylates α-ε-diaminopimelic acid to CO_2 and lysine. This amino acid, first described by Work in acid hydrolysates of *Corynebacterium diphtheriae*(6,7), has since been shown to be present in many bacteria and blue-green algae, but to be absent from other organisms so far examined(8). Evidence is also accumulating to suggest that DAP serves a metabolic role as a precursor in the synthesis of lysine (9). It has been described and isolated from *Myco. tuberculosis*(10,11) where it is an important fraction of the cell wall, but there has been no previous report of a metabolic role in this organism. Rate of decarboxylation of

TABLE II. Effect of Pyridoxal Phosphate on INH Inhibition of DAP Decarboxylase Activity.

INH conc. (μg)	A μg lysine	A % inhibition	B μg lysine	B % inhibition	C μg lysine	C % inhibition
100	13.2	96.6	70.9	82.2	63.9	83.6
10	267.3	31.5	370.4	7.1	316.9	18.9
1	363.5	6.8	382.1	4.2	364.0	6.7
.1	373.6	4.3	396.5	.58	370.3	5.1
0	390.3		398.8		390.3	

A = 1 hr preincubation with INH before addition of substrate and pyridoxal phosphate.
B = 1 hr preincubation with pyridoxal phosphate before addition of substrate and INH.
C = Substrate, pyridoxal phosphate, and INH added simultaneously.

DAP by cell-free extracts of *Myco. tuberculosis* was increased by addition of pyridoxal phosphate, although it was not essential for enzyme activity. This is probably because with the methods employed, the decarboxylase present in the extract is still largely undissociated from its coenzyme. DAP decarboxylase activity was markedly inhibited by INH. Inhibition was greater if the drug was preincubated with the cell-free extract before addition of substrate. The results obtained also clearly indicate a relationship between toxicity of INH and pyridoxal phosphate. Further studies, however, are necessary to determine whether the reaction is a strictly competitive one between INH and pyridoxal phosphate for active sites on the enzyme molecule. The protection obtained by preincubation of the preparations with pyridoxal phosphate prior to addition of INH seem to indicate a competitive relationship.

On the basis of our findings it is suggested that the DAP decarboxylase is important in the metabolism of *Myco. tuberculosis*, serving as an important and probably major pathway in lysine synthesis. The finding that DAP decarboxylase is inhibited by INH, and that this inhibition can be overcome by pyridoxal phosphate, is in accordance with the previous observation in our laboratory that lysine synthesis by *Myco. tuberculosis* is markedly inhibited by culture in the presence of subinhibitory concentrations of isoniazid. Because of the nature of the isoniazid molecule the interpretation of any data relating to its specific mode of action is difficult, but the finding of a pyridoxal phosphate-requiring enzyme whose activity is inhibited by the drug lends additional support to the hypothesis that one of the important points of attack of INH in *Myco. tuberculosis* are those enzyme systems requiring pyridoxal phosphate as coenzyme.

Summary. DAP decarboxylase activity has been detected in cell-free extracts of *Myco. tuberculosis*. Enzyme activity was inhibited by INH and this inhibition could be overcome by pyridoxal phosphate.

1. Pope, H., *Am. Rev. Tuberc.*, 1953, v68, 938.
2. Boone, I. V., Woodward, K. T., PROC. SOC. EXP. BIOL. AND MED., 1953, v84, 292.
3. Yoneda, M., Kato, N., Okajima, M., *Nature*, 1952, v170, 803.
4. Yoneda, M., Asano, N., *Science*, 1953, v117, 277.
5. Lichstein, H. C., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 519.
6. Work, E., *Biochim. biophys. Acta*, 1949, v3, 400.
7. ———, *ibid.*, 1950, v5, 204.
8. Work, E., Dewey, D. L., *J. Gen. Microbiol.*, 1953, v9, 394.
9. Dewey, D. L., Hoare, D. S., Work, E., *Biochem. J.*, 1954, v58, 523.
10. Asselineau, J., Choucroun, N., Lederer, E., *J. Gen. Microbiol.*, 1950, v5, 197.
11. Work, E., *Biochem. J.*, 1951, v49, 17.

Received July 21, 1958. P.S.E.B.M., 1958, v99.

Effect of d-Lysergic Acid Diethylamide on Spinal Reflexes. (24287)

LESLIE E. GEIGER AND PETER CERVONI (Introduced by William H. Chambers)
Clinical Research Division, U. S. Army Chemical Warfare Laboratories, Army Chemical Center, Md.

The alterations in mental function induced by d-lysergic acid diethylamide (LSD) are, presumably, paralleled by modifications of the activity of cells or synapses within central nervous system, whether cortical, subcortical or spinal. LSD, when administered to spinal cats, in doses as small as 10 μg/kg i.v. was found to augment polysynaptic withdrawal and crossed extensor reflexes(1). At higher doses (40-80 μg/kg) depression of the flexion reflex was found. The monosynaptic knee jerk was less consistently affected. After administration of LSD in doses sufficient to cause mental symptoms increased activity of stretch reflexes has, however, been a consistent clinical observation(2). In the following experiments, the effects of LSD were evaluated by measurement of changes in magnitude of the reflex volley in ventral roots rather than by recording of muscular contraction.