Short Communications and Preliminary Notes

EFFECT OF ISONICOTINIC HYDRAZIDES ON ENZYME SYSTEMS*

by

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The structural similarity of the anti-tubercular drugs[1], isonicotinyl hydrazide** and 1-isonicotinyl-2-*iso*propyl hydrazine, to both pyridoxal and the pyridine nucleotides, has suggested that these substances may act by inhibiting an essential enzyme in the organism. Using both microbial and mammalian enzymes, it has been shown that monamine[2] and diamine oxidase[3], guanidine deamidinase[3], and tryptophanase[4] are inhibited by low levels (*ca.* 10^{-4} to 10^{-5} *M*) of INH and IIH. ZATMAN *et al.*[5] made the significant observation that certain DPN-nucleosidases cause an exchange of INH for the nicotinamide portion of DPN, while NEUBERG AND FORREST[6] have observed that INH interferes with yeast glycolysis by forming the hydrazone of acetaldehyde. The present communication summarizes our studies of the effects of INH and IIH*** on a variety of purified enzymes from mammalian sources, prepared and assayed by conventional techniques.

DPN-requiring dehydrogenases, such as malic, alcohol, 3-phosphoglyceraldehyde and lactic, and TPN-requiring dehydrogenases such as *iso*citric and glucose-6-phosphate, are unaffected by 10^{-4} *M* INH or IIH. Likewise, DPN-pyrophosphatase and DPN-ase (pig brain and erythrocytes) are not inhibited. ROGERS *et al.*[7] have shown similarly that DPN-ase from guinea pig lung is unaffected by a series of pyrazinoic amide compounds. Inhibition of a pyridine nucleotide system was observed, however, when lactic dehydrogenase catalyzed the reduction of pyruvate (*viz.* pyruvate + DPNH + $H^+ \longrightarrow$ lactate + DPN^+), as shown in Table I. Both 1 and 10 μmoles of INH produce about 30% inhibition, which is not overcome by raising the enzyme concentration.

TABLE I

INHIBITION OF LACTIC DEHYDROGENASE BY INH

Inhibitor	*Experiment A*		*Experiment B*	
	Velocity	*Per cent of control*	*Velocity*	*Per cent of control*
None	2.93	—	3.57	—
1 μM	1.99	67.9	2.38	66.7
10 μM	2.09	71.3	2.51	70.3

Velocities are measured as the rate of disappearance of DPNH and are expressed as μmoles/liter/min. Experiments A and B were carried out at two different concentrations of enzyme.

A series of enzymes were considered involving the oxidation of reduced pyridine nucleotides' DPNH-cytochrome *c* reductase and diaphorase are not inhibited, although the drugs "interfere'. by reducing both cytochrome *c* and 2,6-dichlorophenol indophenol non-enzymically (*cf.*[7] for a discussion of oxidation potentials of anti-tubercular drugs). The oxidation of TPNH by oxygen or methemoglobin, catalyzed by purified methemoglobin reductase[8], is also unaffected by INH and IIH.

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** The following abbreviations are used in this paper: INH = isonicotinyl hydrazide (Rimifon); IIH = 1-isonicotinyl-2-*iso*propylhydrazine (Marsilid); DPN, DPNH and TPN, TPNH = oxidized and reduced di- and triphosphopyridine nucleotide; DPN-ase = DPN-nucleosidase; and MHb = methemoglobin.

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The latter result, obtained with the purified reductase, is surprising since human erythrocytes, shaken in air for 30 h at 38°, accumulate 5.7 g of MHb per 100 g of IIH-treated cells as compared to 0.9 g in the control sample. This would suggest either a chemical oxidation of hemoglobin (although at a much slower rate than the conventional oxidants such as sodium nitrite) or an inhibition of the methemoglobin reductase.

Other miscellaneous enzymes, which were found to be insensitive to the drugs, include: xanthine oxidase, D-amino acid oxidase, carboxylase, transaminase and adenylic deaminase.

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THE SHAPE OF PARTICLES OBTAINED BY ACID DEGRADATION OF CELLULOSE

by

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In a recent issue of this Journal[1], ROELOFSEN has commented on a paper by S. M. MUKHERJEE and myself[2] in which we claimed to have observed tabular particles as a degradation product of cellulose fibres. The tabular shape of the particles was deduced from measurements made on photographs of metal-shadowed electron microscope preparations; ROELOFSEN rejects our results, presuming that in our specimens the shadowing angle must have varied to such an extent that our measurements were totally meaningless. This presumption is, in fact, completely unwarranted. Our observations were not confined to a small area of a single grid, and one has only to refer to the figures given in our paper for the coefficients of variation to see that wide variations in the shadowing angle could not have occurred. Since, however, ROELOFSEN asks for ocular demonstration that we did not neglect to take what is, after all, an elementary precaution in work of this kind, I would refer him to an electron micrograph which we have published elsewhere[3], in which the shadow of a latex particle is shown and the cellulose particles have the normal appearance described in our paper.

Since there is no doubt at all about the tabular shape of the particles, ROELOFSEN's second argument, that orientation may be due to conjunction of the $(10\bar{1})$ faces of the particles and not to the geometrical form, loses its point. His other comments do not affect us directly as authors of the paper referred to; my personal opinion is that the fibrils in lignified fibres, such as jute, are certainly "ribbon-like". In "pure" cellulose fibres (cotton and ramie) the electron microscopical examination of acid degradation products suggests very strongly that it is the particle, and not the fibril, which is the ultimate morphological entity.

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