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IDENTIFICATION OF TWO METABOLITIES OF ISONIAZID (ISONICOTINOYLGLYCINE AND 1-ISONICOTINOYL-2-ACETYLHYDRAZINE) BY PAPER CHROMATOGRAPHY IN RAT URINE

by

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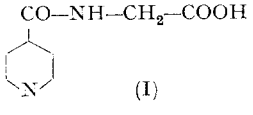
During the comparative study of the metabolism of isonicotinic acid hydrazide (isoniazid) and isonicotinoyl-hydrazino-methanesulfonic acid (Neotizide), the urine of rats, which had been injected with these two drugs, was examined by paper chromatography. It was found that another compound (Compound X) was excreted, besides isoniazid and isonicotinic acid[1].

This substance was detected on the chromatogram not only by the methods already described, that is, by absorption of short wave-length U.V. light (Mineralight Lamp), and yellow colour after treatment with cyanogen bromide and ammonia[1], but also by strong blue violet colour after reaction with benzidine and cyanogen bromide[2], and by the characteristic green colour it gives when treated with picryl chloride and ammonia[3].

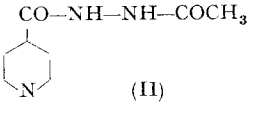
It was seen at once that Compound X was not isonicotinamide, as we supposed at first, because the chromatographic band of the latter has clearly different R_F values with the solvents tried.

It was then thought by analogy with that which occurs in the metabolism of nicotinic acid (which is partly eliminated in the urine combined with glycine as nicotinuric acid), that compound X might be isonicotinoylglycine (I). This hypothesis was confirmed by further research. In fact, when comparing the R_F values observed by paper chromatography in different solvents, compound X was found to behave as isonicotinoylglycine. This substance was prepared by the method of ROHRLICH[4] for nicotinoylglycine. Also the colours resulting from treatment with different reagents appeared to correspond, especially the green colour with picryl chloride and ammonia.

Further confirmation was given when, after elution of the chromatographic band detected by absorption of U.V. light, the eluate was hydrolysed with 6 *N* hydrochloric acid for four hours in sealed tube at 120°. Two paper chromatograms of the hydrolysate were run: one using as a solvent *n*-butanol, saturated with 0.5 *N* aqueous acetic acid, and using as detecting reagents benzidine and cyanogen bromide; and another using phenol: water (80:20) as a solvent, and ninhydrin as detecting reagent. In the former chromatogram isonicotinic acid was found; in the latter chromatogram glycine.



(I)



(II)

Another product of the metabolism of isoniazid was detected in the course of this research. This is a substance which was not noticed in the early experiments[1] because with the solvent used (*iso*amyl alcohol saturated with 0.5 *N* acetic acid) its chromatographic band overlaps that of isonicotinic acid. On the other hand, using a mixture of *iso*propanol and water (85:15) the compound was clearly separated from the other metabolites of the isoniazid, and was identified as 1-isonicotinoyl

2-acetyl-hydrazine (acetyl-isoniazid) (II). This gives the same colour reactions on paper chromatograms as acetyl-isoniazid prepared by synthesis: yellow colour, fluorescent yellow with U.V. light, after treatment with cyanogen bromide and ammonia; light blue colour with benzidine and cyanogen bromide, turning to yellow with yellow fluorescence in U.V. light by exposure to concentrated ammonia vapours.

Further results appear to establish its identity. The compound, when separated from the other metabolites in the urine by paper chromatography, and eluted, always gave the same R_F values as synthetic acetyl-isoniazid in the chromatograms obtained with different solvents (*n*-butanol/0.5 *N* acetic acid; *iso*amyl alcohol/0.5 *N* acetic acid; *n*-butanol/0.5 *N*-ammonia; *iso*propanol/water). The eluted compound gave the reaction of KELLY AND POET[5] with *p*-dimethylaminobenzaldehyde, confirming the presence of a hydrazine group in the molecule. In aqueous solution it gave an U.V. absorption spectrum corresponding to that of acetyl-isoniazid (Fig. 1).

Fig. 2 shows chromatograms of rat urine developed with *iso*propanol: water (85:15) on Whatman paper No. 1 by the descending method. The spots were detected with benzidine and cyanogen bromide. The odd numbers represent the chromatograms of the same urine sample. Four rats were injected with 100 mg/kg of isoniazid, by intraperitoneal route, and their urines collected after four hours. The even numbers correspond to chromatograms of the same urine sample with the addition of standard solutions of:

2, isonicotinoylglycine (A); 4, isonicotinic acid (B); 6, isoniazid (C); 8, acetyl-isoniazid (D).

A more detailed account of this work will be published later.

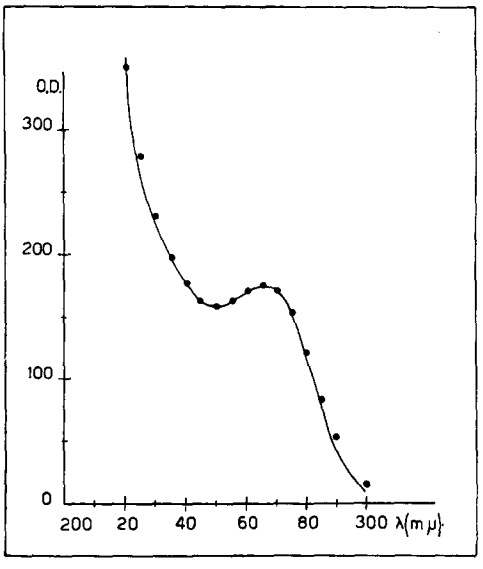


Fig. 1 —— U.V. absorption spectrum of acetyl-isoniazid in aqueous solution (10 μg/ml).
• • U.V. absorption spectrum of the unknown substance eluted from a chromatogram of rat urine after I.P. administration of isoniazid.
O.D. = optical density.

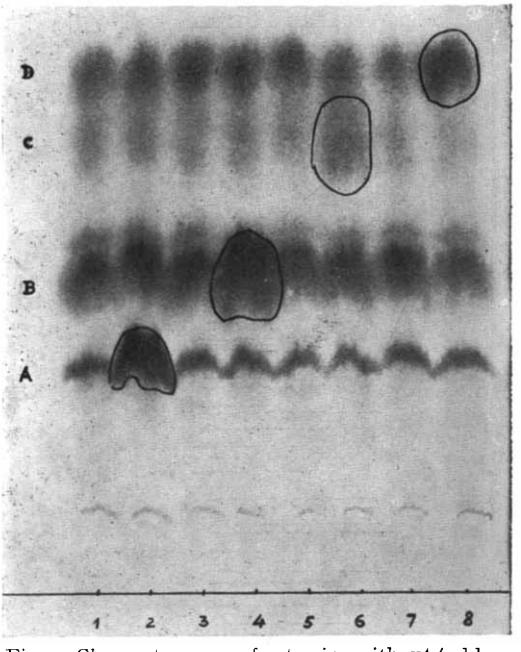


Fig. 2. Chromatograms of rat urine without (odd numbers) and with additions (even numbers; see text).

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