

The fate of ^{14}C -labelled iproniazid in the rat*

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It has been reported by Hess *et al.*¹ that monoamine oxidase inhibition produced by iproniazid (1-*isonicotinyl*-2-*isopropylhydrazine*) persists for 5 to 6 days after measurable (by chemical methods) quantities of the drug have disappeared from the tissues. The inhibition produced has been shown to be of an irreversible type, both *in vitro* and *in vivo*,² and Davison³ has proposed a mechanism involving the whole molecule mediated through the dehydrogenated product. The complex proposed by Davison retained the *isonicotinoyl* moiety of iproniazid and it was thought that iproniazid labelled at the C=O group would be a good tool to confirm this. Accordingly, we have synthesised ^{14}C -labelled iproniazid† with the label at the C=O group and studied its distribution pattern in the rat. Our results confirm the finding of earlier workers that *isonicotinic acid* is the major metabolite of iproniazid and that the drug or its metabolite, *isonicotinic acid*, is not retained in the blood, brain, or liver. Almost all of the injected radioactivity has been recovered, as iproniazid and *isonicotinic acid*, in the urine collected during the subsequent 24 hours. An explanation is suggested for the persistence of inhibitory activity in the light of these data.

METHODS AND RESULTS

Male white rats weighing 300–350 g were given 25 mg of radioactive iproniazid per kg intraperitoneally (0.92×10^6 dis/min per mg) and, at the end of definite intervals of time, blood samples were taken by cardiac puncture. The animals were then sacrificed and the brain and liver removed quickly, washed and chilled. In one case the brain was perfused with physiological saline. All animals were anaesthetised with pentobarbital, and heparin was given prior to sacrifice. There was no significant difference in the amount of radioactivity found in the brain between the perfused and the non-perfused animal. Urine collected from the 24 hr experiment and the homogenates of the brain and liver were assayed for radioactivity and then fractionated. The fractionation process consisted of a preliminary extraction with ether followed by extraction with chloroform (at pH 7.5–8). The chloroform extractives, after removal of the solvent, were taken up in water and chromatographed on paper. The aqueous residue from chloroform extraction was also chromatographed on the same sheet of paper. The paper had three slits 2 mm wide extending longitudinally from just above the line where the test material is applied to about 2 cm from the bottom of the paper. This facilitated application of the chloroform extract, the aqueous residue, and authentic samples of iproniazid and *isonicotinic acid*

TABLE 1. TISSUE DISTRIBUTION OF RADIOACTIVITY FROM ^{14}C -LABELLED IPRONIAZID‡ (EACH VALUE IS A MEAN OF FOUR EXPERIMENTS)

Time	Radioactivity (in dis/min per mg dry wt)		
	Plasma (dis/min per ml)	Brain	Liver
10 min	23,170	78	48
20 min	43,600	102	135
1 hr	29,650	83	96
24 hr	0	0	0
			in this case about 98 per cent the total injected radio- activity was recovered in the urine

‡ Chromatography and autoradiography of the extracts of these tissues, followed by treatment with cyanogen bromide and benzidine, showed the presence of two spots identified as *isonicotinic acid* and iproniazid (by comparison with authentic samples). Quantitative estimations were not done since these studies were primarily directed towards obtaining information concerning the maximum period the drug or its metabolites are retained in the brain.

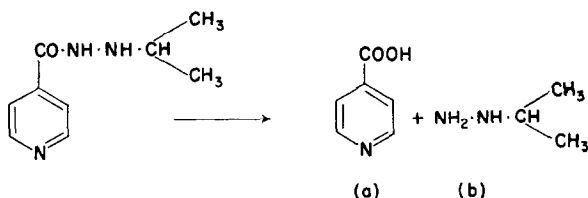
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† The synthesis will be reported elsewhere.

on the same sheet of paper without possible contamination. After development with *n*-propanol-water (4:1), the chromatograms were exposed to Kodak no-screen X-ray film and were subsequently treated with cyanogen bromide and benzidine.⁴ The zone of radioactivity in the autoradiogram corresponded with the color zone produced by cyanogen bromide-benzidine. Iproniazid was detected in the chloroform extract, and *isonicotinic* acid in the aqueous residue. About 98 per cent of the total injected radioactivity was recovered, as iproniazid and *isonicotinic* acid, in the urine collected during the 24 hr period. The results are summarised in Table 1.

DISCUSSION

It will be seen from Table 1 that the radioactivity reaches a maximum in the brain within an hour and disappears completely at the end of 24 hr. Liver and plasma also show a similar pattern. Analysis of the tissue extracts showed that the contribution to the radioactivity came from both iproniazid and *isonicotinic* acid. At the end of a 24 hr period no trace of iproniazid or *isonicotinic* acid could be detected in tissues, either by radio-assay or by chemical tests (CNBr-benzidine). These findings are essentially in agreement with those of Hess *et al.*,¹ using non-radioactive iproniazid, except that in the plasma they were able to detect trace quantities of the drug (less than 10 µg/ml) 48 hr after injection. It is not possible to say whether this is due to the difference in the methods of assay or whether this is related to the high dose of iproniazid used by Hess *et al.* in their studies (195 mg/kg, cf. LD₅₀ 117 mg/kg). The results of these studies indicate that neither iproniazid nor *isonicotinic* acid is stored in the brain, liver or blood. However, it can be seen that *isopropyl* hydrazine (b), or a metabolite of it, if



retained in the tissues will not be detected by the methods used in the present studies or by those used by Hess *et al.* At the dose level of iproniazid we have used, it was not possible to detect *isopropyl* hydrazine or its derivatives in the tissues by ordinary chemical methods. It is known that alkyl hydrazines are much more toxic to the tissues than hydrazine. If the *isopropyl* hydrazine that is liberated in the brain by hydrolytic cleavage causes alteration or destruction of the sites of enzyme action or enzyme synthesis (amine oxidase), it is conceivable that the amine oxidase inhibitory power attributed to iproniazid is due to this effect and thus persists long after the drug or its metabolite has disappeared. When this damage is repaired, the tissue returns to its normal activity. Injuries to areas other than the amine oxidase system are not excluded by this hypothesis. A similar situation in the liver could explain the pathological changes (indistinguishable from viral hepatitis) observed in the liver of human subjects who exhibited the toxic effects of iproniazid. The fact that *isonicotinic* acid hydrazide does not cause any effects comparable to those of the *isopropyl* derivative is also consistent with this reasoning. The recent finding of Koechlin and Iliev⁵ that tissues retain radioactivity for longer periods with iproniazid labelled at the C₂-*isopropyl* group adds interest to this problem. Previous work of Roth⁶ and Barlow⁷ emphasizes the uniqueness of drug entry and distribution in the central nervous system. Autoradiographic studies with the labelled drug designed to show any discrete anatomical distribution, in combination with discrete biochemical distribution and histochemical analysis, should provide additional information regarding the mechanism of action of iproniazid. Such experiments are in progress.

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