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SHORT COMMUNICATIONS

Fate of succinonitrile-1-^{14}C in the mouse

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SUCCINONITRILE is widely used as an antidepressant in several European countries, its clinical use was prompted by the discovery of some specific effects of aliphatic dinitriles on the central nervous system.[1,2]

The pharmacology of this class of substances was extensively investigated as early as 1897,[3] and a few hypotheses appeared on the dynamics of their therapeutic action,[4–6] but no studies were ever published on their metabolism or on the biochemical aspects of their mechanism of action.

We report here some preliminary results on the metabolic fate in the mouse of succinonitrile ^{14}C labelled in the —CN group, after the intraperitoneal injection of one single dose or of repeated doses at regular intervals.

Materials

White male mice, average weight 22 g, were used. The succinonitrile (SDN) ^{14}C labelled in position one with a specific activity of 1·69 mc/mmole was obtained from New England Co., Boston, U.S.A. 100 microcuries of substance, corresponding to 4·74 mg, were dissolved in 1 ml of a 5% SDN solution in twice-distilled, sterile water. This solution was used as such to inject the animals intraperitoneally, and was kept frozen between experiments. All solvents and chemicals used were of reagent grade.

Administration of the drug and urine collection. Groups of four mice in metabolic cages were injected i.p. by means of a microsyringe with 10 μl of SDN solution corresponding to 547·4/μg of substance and to 1 μc of radioactivity (25 mg/Kg. The LD_{50} for the mouse is 150–200 mg/Kg).

After the injection the animals were given no food but water *ad lib.* After 24 hr the urine from each cage was collected, brought to volume and its radioactivity counted on an aliquot by liquid scintillation. In the case of repeated daily administration, the animals were kept on a normal diet and injected every 24th hr for 7 consecutive days with 10 μl of the 5% SDN solution. On the 8th day they were injected with 10 μl of the labelled solution and transferred to the metabolic cages. After 24 hr of fasting the urines were collected as described.

Urine fractionation. Each sample, representing the 24 hr urines of four mice, was brought to 25 ml with distilled water, 5 ml were brought to pH 2 by means of 1 N HCl and extracted three times with 15 ml of $CHCl_3$ each time. The chloroform extract was brought to 50 ml. Two ml of the residue were extracted five times with 15 ml each time of isoamyl alcohol saturated with water at room temperature. Also this second extract was brought to 50 ml. To 1 ml of the second residue a large excess of ammonium thiocyanate was added, plus a 9% ferric chloride solution slightly in excess of its stoichiometric amount. The ferric thiocyanate formed was then thoroughly extracted with water-saturated isoamyl alcohol, reduced with a few drops of a freshly prepared 30% ascorbic acid solution in diethylene glycol, and then brought to volume. Microaliquots of all samples were taken in duplicate by means of calibrated micropipets, and the radioactivity measured by liquid scintillation.

As a mean, 55 per cent of the injected radioactivity was found in the urines after 24 hr. Although there were ample fluctuations from experiment to experiment, no significant differences were observed between the values obtained in the case of single and that of repeated injections.

The first fractionation step, the chloroform extraction of acidified urine, gives the SDN eliminated as such. Its amount represents the 7 per cent of the total radioactivity eliminated in the first 24 hr, and it is not influenced by a previous repeated administration of the drug. 88 per cent of the initial radioactivity was found in the residue, with a 95 per cent yield in this step. This residue was then extracted with a more polar solvent, namely water saturated isoamyl alcohol (i.e. the same solvent used in the next step to extract ferric thiocyanate). A considerable difference was observed in this fractionation step between single injected (77 per cent of radioactivity extracted) and multiple injected (52 per cent of radioactivity extracted) animals. The total recovery in this step was higher than 90 per cent (Table 1).

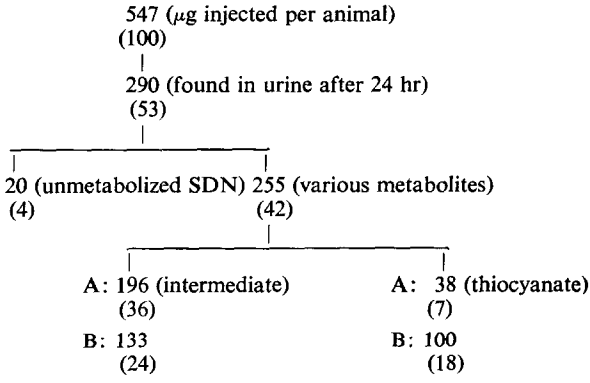
Several logical hypotheses can be formulated on the structure of the metabolites present in such large amounts. If an enzymatic system is present in the organism, capable of converting the —CN group into an —OH, the main intermediate compound could be diethylene cyanhydrin. A second

TABLE 1.

	Chloroform extraction		
	Extract	Residue	Yield:
A	6 ± 1	88 ± 2	93%
B	8 ± 1	88 ± 1	95%
		(100)	
	Isoamyl alcohol extraction		
	Extract	Residue	
A	77 ± 3	15 ± 1	92%
B	52 ± 4	39 ± 4	91%
		(100)	
	Fe^{3+} reaction		
	Isoamyl alcohol extraction		
	Extract	Residue	
A	69 ± 4	13 ± 2	82%
B	77 ± 5	8 ± 1	85%

Distribution of radioactivity in mouse urine after extraction with solvents and reaction with Fe^{3+} All results expressed in percentages. Urines collected 24 hr after i.p. injection of 0·55 mg of SDN (^{14}C labelled in position one) containing 1 μc of activity per animal. A = groups of four mice which have received a single injection. B = groups of four mice which have received repeated injections.

TABLE 2.



Scheme of metabolic fate in the mouse of i.p. injected SDN ^{14}C labelled in position one. Results expressed in micrograms as mean values per animal and percentages of injected amount. A = single injection, B = repeated injections.

possibility is suggested by the great reactivity of the ethylene groups adjacent to the —CN groups. With an oxidative mechanism, the substance produced would eventually be cyanacetic acid, through the formation of a keto-intermediate, which by addition of a molecule of water gives the unstable acetal. In both these postulated reactions there would be the formation of one mole of cyanide per mole of SDN. Thus this amount of —CN ought to be eliminated in the urine as —SCN. In fact, over 70 per cent of the residual radioactivity can be extracted with isoamyl alcohol after reaction with Fe^{3+} in the presence of relatively large amounts of inert carrier —SCN. It can be assumed that the reaction is not quantitative in excess of iron.

This hypothesis of an enzymatic reaction resulting in a liberation of cyanide is substantiated by the fact that the amount of thiocyanate increases considerably in animals treated with repeated doses of SDN, while at the same time the total of intermediate metabolites decreases by the same amount (Table 2).

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Biochemical properties of azapropazone and other anti-inflammatory drugs

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RECENTLY, many attempts have been made to establish relationships between the anti-inflammatory actions of drugs and their biochemical actions on organelles and macromolecules. Such attempts include the actions of the drugs in stabilizing lysosomes[1] and proteins[2] and in uncoupling oxidative phosphorylation.[3] It appears probable that uncoupling oxidative phosphorylation and protein stabilization may be of particular importance for non-steroidal drugs.

In this investigation we have examined the new anti inflammatory drug Azapropazone together with other established drugs for their actions on mitochondria and proteins.

Protein stabilization. A modification of the method of Mizushima and Nakagawa[4] was used. Bovine albumin (1% w/v) was dissolved in phosphate (0·1 M with respect to phosphate) buffered saline at either pH 5·8 or 7·4. The drugs were dissolved in alkaline saline (0·1 N NaOH in 0·9% w/v NaCl) and portions (0·1 ml) added to 2·7 ml of buffered albumin solution in stoppered test tubes. Portions (0·1 ml) of the alkaline saline alone was added to control tubes. The pH of each solution was checked. The absorbancies of the solution at 420 nm were determined and the tubes heated for 1 hr at 70° in a waterbath. After incubation the tubes were cooled in ice–water and the absorbancies again determined at 420 nm. Preliminary experiments had shown that 420 nm was the most sensitive wavelength for determining changes in turbidity due to protein denaturation.

Effect on mitochondrial respiration. Mitochondrial respiration was measured polarographically using a modified Clark oxygen electrode[5] coupled to a Honeywell Brown "Electronik" recorder.

Mitochondria were isolated from rat liver homogenate as the fraction sedimenting between 600 and 8500 *g* for 10 min in an isolation medium—0·25 M sucrose buffered at pH 7·4 with 5 mM Tris–HCl and 1 mM E.G.T.A. (ethylene glycol-bis-(amino ethyl ether)N,N′ tetra-acetic acid). The final suspensions contained the equivalent of 1 g liver to 2 ml of medium.

Portions (0·5 ml) of the mitochondrial suspension were added to 2·5 ml of a respiratory medium in the reaction vessel of the oxygen electrode which was maintained at 30° by a water jacket. The respiratory medium consisted of 0·25 M sucrose–12 mM $MgSO_4$–12 mM phosphate buffer (pH 7·4). Portions (10 μl) of sodium succinate (0·5 M) were added and respiration followed. Drugs were added