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LIBERATION OF CYANIDE FROM SUCCINONITRILE*†

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Abstract—Release of cyanide from succinonitrile (DNS) was investigated *in vivo* and *in vitro*. Administration of DNS to rabbit and rat resulted in a conspicuous increase of thiocyanate excretion in the urine. It was estimated that approximately 60 per cent of DNS μmole administered was transformed to CN^- in the animal body and excreted as SCN^-. Liberation of CN^- from DNS was not found in experiments either with homogenates of rat or rabbit liver or with isolated mitochondrial, microsomal and soluble fractions. By contrast it was demonstrated that rat and rabbit liver slices could catalyze this reaction. Addition of the non-ionic detergent Triton X-100 to the incubation medium almost completely abolished the cyanide liberation from DNS. Moreover it was observed that liver slices from rat pretreated with CCl_4, had lost their ability to release CN^- from DNS.

These data suggest that the integrity of cellular membrane is essential for release of cyanide from DNS.

IT IS known[1] that the administration of aliphatic dinitriles to animals is followed by an increase of urinary thiocyanate excretion. Intoxication by these drugs produces symptoms which are similar to those of cyanide poisoning and are relieved by thiosulphate[1] and hydroxocobalamin.‡ This supports the possibility that liberation of cyanide from aliphatic dinitriles is responsible for their toxicity.

These drugs are employed in some European countries as antidepressive agents. Among dinitriles, succinonitrile (DNS) is less toxic and more stable. For this reason it is the most widely used. Little is known about the metabolism of DNS. Merkow *et al.*[2] observed that DNS administration to the rat resulted in the urinary excretion of about 2 per cent or less of cyanoacetic acid and insignificant amount of SCN^-. Stern *et al.*[3] studying the effects and the fate of several nitriles in slices and homogenates of animal tissues, found that DNS did not show cyanide-like effects nor gave rise to SCN^- formation.

Up to 1966 Marigo and Pappalardo[4,5] reported five cases of death in the course of therapeutical treatment with DNS. The authors revealed in viscera the presence of CN^-. These observations show that the problem of cyanide release from DNS needs more careful evaluation. The purpose of this work is to investigate the release of cyanide from DNS *in vivo* and *in vitro*.

METHODS

All reagents were of analytical grade. Triton X-100 was from Sigma. The experiments were carried out on male rabbits (body wt 2000–3000 g) and male albino rats

* A preliminary report of these studies has been previously presented (*Boll. Soc. It. Biol. Sper.* **47**, 192 (1971)).

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‡ Cima, personal communication.

(body wt 400–450 g). The urine was collected from groups of two rats and from each rabbit every 24 hr before and after the administration of an aqueous solution of DNS (BDH Laboratory, Chemical Division). The urine was filtered before determination of thiocyanate and cyanide. Mitochondria from rat or rabbit liver were prepared according to Hogeboom,[6] microsome and soluble fraction according to Cooper and Brodie.[7]

Liver slices were prepared after killing the animal by decapitation. The liver was removed and washed twice with ice-cold Krebs–Ringer–phosphate buffer, pH 7·4. The slices were cut by hand and pooled on ice-cold Krebs–Ringer–phosphate buffer. They were washed, dried with filter paper, weighed and transferred to vessels containing 4 ml of Krebs–Ringer–phosphate buffer, pH 7·4. The incubation was carried out at 37° in a Dubnoff bath for 4 hr. After incubation thiocyanate and cyanide were determined.

Estimation of thiocyanate and cyanide. Thiocyanate was determined in a 0·2–0·1 ml urine sample diluted to 8 ml with water; 2 ml ferric–nitrate solution (5 g $Fe(NO_3)_3$. 9 H_2O and 5 ml concentrated nitric acid made up to 100 ml) were added according to Rosenthal.[8] The colour was measured at 450 mμ against an urine blank (0·1–0·2 ml urine to 10 ml with water). In the experiments *in vitro* thiocyanate was determined by the method of Rosenthal,[8] as modified by Ruffo.[9] Cyanide was determined colorimetrically after microdiffusion in Conway's cell according to Feldstein.[10]

RESULTS

Liberation of CN^- from DNS in intact animals. Rat and rabbit urine contain detectable amounts of thiocyanate. It can be seen in Fig. 1 that the SCN^- normal excretion on the rabbit is 87 ± 3·47 μmole/24 hr, and that this amount strongly increased after administration of DNS 25 mg/kg, i.v. With rats a similar picture was observed. During the first 24 hr after DNS administration the amount of thiocyanate was increased

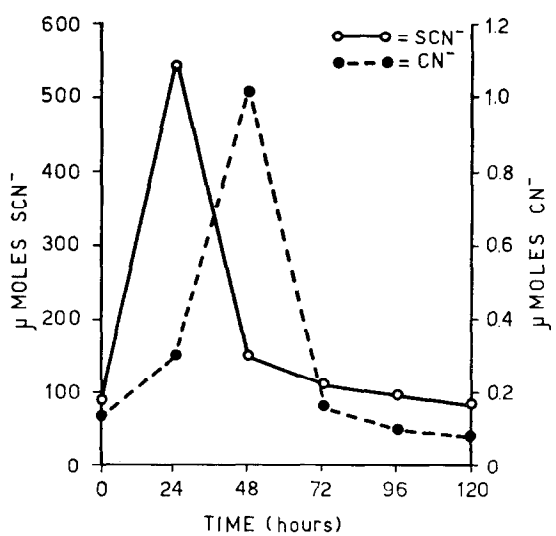

FIG. 1. The urinary excretion of thiocyanate and cyanide following i.v. administration of 25 mg/kg DNS to the rabbit. The values are means of four animals.

about six-times but approximately normal values were detected during the second day. Urinary excretion of free CN^-, usually negligible, was also increased.

Using 25 mg/kg the amount of DNS metabolized to CN^- and excreted as SCN^- by rabbit is approximately 60 per cent (expressed as micromole of CN^-/100 μmole of DNS administered). The same value was observed after administration of 50 mg/kg in rat.

The presence of cyanide was determined in the viscera and in biological fluids of a rabbit injected i.v. with 40 mg/kg DNS which died within 4 hr. The following amounts of CN^- were detected: 0·78 μg/g tissue (wet wt) in the lung, 0·51 μg/g in the brain, 0·41 μg/g in the kidney and heart, 0·22 μg/g in the liver, 1·80 μg/ml in the blood and 2·64 μg/ml in the urine. Untreated rabbit did not contain in the viscera detectable amount of cyanide.

Liberation of CN^- from DNS by rabbit and rat liver slices. Whole homogenates from rabbit or rat liver did not catalyze liberation of CN^- from DNS *in vitro*. The same result was obtained with mitochondrial, microsomal as well as the soluble fractions of rabbit or rat liver homogenates. In contrast it can be seen (Table 1) that rabbit and rat liver slices can catalyze this reaction.

TABLE 1. FORMATION OF CN^- AND SCN^- FROM DNS BY RAT AND RABBIT LIVER SLICES

	Thiosulphate added	mμMole CN^- or SCN^- formed/g tissue wet wt	
		By rat liver	By rabbit liver
CN^-	−	204·17 ± 20·67	124·75 ± 21·50
CN^-	+	9·00 ± 1·35	13·90 ± 3·04
SCN^-	−	124·28 ± 13·23	249·75 ± 32·20
SCN^-	+	354·57 ± 17·11	485·50 ± 48·40
Total CN^- formed	−	328·45 ± 30·90	374·50 ± 29·50
	+	363·57 ± 18·00	499·40 ± 50·30

Each vessel contained 4 ml of Krebs–Ringer–phosphate solution pH 7·4; 5 μmole DNS; when present 5 μmole Na thiosulphate; 500 mg of liver slices wet wt. Incubation 4 hr at 37°. The values are mean of seven separate experiments (rat liver) and eight (rabbit liver) ± S.E.

The cyanide released from DNS appeared in the incubation medium part as CN^- and part as SCN^-. In the presence of added thiosulphate almost all CN^- is transformed to SCN^-. The rate of release and the amount of CN^- formed from DNS were low and not more than 7 per cent of added DNS after 4 hr of incubation under the experimental conditions used. The sluggish liberation of CN^- in liver slices can explain the failure of Stern *et al.*[3] to detect cyanide liberation using the inhibition of oxygen uptake as indication. DNS could not inhibit the respiration of tissue slices because the amount of cyanide released was insufficient to produce inhibitory effect.

Figure 2a shows the relationship between cyanide liberation and the amount of

added DNS. The time-course of the CN^- release from DNS is reported in Fig. 2b. CN^- formation was not found in experiments carried out with rat kidney slices.

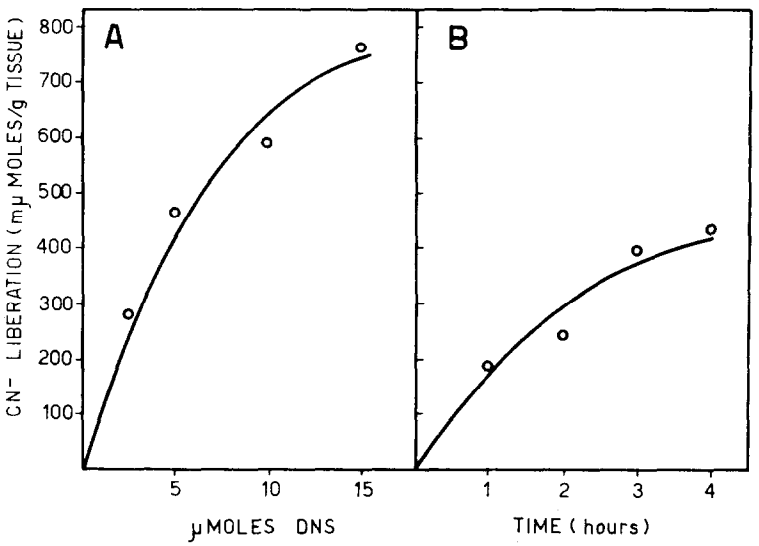

FIG. 2. (a) Liberation of CN^- from increasing amount of DNS by rat liver slices. Experimental conditions as described in Table 1. DNS added at the concentrations indicated, tissue 500 mg. (b) Time course of CN^- liberation. Experimental conditions as described in Table 1.

To investigate whether the activity of slices in contrast to the homogenate reflects a requirement of cellular integrity, experiments were carried out in the presence of agents able to damage cellular membranes. Figure 3 shows that Triton X-100 in the concentration ranging from 0·1 to 2·0 mg/g tissue (wet wt) strongly inhibits the cyanide release from DNS by rat liver slices.

Effect of CCl_4. Preliminary experiments have shown that pretreatment with 2 ml/kg CCl_4 administered s.c. as solution in olive oil, 48 hr before DNS administration caused considerable decrease of DNS toxicity in the mouse, and almost completely abolished the increase of SCN^- urinary excretion in the rat. In agreement with these results it is shown (Fig. 4) that liver slices of rat pretreated with CCl_4, had lost their ability to release CN^- from DNS. Addition of CCl_4 to the incubation medium almost completely inhibits the CN^- release from DNS.

DISCUSSION

The main findings of this paper is the demonstration that DNS can be decyanated *in vivo* and *in vitro*. This conclusion is in agreement with the study of Heymans and Masoin[1] but in contrast with Merkow *et al.*[2] Rabbit and rat transform about 60 per cent of DNS μmole administered to CN^- which is excreted almost completely as SCN^-. This conspicuous amount, when liberated in the human body, can fully account for the deaths in the course of therapeutical use of DNS (usually given at 200–300 mg/day) especially when the enzymatic conversion of cyanide to thiocyanate

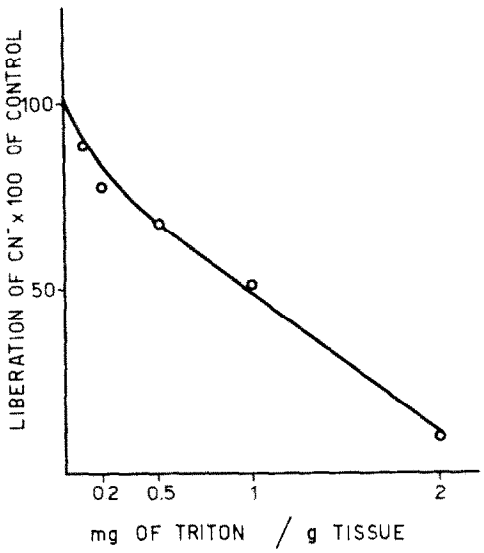

FIG. 3. Effect of Triton X-100 on CN^- release from DNS. Experimental conditions as described in Table 1.

is impaired for some reason (e.g. deficiency of sulphur donors). This possibility calls for serious reason of warning when therapeutic use of such a drug is attempted.

The mechanism of liberation of CN^- from DNS is not clear. It is noteworthy that the reaction is catalyzed by liver slices but not by homogenate and other subcellular

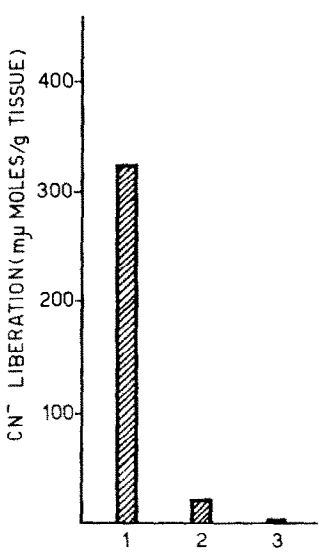

FIG. 4. Effect of CCl_4 on CN^- release from DNS. 1 = Normal rat liver slices. 2 = Liver slices of rat treated s.c. with 2 ml/kg CCl_4. 3 = Normal rat liver slices plus CCl_4 20 μl/g tissue in the incubation medium. Experimental conditions as described in Table 1.

preparations. The different behaviour of slices and homogenate and the inhibitory effect induced by the detergent Triton X-100 are indications that the integrity of cellular membrane is essential for releasing cyanide. The same explanation yields for the action of CCl_4 since it is shown that this agent can damage cellular membranes.[11]

To explain the requirement of cellular integrity two possibilities can be considered: (1) the enzyme or the enzyme-complex that releases cyanide from DNS needs a definite ionic composition lost during homogenization; (2) the enzyme system is located in the cellular membrane and is damaged or changed during homogenization or detergent treatment.

Note—After this paper had been accepted for publication, a short communication appeared (R. CAVANNA and F. POCCHIARI, *Biochem. Pharmac.* **21,** 2529 (1972)) concerning the fate of succinonitrile-1-^{14}C in the mouse. The results are in agreement with our data obtained on the rabbit and on the rat.

REFERENCES

1. J. F. HEYMANS and P. MASOIN, *Archs Int. Pharmacodyn. Thé.* **3,** 77 (1897).
2. L. P. MERKOW, S. H. LIPTON, J. J. LALICH and F. M. STRONG, *Proc. Soc. exp. Biol. Med.* **102,** 728 (1959).
3. J. STERN, H. WEIL-MALHERBE and R. H. GREEN, *Biochem. J.* **52,** 114 (1952).
4. M. MARIGO and G. PAPPALARDO, *Medicina Legale e assicurazioni* **14,** 155 (1966).
5. M. MARIGO and G. PAPPALARDO, *Medicina Legale e assicurazioni*, in press.
6. G. HOGEBOOM, in *Methods in Enzymology* (Eds. S. P. COLOWICK and N. O. KAPLAN) Vol. 1, p. 16. Academic Press, New York (1955).
7. J. R. COOPER and B. B. BRODIE, *J. Pharmac. exp. Ther.* **114,** 409 (1955).
8. O. ROSENTHAL, C. S. ROGERS, H. M. VARS and C. C. FERGUSON, *J. biol. Chem.* **185,** 669 (1950).
9. A. RUFFO, M. ROMANO and G. GIAMPAOLO, *Boll. Soc. It. Biol. Sper.* **29,** 46 (1953).
10. M. FELDSTEIN, in *Toxicology Mechanisms and Analytical Methods* (Eds. C. P. STEWART and A. STOLMAN) Vol. 1, p. 639. Academic Press, New York (1960).
11. R. O. RECKNAGEL, *Pharmac. Rev.* **19,** 145 (1967).