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# PREVENTION OF THE CONVULSIVANT AND LETHAL EFFECTS OF *ISO*NICOTINIC ACID HYDRAZIDE BY PYRUVIC ACID

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**Abstract**—The effect of *iso*nicotinic acid hydrazide upon the concentration of pyruvic acid in the serum of rabbits injected with high doses of the drug, and the prevention of the toxic effects of the hydrazide by means of pyruvic acid were studied.

It was found that *iso*nicotinic acid hydrazide reduces the amount of circulating pyruvic acid, combining with it to form an *iso*nicotinyl-hydrazone. The simultaneous injection of sodium pyruvate prevented both the convulsant and the lethal effects of the hydrazide. This was attributed to the reduction of its concentration to levels under the $LD_{50}$, through the formation of the hydrazone and to the maintenance of the circulating pyruvate at safe concentrations.

## INTRODUCTION

IN A recent paper Balzer *et al.*[1] have shown that *iso*nicotinic acid hydrazide (INH) reduces the levels of $\gamma$-aminobutyric acid (GABA) and glutamic acid (GA) in the brain of mice. The reduction in GABA had been previously related to the inhibition of GA-decarboxilase,[2] probably through the formation of the *iso*nicotinyl-hydrazone of pyridoxal (pyridoxal · INHzone).[3] The simultaneous injection of pyridoxine led to a still greater reduction in GABA levels,[1] as it was to be expected since it would enhance the formation of the INHzone, but GA concentrations reverted to normal values. The toxic effects of INH were not prevented by pyridoxine, and they were attributed to a reduction in the turnover of GA.

A partial protection against high doses of INH was obtained by della Pietra *et al.*[4] with the simultaneous administration of GA, complete protection being obtained with GA plus pyridoxine.

It is known[5] that INH reacts with pyruvic acid to form an hydrazone (pyruvic acid · INHzone), and *in vitro* experiments[6] demonstrated that INH causes a reduction in the amount of pyruvic acid in tissues. This indicates the possibility of INH inhibiting GA biosynthesis through the removal of pyruvic acid and would explain the fact of pyridoxine reverting GA levels to normal values.

The present work was undertaken in order to ascertain the effect of INH upon the levels of pyruvic acid in the blood of rabbits and the prevention of the convulsivant and lethal effects of INH by pyruvic acid.

## MATERIALS AND METHODS

INH and pyruvic acid were injected intravenously into rabbits, and blood samples were collected from the marginal vein of the ear at different times after the injection.

*"Total" pyruvic acid*

We found that the technique of Friedeman and Haugen,[7] when used for the determination of pyruvic acid in the serum of INH-treated animals, assays also for the INH-bound pyruvate, so giving a "total" reading. This would explain the results quoted by Chmelev,[8] who found an accumulation of pyruvic acid in the blood of INH-treated patients.

*"INH-bound" and "free" pyruvic acid*

Pyruvic acid combined as an INHzone was evaluated by spectrophotometry (267 mμ), after being separated from the other metabolic derivatives by means of paper electrophoresis. Samples of 40 μl of serum were applied to a strip of filter paper (Macherey-Nagel no. 261), which was then impregnated with M/15 phosphate buffer

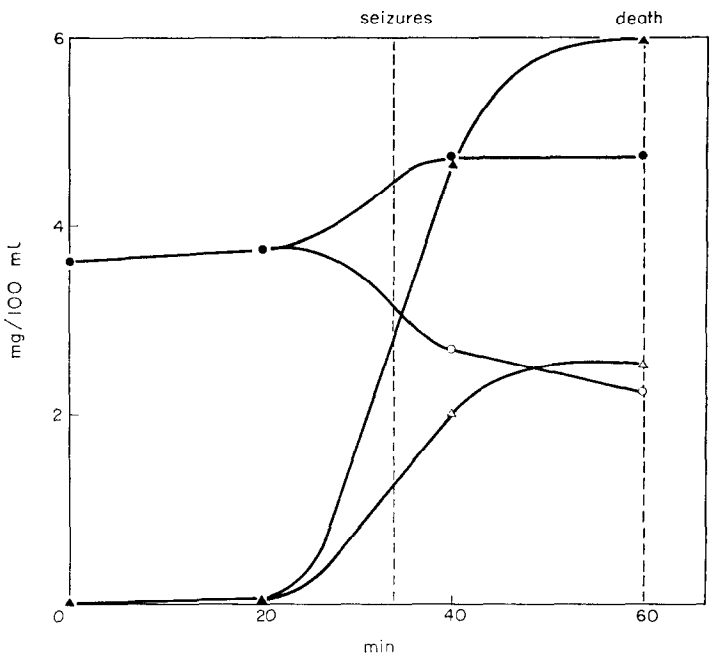


FIG. 1. Effect of an intravenous injection of INH (250 mg/kg body weight) upon the concentration of pyruvic acid in the serum of a rabbit. ●—● = "total" pyruvic acid; ○—○ = "free" pyruvic acid; ▲—▲ = Pyruvic acid . INHzone; △—△ = "INH-bound" pyruvic acid.

pH = 7·0 and submitted to a potential of 300 V during 3 hr. The INHzone of pyruvic acid migrates alone towards the anode, being easily localized by the observation of the dry paper strip under the u.v. lamp. The region of the paper containing the hydrazone was then cut and eluted overnight with water. The volume was adjusted to 3 ml and the absorption was measured at 267 mμ.

The amount of "INH-bound" pyruvic acid was calculated from the concentration found for the INHzone, and the "free" pyruvic acid was evaluated by subtracting the "INH-bound" from the "total" value.

## RESULTS AND DISCUSSION

Fig. 1 shows the concentrations of "total", "free" and "INH-bound" pyruvic acid found in the blood of a rabbit at different times after the injection of 250 mg of INH per kg of body weight. After 1 hr there was a reduction of 39 per cent in the level of "free" pyruvic acid. Seizures started 35 min after the injection, when there was a 17 per cent reduction of circulating pyruvate. The last sample of blood was collected from the dying animal.

Assuming that seizures and death could have been caused by a shortage of available pyruvic acid, we injected it together with INH, when the following results were obtained (Table 1):

TABLE 1. PREVENTION OF THE CONVULSANT AND LETHAL EFFECTS OF INH (500 MG/KG BODY WEIGHT) IN RABBITS BY MEANS OF PYRUVIC ACID

(The theoretical amount of "free" INH was calculated assuming that all the Na pyruvate injected would combine to form the INHzone).

| Rabbit no. | mg/kg body weight | | Effect after . . . min | | |
|---|---|---|---|---|---|
| | Na pyruvate | "Free" INH | Seizures | Death | Observations |
| 1 | — | 500 | 50 | 90 | — |
| 2 | — | 500 | 50 | 70 | — |
| 3 | — | 500 | 30 | 60 | — |
| 4 | 80 | 400 | 80 | 120 | — |
| 5 | 80 | 400 | 30 | 50 | — |
| 6 | 160 | 300 | 60 | 100 | — |
| 7 | 240 | 200 | 40 | 60 | — |
| 8 | 240 | 200 | 50 | 90 | — |
| 9 | 320 | 100 | — | — | None |
| 10 | 320 | 100 | — | — | None |
| 11 | 320 | 100 | — | — | None |

Considering that the lethal dose ($LD_{50}$) of INH is 160 mg/kg body weight,[9] the prevention of the toxic effects of the drug by sodium pyruvate, as shown in Table 1, would depend on the reduction of the circulating "free" INH to levels under the $LD_{50}$, due to the formation of the *iso*nicotinyl-hydrazone.

The acute toxicity of the *iso*nicotinyl-hydrazone of pyruvic acid was studied in mice and in rabbits (unpublished results) and found to be nil even in doses of 10 g/kg of body weight (intraperitoneal injections). This reinforces the possibility of the anti-toxic activity of pyruvic acid in respect to INH being due to the formation of the INHzone, which would prevent the noxious reduction of blood pyruvate and consequent effect upon the metabolism of glutamic acid.

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