Failure of pyruvate to counteract isoniazid toxicity in rabbits

The intravenous LD50 of isoniazid in rabbits is 1·2 mmol kg^{-1}, but it was reported that 3·6 mmol kg^{-1} was without toxic effect when sodium pyruvate (2·6 mmol kg^{-1}) was injected simultaneously. The effect of pyruvate was ascribed to quantitative *in vivo* formation of the non-toxic pyruvate isonicotinylhydrazone (Barreto & Mano, 1961). On thermodynamic grounds we thought this explanation improbable.

To determine equilibrium constants for hydrazone formation, solutions containing isoniazid (0·25 mM) and sodium pyruvate (25 mM) in suitable buffers (0·5M) were incubated at 37 ± 0·5°. Each reagent separately was stable for at least 2 h under these conditions. Free isoniazid was determined at intervals up to 2 h by the method of Dymond & Russell (1970). Below pH 5 the isoniazid concentration fell to a constant value within about 5 min. At pH 5 or above no constant value was observed; an initial first-order decline to 20% or less of the original isoniazid concentration within 30 min was followed by a very much slower decline that was also approximately first-order in isoniazid. In these cases the equilibrium concentration [INH] of isoniazid was estimated by extrapolating the distal, slow-decline portion of the log [INH] *vs* time plot back to zero time. The results were used to derive the equilibrium constant

$$K_{eq} = [\text{hydrazone}]\,[\text{INH}]^{-1}[\text{pyruvate}]^{-1} = (0{\cdot}25 - [\text{INH}])\left\{[\text{INH}].(24{\cdot}75+[\text{INH}])\right\}^{-1}$$

litre $mmol^{-1}$ (Table 1).

In separate experiments using a 10-fold molar excess of pyruvate, the second-order rate constant at pH 7·4, 37°, was found to be $5{\cdot}4 \times 10^{-5}$ litre $mmol^{-1}$ s^{-1} (s.e.m. = $0{\cdot}13 \times 10^{-5}$, n = 16) during the first 2 h.

Let us assume that the rabbits used by Barreto & Mano (1961) contained only 48 ml of blood kg^{-1} (the lowest value recorded; Armin, Grant & others, 1952), that the two compounds were administered in 10% solution, and that neither left the circulation. Then the reaction, if uncatalysed, would not reach equilibrium for several days. Moreover the equilibrium constant (0·3 litre $mmol^{-1}$) at physiological pH is such that the equilibrium amount of isoniazid, namely 1·3 mmol kg^{-1}, would still be approximately equivalent to the LD50. In fact, isoniazid is rapidly diluted by diffusion into all body compartments, and pyruvate is rapidly metabolized. Accordingly, the results of Barreto & Mano (1961) could not be attributed to increased *in vivo* hydrazone formation. However, the possibility remained that pyruvate acted as an antidote to isoniazid by some other mechanism.

New Zealand White rabbits (3–4 kg) of either sex were starved overnight then given isoniazid (10%, w/v) into the right marginal ear vein. Blood samples were taken at 0, 30, 90, and 180 min into heparin and analysed for both free and "total active"

Table 1. *Equilibrium constants for the reaction of isoniazid with sodium pyruvate at 37°.*

pH	Buffer	K_{eq} (litre $mmol^{-1}$)[1]
2·3	Formate	0·79
3·0	,,	1·12
4·0	Acetate	1·23
5·0	,,	0·90[2]
6·0	Phosphate	0·35[2]
7·0	,,	0·30[2]
7·2	,,	0·32[2]
7·4	,,	0·34[2]
7·7	,,	0·36[2]

[1] Results are means of duplicate determinations.
[2] Equilibrium concentration of isoniazid estimated by extrapolation.

Table 2. *Effects of isoniazid and sodium pyruvate in the rabbit.*

Rabbit no.	INH, mmol kg^{-1}	Na pyruvate mmol kg^{-1}	Time (min) First convulsion	Time (min) Death
1, 2	0·29	none	—	—[1]
3	0·44	none	—	—[1]
4, 5	0·58	none	—	—[1]
6, 7, 8	0·91	none	37[1], 48[2], 57[3]	—
9	none[4]	1·82	—	—[1]
10, 11	1·82	none	20, 50	70, 85
12	1·82	1·82[5]	19	65
13, 14, 15, 16	1·82	1·82[6]	20, 20, 31, 39	28, 67, 70, 67
17, 18, 19	1·82	1·82[7]	—	—[1]
20	1·82	1·82[7]	136[8]	—[1]
21, 22	1·82[9]		—	—[1]

[1] Rabbit alive after 3 days.
[2] Broke neck at 120 min during convulsion.
[3] Broke back during convulsion, killed at 36 h.
[4] A volume of physiological saline, equivalent to that of 1·82 mmol kg^{-1} of isoniazid in 10% (w/v) solution, was injected immediately after the sodium pyruvate into the same vein.
[5] Sodium pyruvate was injected immediately after isoniazid, into the same vein.
[6] Sodium pyruvate and isoniazid were injected simultaneously (see text).
[7] Sodium pyruvate was drawn into the syringe containing isoniazid (see text).
[8] One convulsion only.
[9] Sodium pyruvate isonicotinyl hydrazone, 1·82 mmol kg^{-1}, in 10% (w/v) aqueous solution.

isoniazid. The latter includes isoniazid in the form of its hydrazones (Russell, 1972). With a dose of 0·145 mmol kg^{-1} the half-life of free isoniazid was 60 min (standard deviation 12·5 min, n = 12), and isoniazid as hydrazones constituted up to 25% of the total, a value similar to that found in human subjects (Russell, 1972). In two rabbits (half-lives 72 and 60 min, blood [INH] at 90 min 39 and 31 nM, respectively) the experiment was repeated after one week, but sodium pyruvate (10%, w/v) in *ten-fold molar excess* was injected into the same vein 30 min after isoniazid. No change in the slope of the free isoniazid decay curves occurred as a result of the pyruvate injection. The free isoniazid half-lives were 65 and 62 min, blood [INH] at 90 min 37 and 26 nM, respectively.

The results with larger doses of isoniazid (Table 2) were consistent with the LD50 previously reported (Barreto & Mano, 1961). A dose of 1·82 mmol kg^{-1} produced convulsions and death. This outcome was unaffected by simultaneous administration of an equimolar amount of sodium pyruvate (10% w/v) into the contralateral vein. Only when the pyruvate was drawn into the same syringe as the isoniazid, and the solutions were mixed by inverting twice before injection, was protection afforded. Analysis (Dymond & Russell, 1970) of the syringe contents showed that 60% of the isoniazid had reacted before injection could be started, and 96% by the time it was complete (6–9 min). The product, sodium pyruvate isonicotinylhydrazone (1·82 mmol kg^{-1}; Dymond & Russell, 1970), was non-toxic, as reported by Barreto & Mano (1961).

We conclude that sodium pyruvate is not an antidote to isoniazid poisioning.

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Further studies on brain concentrations of amphetamine and its metabolites in strains of mice showing different sensitivity to pharmacological effects of amphetamine

The mechanism by which amphetamine increases the body temperature remains in doubt.

Peripheral (McCullough, Milberg & Robinson, 1970; Jellinek, 1971; Gessa, Clay & Brodie, 1969) central (Matsumoto & Griffin, 1971; Hill & Horita, 1970) and both central and peripheral (Weis, 1973) mechanisms have been suggested because of the known interactions between amphetamine and the catecholaminergic systems.

We have examined the reasons for the insensitivity shown by C_3H mice towards amphetamine-induced hyperthermia.

Charles River CD_1 and C_3H mice (20–25 g) were housed in plastic cages, 6 animals per cage (25 × 25 × 15 cm) at 23° with a relative humidity (60%). All animals whether untreated or pretreated with reserpine (2·5 mg kg i.p., 16 h earlier) received an intraperitoneal injection of (+)-amphetamine sulphate at various doses. Body temperature was recorded before and every 30 min after amphetamine treatment.

Blood was collected following decapitation and the plasma was used for the determination of FFA according to Dole (1956) with minor modifications.

Brains were removed and immediately dissected on dry ice; whole brains and striatal areas were stored at −20° until analysis. Amphetamine and its hydroxylated metabolites (*p*-hydroxyamphetamine and *p*-hydroxynorephedrine) were measured according to Ånggård, Gunne & Niklasson (1970) and Belvedere, Caccia & others (1973) respectively. Homovanillic acid (HVA) was determined in the striatum using the procedure of Korf, Ottema & Van der Veen (1971).

From the results in Table 1, it can be seen that (+)-amphetamine sulphate (7·5 mg kg^{-1}, i.p.) elicits a hyperthermic effect in CD_1 mice, while not significantly increasing the body temperature in C_3H mice. Also, the concentration of homovanillic acid (HVA), the major metabolite of dopamine, in the striatum is increased in CD_1 mice but is unaffected in the C_3H strain. However, the concentrations of amphetamine in whole brain as well as in the striatum are similar for the two strains. Moreover no differences could be observed in concentrations of the *p*-hydroxylated metabolites of amphetamine. These differences in sensitivity do not apply to all the effects of amphetamine on the two strains of mice. For instance amphetamine elicits a similar dose-dependent increase of body temperature in both strains if the animals are pretreated with reserpine (2·5 mg kg^{-1}, i.p. 16 h earlier). Similarly the two strains respond with a comparable increase of plasma free fatty acids (FFA) to (+)-amphetamine sulphate (5 mg kg^{-1}, i.p.).

The data reported here confirm previous findings that C_3H mice are insensitive to the hyperthermic effect of amphetamine (Dolfini, Garattini & Valzelli, 1969a, b; Caccia, Cecchetti & others, 1973; Jori & Garattini, 1973). Concentrations of amphetamine in the whole brain and in the striatum, a site where amphetamine interacts with dopamine stores, have been found comparable for the sensitive (CD_1) and the insensitive (C_3H) strain of mice. Also *p*-hydroxyamphetamine and *p*-hydroxy-