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STUDY OF THE METABOLISM AND EXCRETION OF T-2 TOXIN, A TRICHOTHECENE FUSARIOTOXIN, IN RABBITS

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Introduction

As other mycotoxins, T-2 toxin is chemically stable and resists the action of external factors. Recently, however, it has come to light that in a biologically active environment the toxin is metabolized /decomposed or transformed/ rather quickly. Wei et al. /1971/ reported that in a slightly alkaline medium T-2 toxin is hydrolysed into HT-2 toxin, T-2 triol and T-2 tetraol. The 12, 13-epoxy group itself remains intact. In Fusarium nivale and F. solani cultures Yoshizawa and Morooka /1975/ demonstrated, in addition to T-2, also HT-2 toxin. A similar derivative was isolated from T-2 solution treated with human and bovine liver homogenate (Ohta et al., 1977; Ellison and Kotsonis, 1974/.

Robison et al. /1979/ and Mirocha and Robison /1976/ studied the metabolism of T-2 in 8-11 weeks old broiler chickens. About half of the excreted toxin quantity was T-2 toxin, while neosolaniol, HT-2 toxin and T-2 tetraol constituted the other half.The metabolizing capacity of the liver varies by animal species /Ohta et al., 1978/. Trichothecene mycotoxins including T-2 toxin are metabolized in embryonated hen's eggs /Bata et al., 1983/ as well. Furthermore, F. sporotrichioides, the strain which produces T-2, catabolizes the toxin parallel to synthesizing it /Sándor et al., 1984/.

There is a scarcity of data in the literature on the metabolism of T-2 in rabbits. Lucisano et al. /1983/ performed subacute toxicity studies of

T-2 in rabbits, but failed to study the metabolism of the toxin. In preliminary experiments we found that T-2 is metabolized also in rabbits,but less repidly than in other animal species /Ványi et al., 1986; Ványi and Bata, 1987/. The rabbits excrete the toxin in the faeces and to a lesse extent in the urine. T-2 toxin and its metabolites are excreted in the milk as well.

Materials and methods

To produce T-2 toxin, HT-2 toxin, and neosolaniol, F. sporotrichioides was grown on rice in three successive temperature-periods.

T-2 tetraol and T-2 triol toxins were obtained by hydrolysis of HT-2 toxin.

Chemical analysis was performed by capillary gas chromatography as described earlier /Ványi et al., 1982/.

Rabbit experiments

Acute toxicity study. Growing rabbits of 2 kg body mass were used in the experiments according to the following design.

Number of rabbits	Toxin dose per animal	Toxin dose per kg body mass
2	2 mg	1 mg
2	4 mg	2 mg
2	8 mg	4 mg
2	12 mg	6 mg
2	16 mg	8 mg
2	20 mg	10 mg
2	30 mg	15 mg

Further two rabbits were treated with solvent /dimethyl sulfoxide/ only, and two untreated rabbits served as controls.

Forty-eight hours after oral toxin administration the rabbits were killed by bleeding. From these rabbits, and from those that had died in the meantime, samples were taken from the stomach contents, caecal contents, and faeces for chemical analysis. The samples were assayed for T-2 toxin, HT-2 toxin, neosolaniol, T-2 triol, and T-2 tetraol by capillary gas

chromatography.

Subacute toxicity study and study of toxin metabolism.

Thirteen rabbits weighing 2 kg. received 1 mg/kg body mass T-2 toxin per os daily. Another thirteen rabbits were treated with the toxin-free solvent and served as controls. One treated and one control rabbit were killed by bleeding 6 and 12 h after treatment and then at the same time daily. The stomach contents, caecal contents, faeces, and liver were assayed for toxin concentration.

Results and discussion

The toxin concentration of the stomach contents, caecal contents, and faeces, as determined in the acute toxicity experiment, is shown in Table I. The toxin concentration is proportional to the amount of toxin ingested.

It can be seen from the table that proceeding towards the end digestive tract the amount of T-2 toxin increases.

The T-2 toxin, HT-2 toxin, neosolaniol, T-2 triol and T-2 tetraol concentrations of the faeces and liver, as determined in the subacute toxicity experiment, are shown in Fig. 1 and Fig.2.

From Tables I it appears that the ingested T-2 toxin remains unchanged in the stomach and is passed on unaltered. In the stomach contents practically no metabolites of T-2 toxin can be detected. On the other hand, in the caecal contents /Tables I/ all metabolites of the toxin are demonstrable. The same holds for the faeces /Tables I and Fig.1./, with the exception that in the latter the quantity of metabolites has increased significantly. In rabbits receiving 10 or 15 mg toxin per kg body mass, the rate of toxin degradation is insignificant. These data suggest that in the rabbit caecum the intensive digestive processes are accompanied by substantial toxin catabolism. This toxin-degrading capacity, however, seems to get exhausted around a toxin dose of 4-5 mg/kg body mass the proportion of metabolites does not rise further. In animals receiving even higher doses /10 or 15 mg/kg body mass/ the proportion of metabolites decreases, indicating that the bulk of T-2 toxin is excreted in the faeces unchanged.

The toxin and metabolite concentrations measurable in the liver /Fig.2/ deserve particular attention. The 1 mg/kg body mass toxin dose ingested daily in the first three days in mostly metabolized in the liver, and only about

one-fourth remains unchanged. Beyond day 3 the increased toxin load results in reduced toxin-metabolizing capacity of the liver. On day 10 more than 80 % of the toxin quantity demonstrable in the liver is present as unchanged T-2 toxin. The metabolism of T-2 toxin causes a marked reduction in its biological activity. Thus, if most of the toxin leaves the liver unchanged, the organism will have higher relative sensitivity to it. Prolonged administration of the toxin enhances to organism's sensitivity. The relationship between the duration of toxin administration and the increase in sensitivity is much stronger than linear.

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Table I

Toxin concentrations measured in animals of the acute toxicity experiment (mg/100 g)

Toxin ingested (mg/kg)	T-2 toxin	HT-2 toxin	Neosolaniol	T-2 triol	T-2 tetraol
		Stomach contents			
1	0	0	0	0	0
2	0,2	0,1	0	0	0
4	0,5	0,1	0	0	0
6	0,8	0	0	0	0
10	3,8	0	0	0	0
15	6,3	0,2	0	0	0
		Caecal contents			
1	0,6	0,1	0,2	0,1	0
2	1,4	0,3	0,3	0,2	0
4	3,2	0,6	0,6	0,6	0
6	4,5	0,7	0,6	0,6	0,1
10	5,6	0,5	0,5	0,4	0,1
15	10,3	0,1	0,1	0	0
		Faeces			
1	3,2	2,3	1,9	1,5	0
2	4 8	3,2	3,1	2,4	0
4	7,2	4,7	4,2	3,2	0,4
6	9,2	5,7	4,3	3,0	0
10	12,2	3,5	2,1	2,3	0
15	10,0	1,2	0,7	0,3	0

Fig.1

Toxin concentration of the faeces (mg/kg) in rabbits getting 1mg T-2 toxin/kg bodyweight

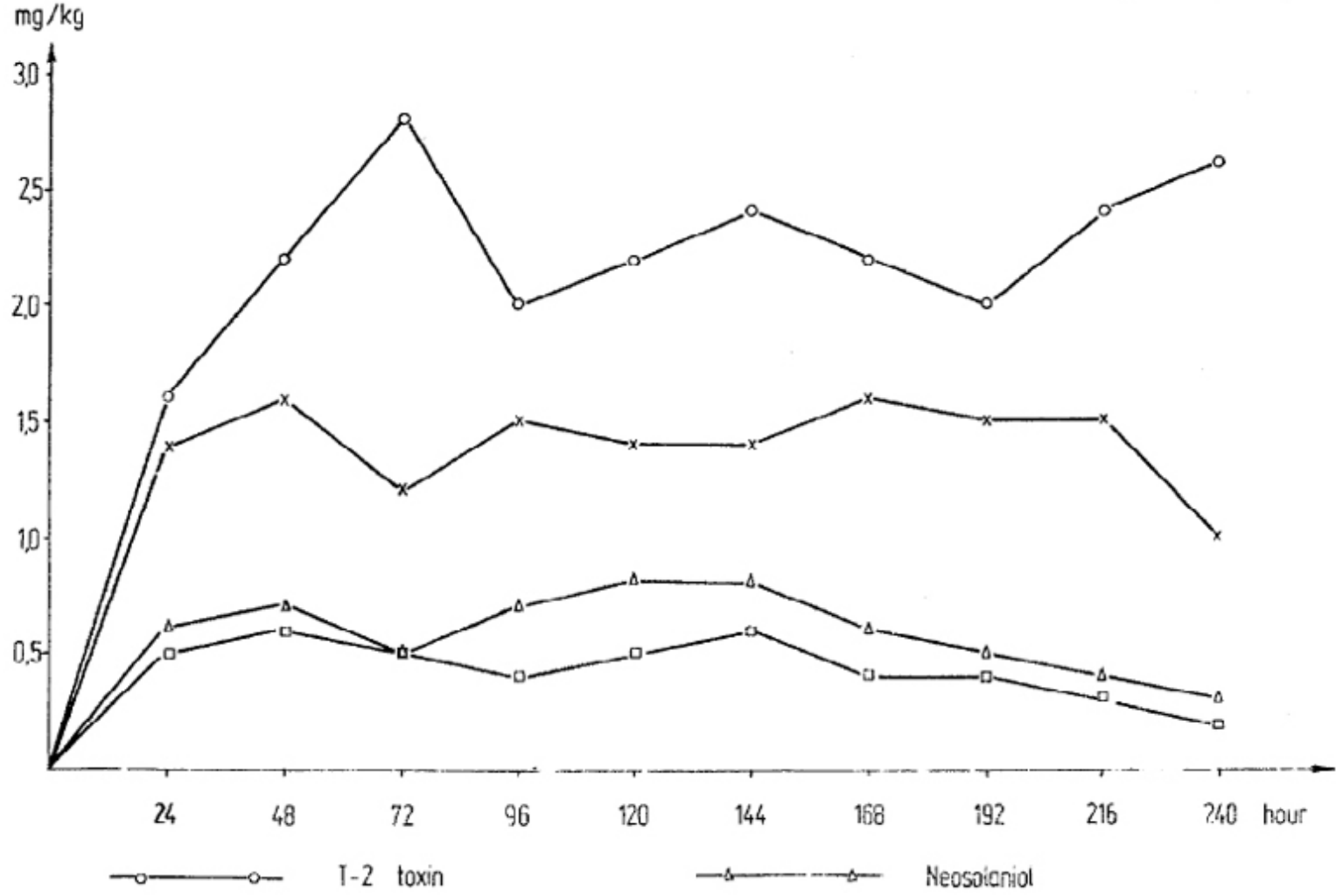
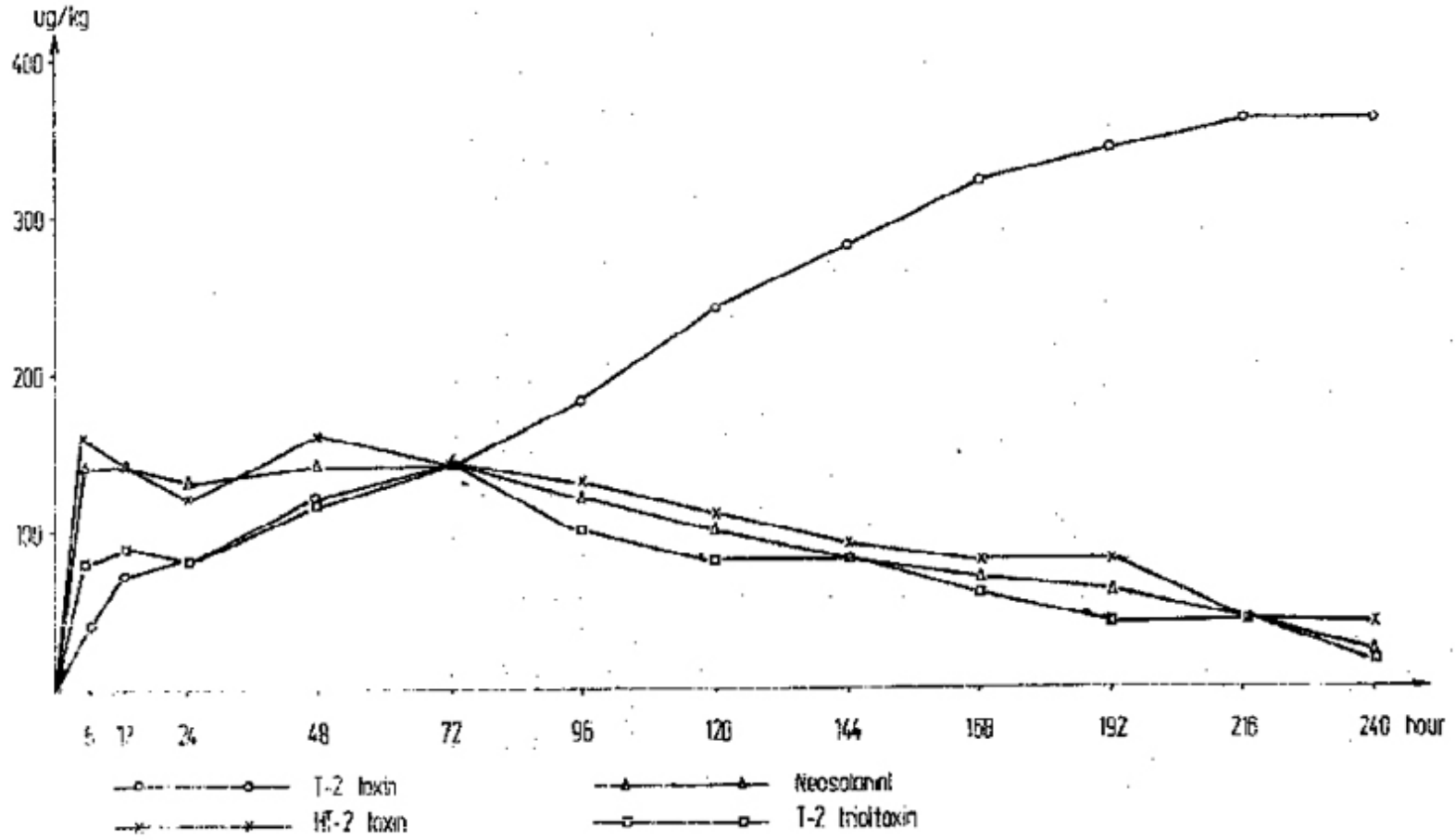


Fig. 2

Toxin concentration of the liver (µg/kg) in rabbits getting 1mg T-2 toxin/kg bodyweight



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THE METABOLIZATION AND ELIMINATION OF T-2 TOXIN FROM RABBITS

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Acute and subacute toxicological investigations /with 18 and 28 animals respectively/ were carried out on rabbits of 2-2,5 kg bodyweight with per os dosing of uncleaned raw-extract of rice contaminated with T-2 toxin. The quantity of T-2 toxin, HT-2 toxin, neosolaniol, T-2 triol, and T-2 tetraol of the stomach content, blind gut content, faeces and liver were analysed in both experiments. The results are summarized in tables. The more important results are as follows:

- the toxin content of the stomach, blind gut and faeces is proportional to the quantity of the ingested toxin and it increases towards the rear intestine section
- the metabolization capacity of the liver decreases continuously, the sensitivity of the organismus increases.

DIE UNTERSUCHUNG DER METABOLISATION UND ELIMINATION DES T-2 TOXINS IN KANINCHEN

In Kaninchen von 2-2,5 kg Körpergewicht wurden akute /18 Tiere/ und subakute /28 Tiere/ toxikologische Untersuchungen durchgeführt mit einmaliger per oral Dosierung eines T-2 Toxin-haltigen ungereinigten Reisextraktes. In beiden Experimenten wurden die T-2 Toxin, HT-2 Toxin, Neosolaniol, T-2 triol-Toxin und T-2 tetraol-Toxin im Mageninhalt, im Blinddarminhalt, im Kot und in der Leber bestimmt. Die Ergebnisse sind in Tabellen zusammengefasst. Die wichtigsten Ergebnisse sind die Folgenden:

- der Toxininhalt im Magen, im Blinddarm und im Kot ist verhältnissgleich zu der eingegebenen Toxinmenge und diese Menge erhöht sich in die Richtung des hinteren Darmabschnittes
- die Metabolisations-Vermögen der Leber vermindert sich fortlaufend, die Empfindlichkeit des Organismus steigert sich.