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EFFECT OF LONG-TERM FEEDING OF SUBLETHAL QUANTITIES OF T-2 TOXIN UPON THE OVARIAN ACTIVITY OF THE RABBIT

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Abstract

The authors examined the effect of 4-7 week feeding of naturally infected wheat grains containing 0.284 mg T-2 toxin/kg on the health and reproductive status of sexually mature, virgin female rabbits. The toxin intake altered the immune system, the alanine-aminotransferase, alkaline phosphatase and cholinesterase activities and glomerular filtration (evaluated by serum creatinine concentration). The T-2 feeding also influenced the ovarial activity, measured using GnRH-stimulation of the ovulation and measurement of the individual progesterone profile.

Introduction

The relatively high (>1.0 mg/kg) T-2 toxin concentration in the feed has an serious adverse effect on the cyclic function of the ovaries, inhibits the ovulation and/or the formation of the corpus luteum (CL). The majority of the concerning knowledge are derived from morphological studies (Shull and Cheeke, 1985) and there are only few trials using the dynamic, endocrinological method.

The occurrence of relatively low toxin concentrations is frequent in feeds (Scott, 1978; Bauer, 1988; Russel et al., 1991). After the intake of such a feed no acute, pathognostical symptoms appear and the toxin uptake remains undiscovered. In lack of "spectacular" signs the sublethal mycotoxin exposure can often last for a longer period of time (Smith, 1981). Concerning the reproductive consequences of the described toxin intake, we have practically no data. In the present experiment - using naturally infected wheat grains - we wanted to study the effect of the long-term (4-7-week) sublethal (Fekete et al., 1989) T-2 toxin intake on the reproductive functions of the domestic rabbit.

Materials and Methods

Twenty, young New Zealand White, sexually mature, virgin New Zealand White rabbits weighing about 3.5 kg were individually penned and divided into two groups. The daily ration

of Group 1 (desinated as: "T-2 group") contained 100 grams of naturally infected wheat grains (0.284 mg/kg T-2 toxin; no F-2 toxin detectable; no analysis for other toxins) and 50 grams alfalfa pellets. The HPLC-analysis was performed as described by Lepom et al. (1990). Group 2 (in the following: Control group) received 100 grams toxin-free wheat and 50 grams alfalfa pellets. The wheat contained 87 % dry matter, 13.8 % crude protein, 1.8 % ether extract and 2.2 % crude fiber, according to the proximate analysis (A.O.A.C., 1975). Drinking water was offered ad libitum. Housing, management and care of animals met animal welfare regulations (USDA, 1985).

On Day 32 of the T-2 toxin exposure blood samples were taken from the 8 surviving T-2 and 10 control animals via the marginal ear vein and serum enzymes and other metabolic parameters were measured. The serum parameters were measured by means of an Eppendorf 1101 M, using commercialized test collections and prescribed kinetic (optimalized) methods of Boehringer and Sons Co.

To evaluate the functional activity of the ovaries, 4 control and 3 T-2 rabbits were killed with penthobarbiturate and necroscopied. The macroscopically detectable ovarian formations were registered and the ovaries fixed in neutral-buffered 8 % formalin solution for histological examination. After paraffin embedding and hemalaun-eosin staining, the sections were evaluated.

The remaining animals (T-2 group: 5; Control: 6) for the triggering of ovulation and the subsequent pseudopregnancy were treated by a single intramuscular injection of 2 μg GnRH-analogue (Gonadotropine Releasing Hormone: D-Phe6-GnRH; Ovurelin inj. ad us. vet.R, Reanal) per animal. Before (Day 0) and after (Days 1, 2, 3, 5, 7, 9. 12, 15, 17 and 19) the GnRH-treatment, for detection of ovulation and the function of the pseudopregnancy corpora lutea (CLps), blood samples were collected and the progesterone concentration was measured using radioimmunassay (RIA), according to the procedure of Bahr et al. (1980). After the last bleeding (Day 19) the rabbits were killed. The necroscopy and the histological study were carried out as described above.

All statistical analyses were performed using the statistical software package SPSS (Norusis and SPSS Inc., 1988).

Results and Discussion

Two of the T-2 animals died during the first 32 days of the toxin exposure, one additional on Day 8 of the GnRH-treatment. In all cases an acute, fibrinous-purulent peritonitis and pneumonia characterized the necroscopical picture. Staphylococcus aureus grown out in pure colony from these altered tissues. Since there were no mortality in the Control group, the phenomenon drew attention to the possible impairment of the immune system. (It is worth mentioning, that in a control animal, No 17, killed at the end of the experiment, there was also a peritonitis.) Otherwise, the T-2 toxin is known as a typical immunosupressive agent (Prier et al., 1980; Taylor et al., 1989).

Enzyme activities and metabolites concentrations on Day 32 of the T-2 exposure are given in Table 1 and Figures 1 and 2. The two liver-cell transaminase activities were slightly (AST = aspartate-aminotransferase, GOT) and significantly ($p < 0.05$: ALT = alanine-aminotransferase, GPT) higher at the T-2 animals compared to the controls. The control AST and ALT activities are in the upper third of the physiological range (Kozma et al., 1974). The damage of the liver-cell membranes must have been only discrete, because there were not differences between the two groups LDH (lactate dehydrogenase) activity. The concentration of two enzymes, gamma-GT (gamma-glutamyl-transferase) and ALP (alkaline phosphatase), primarily showing the conditions of the liver and bile ducts (acute or chronic hepatitis, intrahepatic cholestasis), was higher at the T-2 animals. The differences are not significant ($p > 0.1$), but this tendency is the same as in case of the transaminases. Gentry and Cooper (1981) reported also, that the orally administered T-2 toxin tended to increase serum ALT and decline ALP activity.

Owing to the T-2 exposure the cholinesterase (CHE) activity decreased by 50 % ($p < 0.1$), but the standard deviations are great (CV = 57 and 49 %). This shows the damage of the parenchyme of the liver (Goreczky and Sós, 1983), which can be explained with the direct action of T-2 toxin, i.e. the inhibition of the protein synthesis of eucaryote cells (McLaughlin, 1977).

Concerning the activity of the hydroxy-butyrate-dehydrogenase (HBDH) and MDH (maleate dehydrogenase), the tendency is the same, as in case of the tranaminases, ALP and gamma-GT, i.e. the values of the control group are lower, but the differences are not significative, either statistically or physically.

The creatinine concentration is significantly ($p < 0.02$) higher in the T-2 rabbits, referring to the damage of kidney functions (Finco, 1989). It may show also some muscle protein mobilization. It is surprising that at the same time serum urea-N (BUN) levels of T-2 animals are hardly higher, than at the control. There were no differences in the glucose, total cholesterol and triglycerid blood concentrations either. The lesions must have been really fine, because during the necroscopy the liver and the kidneys seemed to be healthy.

The state of the ovaries on Day 32 of the T-2 exposure. The morphological examination of the ovaries of the three T-2 and 4 control rabbits did not reveal differences. On the ovaries of the animals of both groups the number and the type (developing, i.e. < immmature, i.e. > 1 mm and atrophic) of follicles were the same.

Ovarian response on the GnRH-treatment. There were five T-2 and six control animals (Table 2, Figure 3). The individual progesterone profile of the three T-2 and each control animals refers to ovulation and subsequent CLps-formation. The CLps-function in each control and two T-2 (No 39 and 40) animals corresponded to the expectations, i.e. after the GnRH-treatment the progesterone level sharply increased and after Days 12-15 decreased (Zöldág, 1990; Marcinkiewicz et al., 1992). The values of Days 17-19 were characteristic of the follicular phase. In a T-2 rabbit (No 34) the trifling rise of the progesterone concentration reflects impaired ovarian functions and on Day 9 the luteal activity ceased. Two T-2 animals (No

37 and 38) showed continously low (<2.0 mmol/l) progesterone level, i.e. they did not have an ovulation after the GnRH-treatement or there were not any luteinisation during and after the ovulation. The rabbit No 37 died on Day 8 after the GnRH-treatment.

The pathological examination (necroscopy and histology) on Day 19 has found a lot of follicles of different development and large CLps-s on the ovaries of each control animals. In the ovaries of the two T-2 rabbits (No 34 and 38) one could not macroscopically detect any CLps. The histological study showed some underdeveloped, atrophied luteal formations. The findings practically were the same in case of the T-2 rabbit (No 37), died on Day 8 of the GnRH-treatment. The macro- and microscopical pictures of the two further T-2 animals (No 39 and 40), likely to the control, gave evidence of the physiological ovarial functions.

Conclusions

On the basis of the present experiment, it renders possible, that the naturally occurred, sublethel, continous T-2 exposure is not neutral to the health state of the monogastric animals. The problem may have human health consequences, too.

The continous, sublethal T-2 intake alters the immune system, the liver, the kidney's glomerular function and the ovarian activity.

The combining of the sublethal T-2 toxin intake with some specific agent (e.g. Staphylococcus aureus) may result in mortality.

One cannot exclude that some of the described alterations (e.g. kidney) partly may be attributed to other unrevealed mycotoxins. Consequently, to certify the present statements, one has to repeat this trial, with the same design, but using isolated, identified toxins.

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References

A.O.A.C. 1975. Official method of analysis. 12th Ed., Association of Official Analytical Chemists. Washington, D.C. pp. 129-146.

Bahr, J.M., Gardner, R. and Shahabi, N. 1980. Follicular steroidogenesis: effect of reproductive conditions. Biol. Reprod. 22:817-826.

Bauer, J. 1988. Diseases and depression of performance of pigs by mycotoxins. (in German) Tierårztl. Prax. Suppl. 3:40-47.

Cheeke, P.R. and Shull, L.R. 1985. Natural toxicants in feeds and poisonous plants. AVI Publishing Compagny, Inc. Westport, CT, pp. 393-477.

Fekete, S., Tamás, J., Ványi, A., Glavits, R. and Bata, Á. 1989. Effect of T-2 toxin on feed intake, digestion and pathology of rabbits. Lab. Anim. Sci. 39:603-606.

Finco, D.R. 1989. Kidney function. In: Kaneko, J.J. (Ed.): Clinical biochemistry of domestic animals. Fourth ed. Academic Press Inc. New York-London-Tokyo, pp. 496-542.

Gentry, P.A. and Cooper, M.L. 1981. Effect of Fusarium T-2 toxin on hematological and biochemical parameters in the rabbit. Can. J. Compar. Med. 45:400-405.

Goreczky, L. and Sós, J. 1983. Clinico-chemical manuel (in Hungarian) 4th ed. Medicina. Budapest. pp. 161-163.

Kozma, C., Macklin, W., Cummins, L.M. and Mauer, R. 1974. Anatomy, physiology and biochemistry of the rabbit. In: Eeisbroth, S.H., Flatt, R.E. and Kraus, A.L. (Eds.): The biology of the laboratory rabbit. Academic Press. New York, p. 618.

Lepom, P., Knabe, O. and Baath, H. 1990. Occurrence of Fusarium species and their mycotoxins in maize. (in German) Arch. Anim. Nutr. 10:1005-1012.

Marcinkiewicz, J.L., Moy, E.S. and Bahr, J.M. 1992. Change in responsiveness of rabbit corpus luteum to prostaglandin F-2α during pregnancy and pseudopregnancy. J. Reprod. Fertil. 94:305-310.

McLaughlin, C.S., Vaughan, M.H., Cambell, I.M., Mer Vei, C., Stafford, M.E. and Hansen, B.S. 1977. Inhibition of protein synthesis by trichothecenes. In: Rodrick, J.V., Hesseltine, C.W. and Mehlman, M.A. (Ed.): Mycotoxins in human and animal health. Pathotox. Publ. Park Forest South, IL. pp. 263-273.

Norusis, M.J. and SPSS Inc. 1988. SPSS/PC+ V2.0. SPSS Inc.
Chicago,

Prier, A.C., Richard, J.L. and Cysewski, S.J. 1980. Implications of mycotoxins in animal disease. J. Am. Vet. Med. Assoc. 176:719-724.

Russel, L., Cox, D.F., Larsen, G., Bodwell, K. and Nelson, C.E. 1991. Incidence of molds and mycotoxins in commercial animal feed mills in seven midwestern states, 1988-1989. J. Anim. Sci. 69:5-12.

Scott, P.M. 1978. Mycotoxins in feeds and ingredients and their origin. J. Food Protect. 41:385-398.

Smith, J.E. 1981. Mycotoxins and animal health. Roche. Basel.

Taylor, M.J., Smart, R.A. and Sharma, R.P. 1989. Relationship of hypothalamic-pituitary-adrenal axis with chemically induced immunomodulation. I. Stress-like response after exposure to T-2 toxin. Toxicol. 56:179-195.

USDA, Animal and plant health inspection service. 1985. Laboratory Animal Welfare Act (PL89-544). Subchapter A-Animal Welfare. US Government Printing Office, Hyattsville, MD. pp. 38-45.

Zöldág, L. 1990. Reproduction of the domestic rabbit. In: Vetési, F. (Ed.): Hygiene of the domestic rabbit (in Hungarian). Mezôgazd. Kiadó. Budapest, pp. 62-78.

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Table 1.

The activity of some serum enzymes and the values of some blood constituents at the time of GnRH treatment

	Control (n = 10)	Exposed to T-2 (n = 8)
	MEAN ± SD (range)	
Serum enzymes, IU/l		
AST	14.0 ± 8.3 (7.0 - 31.0)	20.2 ± 11.9 (9.0 - 44.0)
ALT	31.4 ± 13.6 (14.0 - 54.0)	50.1 ± 23.8* (17.0 - 70.0)
gamma-GT	5.9 ± 3.5 (2.6 - 11.6)	8.0 ± 6.2 (3.3 - 21.0)
ALP	142.0 ± 48.0 (68.0 - 251.0)	158.0 ± 49.0 (103.0 - 245.0)
LDH	124.0 ± 26.0 (76.0 - 172.0)	116.0 ± 43.0 (50.0 - 195.0)
CHE	520.0 ±301.0 (151.0 - 907.0)	277.0 ± 135.0 (160.0 - 534.0)
HBDH	56.0 ± 17.0 (24.0 - 91.0)	67.0 ± 34.0 (25.0 - 121.0)
HDH	448.0 ± 75.0 (338.0 - 550.0)	554.0 ± 128.0 (344.0 - 717.0)
Blood constituents, mmol/l		
Glucose	5.5 ± 0.3 (5.0 - 5.8)	5.5 ± 0.4 (4.9 - 5.9)
Total cholesterol	3.82 ± 0.53 (2.91 - 4.54)	3.93 ± 0.80 (2.03 - 4.85)
Creatinine	0.124 ± 0.016(0.097- 0.151)	0.152± 0.029** (0.125- 0.211)
Urea-N	8.95 ± 3.55 (0.99 -13.30)	9.37 ± 2.90 (6.46 -12.98)
Trigliceride	1.53 ± 0.78 (0.91 - 3.55)	1.35 ± 0.34 (0.80 - 1.98)

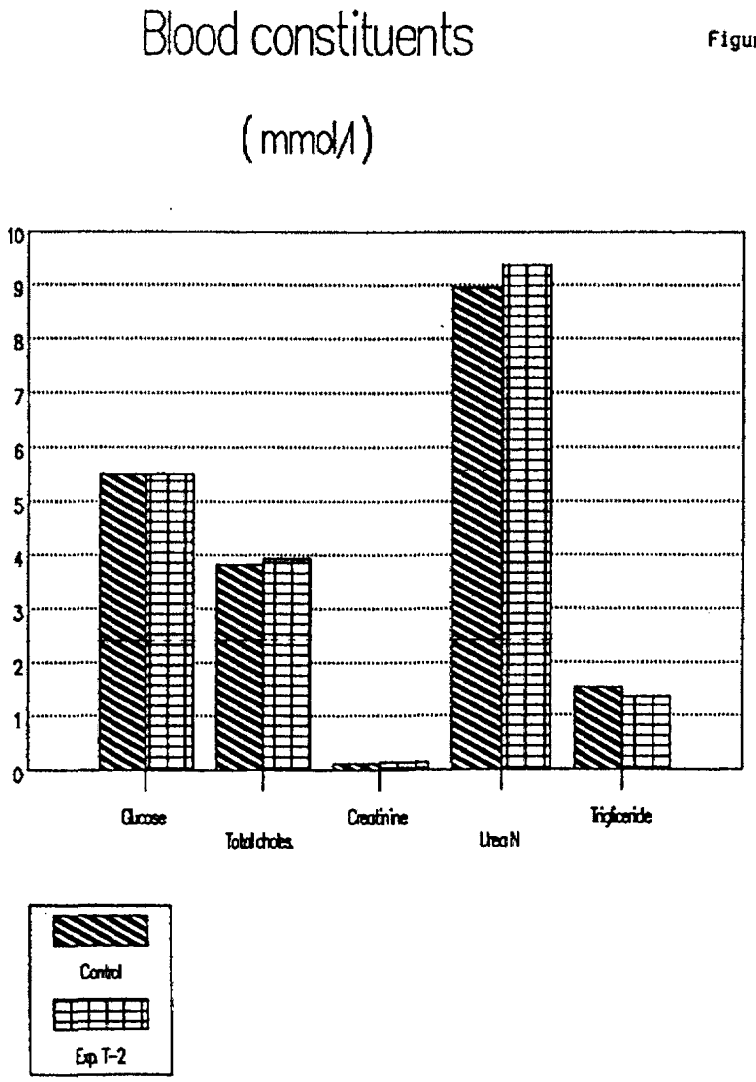
Remarks: *p<0.05, **p<0.02

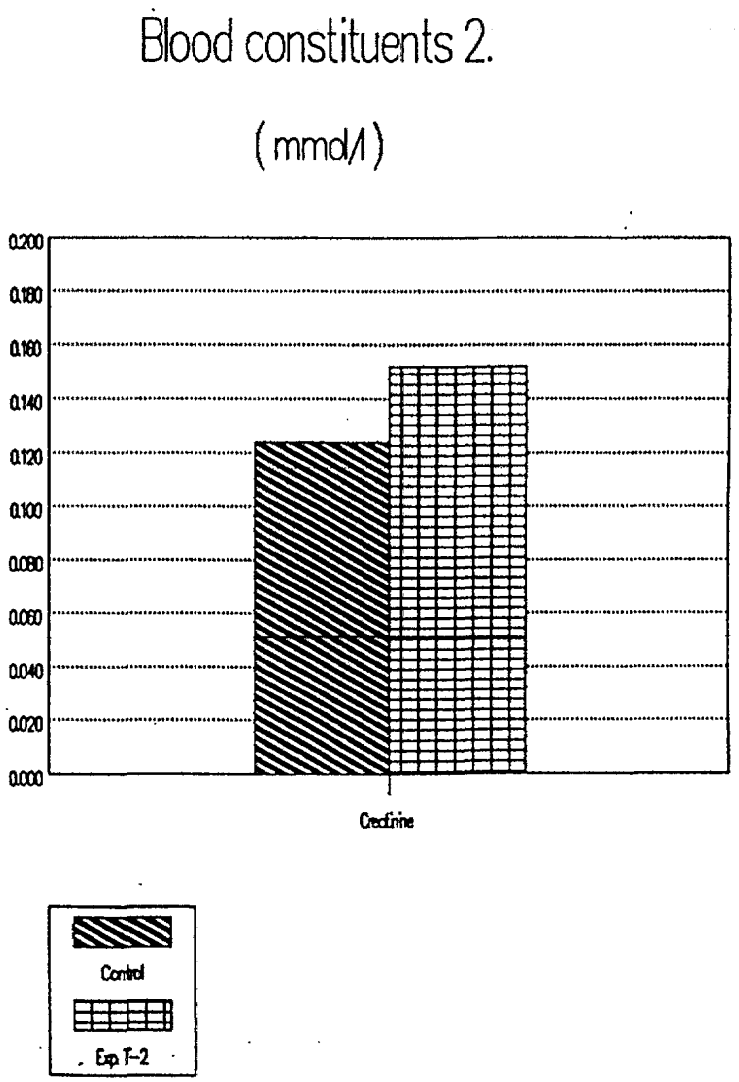
Table 2.

Blood plasma progesterone concentration, nmol/l

	0 GnRH	1	2	3	5	7 (day)	9	12	15	17	19
Control											
14	0.31	0.41	0.44	2.80	16.3	27.7	26.8	20.6	18.4	8.23	4.77
15	1.01	0.82	2.81	4.08	13.7	26.8	27.6	27.8	15.7	4.31	2.31
16	1.04	1.08	2.01	5.72	10.5	16.3	18.6	15.9	13.4	6.62	1.63
17	0.67	1.42	2.38	3.23	12.4	24.2	18.8	13.7	6.28	0.43	1.18
19	0.88	0.92	1.99	6.01	11.7	21.3	24.4	21.2	3.88	1.26	0.38
20	0.71	1.41	1.87	8.08	14.7	18.3	14.8	14.3	12.5	1.87	1.41
Exposed to T-2											
34	0.82	0.92	1.33	1.98	3.80	7.21	0.66	0.82	0.41	0.73	1.12
37	0.61	0.48	0.83	0.55	0.81	1.27	died	-	-	-	-
38	1.17	0.88	1.07	0.95	0.80	0.59	1.34	1.52	0.98	0.87	0.81
39	0.37	1.19	1.85	5.17	18.6	29.2	19.3	15.5	10.7	4.23	3.31
40	0.41	1.07	2.35	4.58	13.1	24.4	21.2	21.8	15.2	3.97	0.56

Figure 1


Blood constituents
(mmol/l)
10
9
8
7
6
5
4
3
2
1
0
Glucose
Total chotes.
Creatinine
Urea N
Triglyceride
Control
Exp T-2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(mmol/l)
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0.160
0.140
0.120
0.100
0.080
0.060
0.040
0.020
0.000
Creatinine
Control
Exp T-2

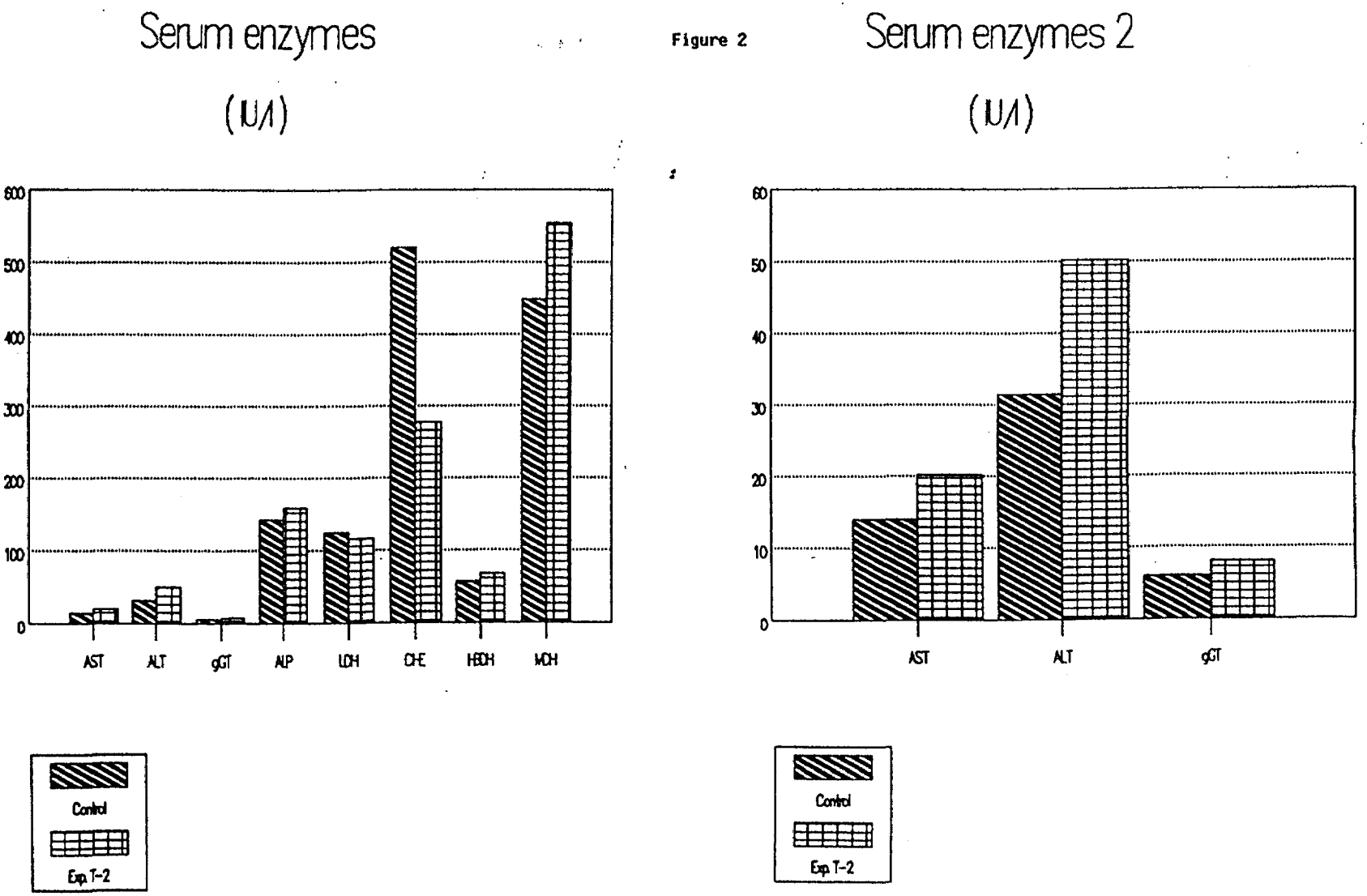
Serum enzymes
(U/l)
600
500
400
300
200
100
0
AST
ALT
gGT
ALP
LDH
CHE
HBDH
MDH
Control
Exp.T-2
Serum enzymes 2
(U/l)
60
50
40
30
20
10
0
AST
ALT
gGT
Control
Exp.T-2

Figure 2

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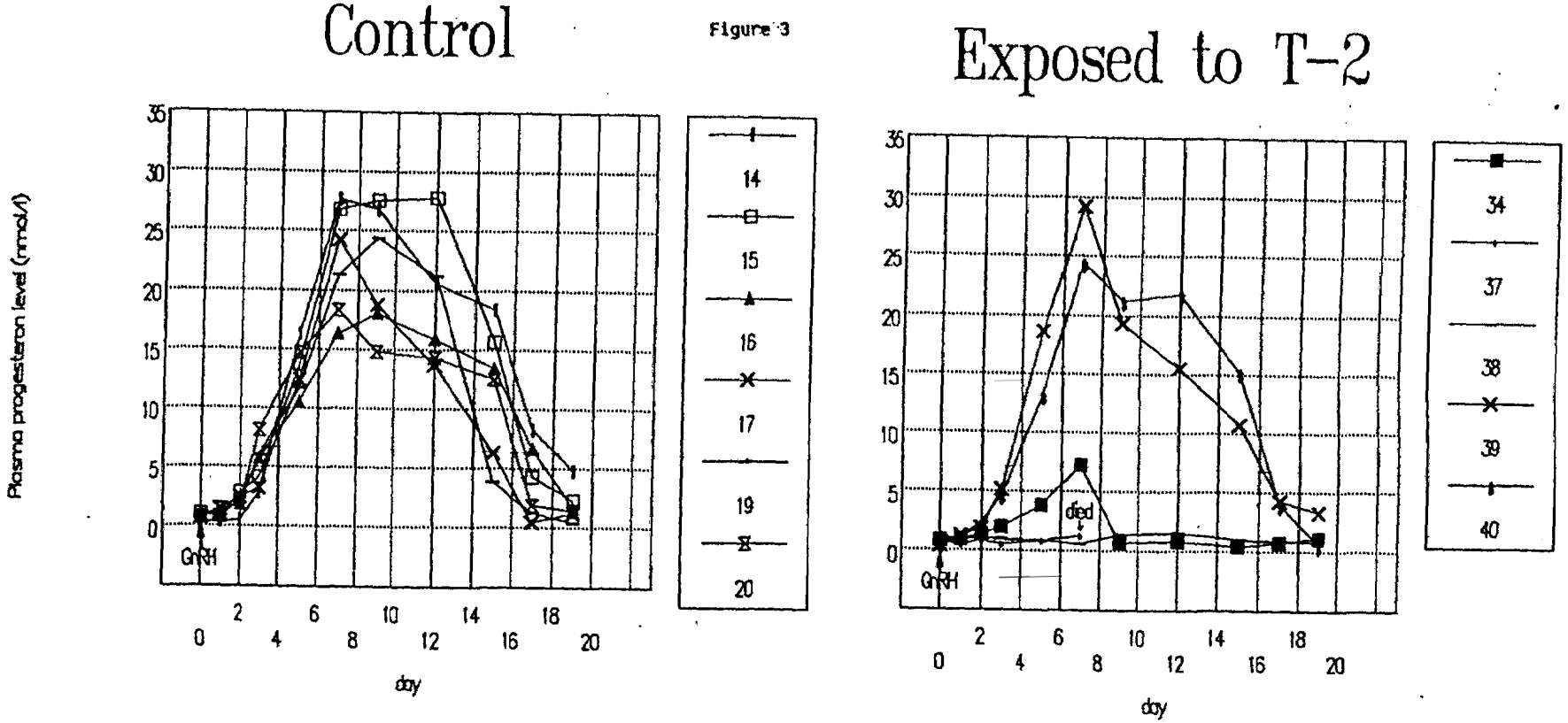

Figure 3