SZENT-GYÖRGYI ALBERT ORVOSTUDOMÁNYI EGYETEM
KÖZEGÉSZSÉGTANI ÉS JÁRVÁNYTANI INTÉZET
Igazgató : Dr. DÉSI ILLÉS egyetemi tanár
6720 SZEGED
Dóm tér 10.
Telefon : (62)311-954
Telex : 82 441 szote hm

DEPARTMENT OF HYGIENE AND EPIDEMIOLOGY
ALBERT SZENT-GYÖRGYI UNIV. MED. SCHOOL
Director : Prof. I. DÉSI M. D., Ph. D., D. Sc. (med.), M. Sc. (techn.)
Dóm tér 10.
SZEGED
H–6720 HUNGARY
Tel. : (36-62)11-954 Telex : 82 441 szote hm

Cumulative Toxicological Investigation of Humet-R Solution

Responsible Leader: **Prof. Illés Dési, MD., DSc.**
Leader of the Department

SZOTE
Department of Public Health

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The cumulative toxicological investigation was performed on *"Humet-R"* solution in order of the Horizon-Multiplan KKT. by the Department of Public Health, SZOTE, especially accordingly the effects on haematologically criteria.

Methods

1. Material

Investigation was performed at the "Humet-R" solution send by the customer. The LD_{50} was according determination of OETI larger than 10 g/ttkg.

2. Experimental animals

For the experiments 10-10 male Wistar rats as controls or treated animals were used, respectively. The animals were kept in an animal's house under standard conditions, in plastic boxes 5 animals per box. Light-dark cycle was 12:12 hours, standard show (Lati) and tap-water *ad libitum*.

3. Treatment

The cumulative treatment occurred according the method of Lim et al. The rats received on 4-4 successive days for a time-intervall of 24 days the substance *per os* at the LD50 dose (10.0 g/ttkg) in steps of 9.0; 13.5; 20.0; 30.0; 45.0 or 68.0% , respectively. Treatment occurred every day at the same time in a volume of 5.0 ml.

The animals were continuously observed, observable changes registered, the body-weight gain before, during and after treatment every fourth day controlled.

After the observation period of 24 days, the animals were killed at day 25 and their organs (liver, heart, kidneys, lung, spleen, thymus, renals) measured and investigated for pathological signs. The relative organ weight was calculated.

4. Haematological investigations

Haematological investigation of blood before treatment occurred by vena punction and after treatment by blood sampling from the *v. cava inferior*.

The number of erythrocytes, leucocytes, the volume of the erythrocytes, the haematocrit, number of thrombocytes, the volume of thrombocytes, was estimated by automatic techniques using the Picoscale-5 (Medicor) equipment. Ferrum of the serum was measured by a special kit.

5.Determination of thyroid hormones (T_3-T_4)from blood plasma

Determination of thyroid hormones were carried out by standard immunoassay kits, in the case of T_3 not by isotops, but by the alternativ "DELFIA" (Wallace, Oy, Finland) and for T_4 by RIA ((Izinta MTA, Isotop Institute, Budapest).

6. Histological investigations

Histological investigations were carried out using tissue of the liver, spleen, kidneys, and lung by means of basic histology (HE), haemosiderin determination based on Berlin blue reaction (staining). On kidney tissue PAS reaction was used, too.

6. Statistics

Processing of the data occurred when useful (e.g. for haematological results) or for the self-control experiment by a one-sample t-test. In cases of relative organ weight, control vs. treatment data were compared by the use of the 2-sample t-test.

Results

It was stated, however, that on the basis of body weight gain of the animals (Table 1) the treated rats at any time of the experiment did not show any marked difference vs. the untreated controls.

The relative organ weight - exclusively the significantly increased organ weight of the liver - did not show differnces between treated and untreated animals (Table 2). As mentioned, the increased relative weight of the liver, independently of the grade of significance, reflect the fact that even this small difference is not a sign of serious poisoning.

The haematological data (Table 3) did not show any significant increase of of the values of the primary data, but there was in the treated group an even small increase in the number of erythrocytes ($p<0.1$). Both, in the controls and in the treated group occurred a significant decrease of the number of leucocytes ($p<0.01$), of the haematokrit ($p<0.1$), and the volume of erythrocytes ($p<0.001$).

There were no differences in the qualitative data of the blood samples and the primary data were not different to the results at the end of the experiment (Table 4).

Compared to the primary values, in treated animals the blood content of ferrum was significantly decreased ($p<0.01$). But also in the control group decreased this value significantly (Table 5).

The blood plasma values for thyroid T3 of the rats were during the treatment period markedly decreased in both the controls and the treated animals. The decrease was in every group approximately the same (Table 6). For the functioning of the thyroid gland the T4 is escpecially informative; here

during the treatment period no changes were found in the controls as well as in the treated animals.

The data of the histological investigation did not show any significant difference between the treated rats and the control animals. Only the haemosiderosis was increased in the treated group vs. the controls. In some cases, the treatment results in moderate changes of lung tissue (peribronchial lymphocytic infiltration), but something like also was seen in control animals too.

Appreciation

Humet-R solution did not affect significantly following cumulative toxicological investigation basis values of the treated animals. There were no developmental diminishing effect on body-weight gain, relative organ weight of the liver or some of the other organs. Only in contrast to the controls a significant tendency was observable in haematological data of the treated rats (leucocytes, haematokrit, erythrocytes, and serum content of ferrum), but these findings did not indicate, however, the statement of serious toxicological effects in the treated animals.

The same is true for data acquired by blood sampling at two different time points (vena puncture of the tail vene or the puncture of the *v. cava inferior*). The test substance shows together two, biologically correlating effects, an increase of the number of erythrocytes, on one hand, and a decrease of the ferrum content of the serum significantly to the controls, on the other.

On the basis of the data, Humet-R solution did not express any toxic effect(s) in a cumulative intoxication experiment.

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László Nagymajtényi, MD. DSc.
Sen. Res. Adviser

Prof. Illés Dési, MD., DSc.
Head of the Department

To
Gabor Vetro, MD.
SZOTE
Department of Public Health

Protocol

Wistar rats were treated with "Humet-R" solution and their different organs investigated. The organs arrived in determined and signed glases. The organs were as follows: liver (M), spleen (L), Lung (T), and kidney (V); histological investigation occurred as general histology on Berlin blue staining (HE) for hemosiderin effects. Kidney histology additionally with PAS reaction occurred on the net of the basal membrane.

Results

I. controls

C21
M: no signs (n.s.)
L: slight hemosiderosis (+)
T: n.s.
V: n.s.

C22
M: n.s.
L: slight hemosiderosis (+)
T: n.s.
V: n.s.

C23
M: n.s.
L: slight hemosiderosis (+)
T: n.s.
V: n.s.

:osis (+)
lymphocytic infiltration with little

:osis (+)
lymphocytic infiltration with little
:oles groups of foamy cytophagic
ne little nodes siderophagic cells

:osis (+)
lymphocytic infiltration with little

:osis (+)
lymphocytic infiltration with little

:osis (+)
nteralveolar septum (acute emphysema)

:osis (+)
lymphocytic infiltration with little

:osis (++)
tissue 1-2 hemosiderin containing

TABLE I. CHANGES OF BODY-WEIGHT GAIN OF HUMET-R TREATED RATS

DAY OF MEASUREMENT	CONTROLS	TREATED GROUP
0.	253,50 ± 2,38	255,50 ± 3,11
4.	276,00 ± 3,14	298,50 ± 13,60
8.	307,00 ± 4,89	316,50 ± 11,18
12.	332,50 ± 7,38	319,00 ± 7,59
16.	361,00 ± 10,11	335,00 ± 11,60
20.	366,50 ± 11,26	340,00 ± 11,64
24.	375,50 ± 10,99	352,50 ± 11,98
28.	383,50 ± 11,35	353,00 ± 12,32

THE RELATIVE ORGAN WEIGHT OF TREATED RATS

	RELATIVE ORGAN WEIGHT $\bar{X}$±S.E.M.						
	THYMUS (g/100 g)	LUNG (g/100 g)	HEART (g/100 g)	LIVER (g/100 g)	SPLEEN (g/100 g)	KIDNEYS (g/100 g)	ADRENALS (mg/100 g)
CONTROLS	0,13 ±0,01	0,51 ±0,01	0,26 ±0,01	3,10 ±0,04	0,34 ±0,01	0,55 ±0,01	12,43 ±0,56
TREATMENT	0,13 ±0,01	0,54 ±0,03	0,27 ±0,01	3,33* ±0,05	0,32 ±0,01	0,57 ±0,02	13,70 ±0,36

* p (0.05

TABLE 2.

HEMATOLOGICAL PARAMETERS OF THE ANIMALS TREATED BY HUMET-R

(X ± S.E.M.)

		WBC ($\times 10^6$/ml)	RBC ($\times 10^9$/ml)	MCV (μ^3)	PLT ($\times 10^6$/ml)	MPV (μ^3)	HAEMATOCRITE (%)	THROMBOCRITE (%)
CONTROLS	DAY 0	7.92 ± 0.50	8.42 ± 0.29	56.8 ± 1.08	269.2 ± 15.27	14.32 ± 0.46	100.0 ± 3.52	100.0 ± 6.52
	DAY 28	5.21** ± 0.56	7.77 ± 0.15	51.39*** ± 0.69	268.6 ± 15.19	14.21 ± 0.43	83.6** ± 2.30	100.0 ± 7.53
TREATED GROUP	DAY 0	8.11 ± 0.77	7.24 ± 0.18	63.85 ± 1.54	263.2 ± 21.03	18.41 ± 0.83	100.0 ± 3.65	100.0 ± 10.08
	DAY 28	6.25** ± 0.92	7.95* ± 0.18	54.16*** ± 0.74	274.0 ± 16.92	18.09 ± 0.84	93.3* ± 1.15	102.1 ± 7.91

* $p < 0.1$ ** $p < 0.01$ *** $p < 0.001$

TABLE 3.

QUANTITATIVE BLOOD VALUES OF RATS FOLLOWING HUMET-R TREATMENT

($\bar{X} \pm$ S.E.M.)

		jn	st	sg	Eo	bt	Mo	MNS
CONTROLS	DAY 0	0,10 ±0,10	2,10 ±0,34	17,40 ±1,11	0,40 ±0,16	0,10 ±0,10	0,40 ±0,16	79,70 ±1,10
	DAY 28	0,30 ±0,21	2,60 ±0,34	17,00 ±1,05	0,50 ±0,16	0,10 ±0,10	0,20 ±0,13	79,40 ±1,19
TREATED GROUP	DAY 0	0,20 ±0,13	2,40 ±0,52	21,20 ±1,22	0,90 ±0,23	0,10 ±0,10	0,50 ±0,22	74,70 ±1,24
	DAY 28	0,00	2,70 ±0,65	19,90 ±1,26	0,70 ±0,26	0,10 ±0,10	0,20 ±0,13	75,70 ±1,39

TABLE 4.

FERRUM VALUES OF SERUM IN HUMET-R TREATED RATS

	CONTROLS	TREATMENT
	(µmol/l) $\bar{X} \pm SE$	(µmol/l) $\bar{X} \pm SE$
DAY 0	43,28 ±2,66	47,12 ± 2,08
DAY 28	37,06 * ±1,71	38,69 ** ±1,71

TABLE 5.

* p (0,1
** p (0,01

T_3 MEASUREMENTS OF RATS TREATED WITH HUMET-R

	CONTROLS	TREATMENT
	(nmol/l) $\bar{X} \pm SE$	(nmol/l) $\bar{X} \pm SE$
DAY 0	0.62 ± 0.047	0.86 ± 0.065
DAY 28	0.49 ± 0.031	0.63 ± 0.037

$^{*}p < 0.05$

TABLE 6.

T_4 MEASUREMENTS OF RATS TREATED WITH HUMET-R

	CONTROLS	TREATMENT
	(nmol/l) $\bar{X} \pm SE$	(nmol/l) $\bar{X} \pm SE$
DAY 0	72.69 ± 1.514	80.82 ± 4.449
DAY 28	72.62 ± 2.862	84.04 ± 2.641

TABLE 7.