Study on the effects of different formulations of humic acid bound with iron and other micro elements in iron deficient rat pups

Országos Munka- és Üzemegészségügyi Intézet
Budapest
1997.

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Introduction

Iron is required in several metabolic processes and pathways in mammals. Iron deficiency therefore adversely affects, among others, the haematopoiesis, including the synthesis of haemoglobin, the integrity and function of the epithelial tissues, the synthesis of myoglobin, the metabolism of catecholamines and the thyroid hormones, the regulation of the body temperature, the physical fitness and working capacity of the individual (Bernát, 1973; Baynes and Bothwell, 1990). Iron deficiency affects also drug metabolism both in man (Becking, 1972;) and animals (Vitale et al., 1977; Jagedeesan et al., 1994). Biochemical and morphological signs of iron deficiency are influenced by several factors (age, tissue, degree and duration of the deficiency) (Bernát, 1973.). Development of the characteristic microcytic, hypochromic anaemia indicates the late phase of iron deficiency, the total depletion of iron stores (Bernát, 1973.; Wintrobe, 1981).

Iron deficiency is probably the most common form of nutritional deficiency and the most common cause of anaemia in the medical practice. It occurs mainly due to insufficient intake, excessive loss or increased demand for iron. Members of the population predisposed to the development of iron deficiency are women, infants, children and athletes. The proper treatment of iron deficiency has great health and economic impacts. Despite the numerous preparation available, treatment is not always easy.

The aim of the present study was to determine the effectiveness of different formulations of humic acids with bound iron and other micro elements (HME) in the treatment of iron deficient rat pups. Experimental iron deficiency was induced according to Masini et al. (1994) in the pups by

Complete blood cell counts - red blood cells (RBC), white blood cells (WBC), platelets (Thr.), haemoglobin (Hb.), haematocrit (Htk.), mean cell volume (MCV), mean cell Hb. (MCH), mean Hb. concentration (MCHC) were determined by MS8, a 18 parameter Haematological analyser (Melet-Schloesing, France). For IQC 3 levels of CBC-8 control blood (R&D system inc.) Zinc-protoporphyrin/hem ratio (ZP) was determined in whole blood with an AVIV Hematofluorometer. For IQC 3 levels of AVIV control blood (AVIV Biomedicals inc.) were used. Serum iron concentrations, total iron binding capacity (TIBC), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) activities, and triglyceride (Trigl.) concentrations were estimated with AL Plus clinical chemistry analyser and AL Instruments Ltd test kits. For IQC: Elextrol N and P control sera (AL Instruments Ltd) were used.

Statistical analysis. Arithmetic means and SEs were calculated. The group means were compared using Students t-test.

Results

Maternal adverse effects

There was no maternal loss during pregnancy and lactation, neither in the group on normal diet, nor in the group on iron deficient diet. Signs of iron deficiency such as hair loss, muscular weakness or decreased activity could not be observed. Food consumption was less in the iron deficient group than in the control (Table 1), but the relative body mass gain during pregnancy did not differ (Table 2). During lactation, however, body mass gain of dams fed by iron-free diet was significantly smaller compared to the control, despite similar food consumption (Tables 3, 4).

Postnatal observations during lactation

Iron deficiency did not affect the process of pregnancy and delivery, as well as, the average litter size (Table 5). The average body mass of the offspring

of iron deficient mothers was significantly lower at birth than that of the controls and the difference increased during lactation up to 60 % (Fig. 1; Table 5).During the first postnatal week 13 % of the iron deficient pups died. These pups showed general weakness, greyish, transparent skin, lack of movement, somnolence. In the third postnatal week lethality was again high: 11 % of the pups died (Table 5). Data of postnatal development are shown in Table 6.

Postnatal observations during treatment

During the first 8 days of treatment deaths occurred in every group. Some of the physically weak pups did not survive the stress caused by the administration of treatment. During the later period of the treatment only one animal died due to technical reason. The body mass of the offspring of the iron deficient mothers increased on the effect of the treatment both with all forms of HME and Aktiferrin (Fig. 2). The values of body mass gain were the same in the group treated with DHS during the first week and in the groups treated with Gran. I Gran. II and dried preparation during the second week as in the group treated with Aktiferrin, respectively. At the end of the third week the treatment with all forms of HME resulted in greater body mass gain than treatment with Aktiferrin (Fig. 3).

Results of the clinical laboratory measurements

Before treatment

On day 0 of the iron repletion treatment there was a great difference in the mean values of the laboratory parameters of control and iron deficient pups (Fig. 4). In the iron deficient group the mean number of RBC was half, Hb. and Htk. were one third, MCV, MCH and WBC were two thirds of those of the control group, respectively. The mean serum iron level was only about 10 % of the control value, while the TIBC value was twice higher than in the controls. This resulted in a transferrin saturation about 5 % of the

control value. The ZP value was three times higher in the iron deficient group compared to the controls.

The effects of treatment

RBC (Fig. 5) increased in all treated groups and already on day 7 of treatment the mean values (with the exception of the group treated with the dried preparation) were significantly different from the mean of the untreated group, but did not reach the mean value of the control - except the Gran. II group. On day 14 all groups were significantly different from the untreated group and the groups treated with Gran. II and III were not different statistically from the control. On day 21 none of the treated groups differed significantly from the control.

WBC (Fig. 6) increased in all treated groups and on day 7 the mean values of the groups treated with DHS and the dried preparation, on day 14 the mean values of all groups were significantly different from the mean of the untreated group. On day 21 none of the treated groups differed significantly from the control.

Thr. did not change significantly in any of the groups during treatment.

Hb. (Fig. 7) increased in all treated groups but there was a characteristic difference in the rate of increase among the groups. On the effect of DHS and Aktiferrin the mean Hb. reached the control value already on day 7 of treatment. In the other three groups the rate of Hb. increase was slower: on day 7 of the mean values (with the exception of the group treated with the Gran. III) were significantly different from the mean of the untreated group, but did not reach the mean value of the control. On day 14 all groups were significantly different from both the untreated and the control groups and although the slow increase continued, the statistically significant difference from the control persisted on day 21 too.

Htk. (Fig. 8). The change of Htk. went parallel with that of Hb. during the treatment: sudden increase and reaching the control level on day 7 on the

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effect of DHS and Aktiferrin, gradual increase and reaching the control level by day 21 in the rest of the groups.

MCV (Fig. 9). The change of MCV was also characteristically different in the DHS and Aktiferrin groups in comparison to the other three groups. In the DHS and Aktiferrin treated groups the mean value of MCV nearly doubled during the first 7 days of treatment and became significantly higher than the control value, then it decreased slowly, but remained above the control value till day 21. In the other groups a slower increase was experienced, the groups treated with Gran. II and the dried preparation reached the control level by day 7, the Gan. III group by day 21.

MCH (Fig. 10). In the DHS and Aktiferrin treated groups the mean value of MCH nearly doubled during the first 7 days of treatment becoming significantly higher than the control value and remained at the same level till day 21. On day 21 there was no significant difference between the mean values of the control, DHS and Aktiferrin groups, because the control mean slightly increased between days 7 and 21.

Serum iron concentration (Fig. 11) showed great inter-group and within the groups inter-individual variation. After an initial rise the mean value of the Aktiferrin group on day 7 did not differ statistically from the control, by day 14 it decreased to the level of the untreated group, and on day 21 it reached again the control value. The mean values of the DHS and the dried preparation groups rose to the half way between the control and by day 7, and remained there till day 14, then increased and reached the control value in the dried preparation group, but not in the DHS group. The mean value of group Gran. II remained as low as that of the untreated group till day 7, then gradually increased, until it reached the control value on day 21. The mean value of group Gran. III remained as low as that of the untreated group till day 14, then sharply increased, to the control value by day 21.

TIBC (Fig. 12). decreased sharply in all treated groups and on day 7 the means did not differ from the control mean. On days 14 and 21 all values remained around the control mean.

Saturation of transferrin (Sat.) (Fig.13). Changes of Sat. were parallel with that of serum iron concentration. On day 21 only the mean of the DHS treated group differed significantly from the control mean.

ZP (Fig.14) decreased gradually in all treated groups during the 3 week period, but all mean values were still significantly higher than the control mean on day 21.

GOT activity (Fig. 15). On day 21 the mean was twice higher in the untreated group than in the control. The mean values of the treated groups were between those of the control and the untreated groups and were significantly different from both.

GPT activity (Fig. 16). On day 21 the mean was significantly lower in the untreated group than in the control.

Trigl. concentration (Fig. 16). On day 21 the mean of the untreated group was half of that of the control. Among the treated groups the mean values of the DHS and Gran.II groups were not different from that of the control, while the mean values of the other three groups were not different from that of the untreated group.

Discussion

The effectiveness of the HME preparations was tested on the iron deficient experimental rat model developed by Masini et al (1994) by rearing the dams and their offspring of iron-free diet.

The degree of iron deficiency in the offspring was characterised by the presence of severe microcytic, hypochromic anaemia (Wintrobe, 1981), hyposideraemia and high ZP indicating the lack of iron at tissue level in the

bone marrow (Labbe and Rettmer, 1989), furthermore by the decreased body mass gain and increased lethality.

During the third week of treatment, RBC sharply increased in the untreated group. This could be considered a compensational attempt of the organism induced by the critically low Hb. concentration and consequent tissue hypoxia.(Bernát, 1973). The volume (MCV) and Hb. content (MCH) of these red cells, however, remained low and characteristic to iron deficiency (microcytosis and hypochromasia).

Iron repletion to the pups was started at weaning. Effectiveness of the HME preparations was compared to the effectiveness of Aktiferrin syrup, an official medicinal preparation.

All forms of HME tested proved to be effective, there were, however, differences in the size and time course of the changes elicited by the different preparations.

The best result was achieved by treatment with DHS. Regarding the haemopoietic and hepatic effects it proved to be equal to Aktiferrin. Both preparations caused explosion-like increase in Hb. synthesis, MCV, MCH and Htk. Production of the new bigger red cells (macrocytosis), containing more Hb, resulted in reaching the normal control value for Hb. already on day 7, despite that RBC number at that time was still significantly lower than in the controls. During the first week of treatment the serum iron concentration did not reach the control level, indicating the immediate utilisation of iron absorbed. This also indicates that during the first phase of iron repletion serum iron is not the choice of parameter for evaluation of the effectiveness of treatment. Serum iron normalised only during the third week of treatment, after completion of the regeneration process. The greatly elevated serum GOT activity in the untreated group, indicating damage to liver and/or muscle cell mitochondria (Masini et al. 1994), improved similarly on the effect of both DHS and Aktiferrin. Regarding other effects, DHS proved to be superior to Aktiferrin. The decreased serum triglyceride

concentration in the iron deficient pups normalised on the effect of DHS, but not of Aktiferrin treatment. Body mass gain of the pups was also better in the DHS group, compared to the Aktiferrin treated group. This indicates that DHS had a better influence on impaired metabolic processes due to iron deficiency.

All the other HME preparations were effective in iron repletion. The main difference between them and DHS was in the time course of the processes. The improvement of the parameters was slower, more gradual, macrocytosis did not appear. Nevertheless, on day 21 the results of haematological parameters were essentially the same as with DHS or Aktiferrin. With the exception of Gran. II, these preparation had no influence on the low triglyceride level.

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Table 1

Relative food consumption of the pregnant rats

Relative food consumption of pregnant rats on normal diet*

Cage identification number	Number of pregnant rats	Day 7 of pregnancy (g)	Day 14 of pregnancy (g)	Day 21 of pregnancy (g)
1a	3	9.49	9.58	7.66
1b	3	9.63	10.62	5.34
Mean:	(n = 6)	**9.56**	**10.10**	**6.50**
± SE		± 0.07	± 0.52	± 1.16

Relative food consumption of pregnant rats on iron deficient diet*

Cage identification number	Number of pregnant rats	Day 7 of pregnancy (g)	Day 14 of pregnancy (g)	Day 21 of pregnancy (g)
2a	3	7.63	7.77	6.58
2b	3	7.65	8.33	6.77
3a	4	10.25	7.86	6.63
3b	2	8.48	8.64	6.32
4a	4	7.45	8.03	6.45
4b	1	8.12	10.71	6.45
5a	4		9.25	6.71
5b	2	8.02	7.89	5.82
6a	4	11.29		6.69
6b	2			6.59
7a	4	7.56	8.44	6.77
7b	2	9.08	8.18	6.02
Mean:	(n = 35)	8.55	* 8.51	6.48
± SE		± 0.41	± 0.28	± 0.09

* Daily food consumption: g food/100 g body mass
Mean: arrithmetic mean
± SE = ± Standard Error; *:$p < 0.05$
Shadowed areas: food was not measured due to technical causes (falling out).

Table 2

Body mass and relative body mass gain of the pregnant rats

Pregnant rats on normal diet

Cage identification number	Identification number of rats	Body mass (g)				Relative body mass gain (%)		
		Pregnancy				Pregnancy		
		Day 1	Day 7	Day 14	Day 21	Days 1 - 7	Days 7 - 14	Days 14 - 21
1a	7697	230	250	280	340	8.70	12.00	21.43
1a	7797	220	240	265	330	9.09	10.42	24.53
1a	7897	220	255	290	360	15.91	13.73	24.14
1b	10297	210	225	270	345	7.14	20.00	27.78
1b	10397	230	250	280	370	8.70	12.00	32.14
1b	10497	230	250	285	375	8.70	14.00	31.58
n = 6	átlag:	223.33	245.00	278.33	353.33	9.70	13.69	26.93
	± SE	± 3.33	± 4.47	± 3.80	± 7.26	± 1.27	± 1.37	± 1.76

Relative body mass gain: the differences of body masses measured on days 1 and 7, 7 and 14, 14 and 21, respectively, expressed as percentages of body masses measured on days 1, 7 and 14, respectively.
Mean: arrithmetic mean
± SE = ± Standard Error

Pregnant rats on iron deficient diet

Cage identification number	Identification number of rats	Body mass (g)				Relative body mass gain (%)		
		Pregnancy				Pregnancy		
		Day 1	Day 7	Day 14	Day 21	Days 1 - 7	Days 7 - 14	Days 14 - 21
2a	7997	195	220	245	305	12.82	11.36	24.49
2a	8097	235	260	290	350	10.64	11.54	20.69
2a	8197	225	250	280	355	11.11	12.00	26.79
2b	10597	230	245	285	350	6.52	16.33	22.81
2b	10697	210	220	255	340	4.76	15.91	33.33
2b	10797	210	230	275	330	9.52	19.57	20.00
3a	8297	210	225	255	320	7.14	13.33	25.49
3a	8397	230	255	280	355	10.87	9.80	26.79
3a	8497	235	250	280	360	6.38	12.00	28.57
3a	8597	205	230	260	325	12.20	13.04	25.00
3b	10897	220	240	280	345	9.09	16.67	23.21
3b	10997	215	235	270	325	9.30	14.89	20.37
4a	8697	215	230	250	320	6.98	8.70	28.00
4a	8797	225	240	270	330	6.67	12.50	22.22
4a	8897	200	225	250	320	12.50	11.11	28.00
4a	8997	240	265	280	350	10.42	5.66	25.00
4b	11197	225	240	280	365	6.67	16.67	30.36
4b	11197	225	240	280	350	10.87	9.80	25.00
5a	9097	230	255	280	305	10.53	9.52	32.61
5a	9197	190	210	230	375	12.77	13.21	25.00
5a	9297	235	265	300	350	6.38	12.00	25.00
5a	9397	235	250	280	300	10.53	21.43	17.65
5b	11297	190	210	255	315	6.82	10.64	21.15
5b	11397	220	235	260	325	16.67	8.16	22.64
6a	9497	210	245	265	380	10.42	13.21	26.67
6a	9597	240	265	300	380	10.42	13.21	26.67
6a	9697	240	265	300	325	11.90	12.77	22.64
6a	9797	210	235	265	355	6.67	18.75	24.56
6b	11497	225	240	285	325	6.82	14.89	20.37
6b	11597	220	235	270	310	13.16	13.95	26.53
7a	9897	190	215	245	320	12.82	13.64	28.00
7a	9997	195	220	250	375	8.00	14.81	20.97
7a	10097	250	270	310	345	6.82	14.89	27.78
7a	10197	220	235	270	380	15.22	13.21	26.67
7b	11697	230	265	300	340	8.70	14.00	19.30
7b	11797	230	250	285	340.00	9.69	13.23	24.87
n = 35	Mean:	219.57	240.71	272.43	± 3.89	± 0.48	± 0.55	± 0.62
	± SE	± 2.71	± 2.87	± 3.16				

Table 3

Relative food consumption of the lactating rats

Relative food consumption of lactating rats on normal diet*

Cage identification number	Day 7 of lactation (g)	Day 14 of lactation (g)	Day 21 of lactation (g)
1a	7.19	18.43	28.11
1c	5.81	18.53	28.13
1d	9.68	18.27	27.22
1e	8.39	19.05	21.96
1f	4.64	18.76	25.47
Mean:	7.14	18.61	26.18
± SE	± 0.90	± 0.14	± 1.16
n = 5			

* Daily food consumption: g food/100 g body mass.
Mean: arrithmetic mean
± SE = ± Standard Error

Table 3 (continued)

Relative food consumption of lactating rats on iron deficient diet *

Cage identification number	Day 7 of lactation (g)	Day 14 of lactation (g)	Day 21 of lactation (g)
2a	7.87	15.66	22.31
2b	6.41	18.74	32.45
2c	10.32		
2d	11.20	20.37	30.83
2e	10.37	12.76	25.28
2f	20.29	19.59	23.20
3a	7.48	16.21	22.19
3b	5.10	13.30	22.25
3c	9.02	13.77	26.07
3d	8.88	24.37	35.29
3e	8.32	14.15	31.71
3f	8.24	16.97	24.00
4a	11.67	21.02	29.93
4b	3.35		
4d	7.85	13.51	28.57
4f	6.26	14.29	18.78
5a	12.05	17.72	29.65
5b	4.17	8.20	12.32
5c	9.28	12.92	25.56
5d	8.37	12.64	20.49
5f	7.92	14.57	17.86
6a	7.02	13.33	20.83
6b	8.05	12.11	25.51
6c	9.18	13.30	27.65
6d	9.05	14.00	27.41
6e	7.89	14.02	17.75
6f	9.58	12.52	19.14
7a	10.00	14.69	15.93
7b	17.41	15.58	27.01
7c	10.04	24.94	36.87
7d	10.68	14.93	15.31
7e	7.98	12.94	23.50
7f	11.35	11.62	11.33
Mean:	9.17	16.00	22.81
± SE	± 0.56	± 2.34	± 4.49
n	33	31	31

Shadowed areas: measurement was not done due to the death of the offspring

Table 4

Body mass and relative body mass gain of the pregnant and lactating rats

Rats on normal diet

Cage identification number	Identification number of rats	Body mass (g)				Relative body mass gain (%)		
		Lactation				Lactation		
		Day 1	Day 7	Day 14	Day 21	Days 1 - 7	Days 7 - 14	Days 14 - 21
1a	7697	286	310	310	310	8.39	0.00	0.00
1c	7897	285	305	335	310	7.02	9.84	-7.46
1d	10297	266	295	315	320	10.90	6.78	1.59
1e	10397	274	305	340	330	11.31	11.48	-2.94
1f	10497	296	320	350	340	8.11	9.38	-2.86
	Mean:	281.40	307.00	330.00	322.00	9.15	7.49	-2.33
	± SE	± 5.19	± 4.06	± 7.58	± 5.83	± 0.84	± 2.02	± 1.55
	n =	5						

Relative body mass gain: the differences of body masses measured on days 1 and 7, 7 and 14, 14 and 21, respectively, expressed as percentages of body masses measured on days 1, 7 and 14, respectively.
Mean: arrithmetic mean
± SE = ± Standard Error

Table 4 (continued)

Rats on iron deficient diet

Cage identification number	Identification number of rats	Body mass (g)				Relative body mass gain (%)		
		Lactation				Lactation		
		Day 1	Day 7	Day 14	Day 21	Days 1 - 7	Days 7 - 14	Days 14 - 21
2a	7997	240	250	270	255	4.17	8.00	-5.56
2b	8097	302	300	310	280	-0.66	3.33	-9.68
2c	8197	270	270			0.00		
2d	10597	232	240	265	240	3.45	10.42	-9.43
2e	10697	281	270	290	275	-3.91	7.41	-5.17
2f	10797	270	265	260	245	-1.85	-1.89	-5.77
3a	8297	239	265	255	260	10.88	-3.77	1.96
3b	8397	270	290	290	275	7.41	0.00	-5.17
3c	8497	285	285	265	250	0.00	-7.02	-5.66
3d	8597	265	250	260	250	-5.66	4.00	-3.85
3e	10897	272	260	265	235	-4.41	1.92	-11.32
3f	10997	258	245	260	240	-5.04	6.12	-7.69
4a	8697	252	250	280	245	-0.79	12.00	-12.50
4b	8797	257	255			-0.78		
4d	8997	284	280	270	265	-1.41	-3.57	-1.85
4f	11197	272	230	270	255	-15.44	17.39	-5.56
5a	9097	287	270	270	260	-5.92	0.00	-3.70
5b	9197	235	245	260	250	4.26	6.12	-3.85
5c	9297	300	285	290	280	-5.00	1.75	-3.45
5d	9397	272	240	280	250	-11.76	16.67	-10.71
5f	11397	260	245	265	255	-5.77	8.16	-3.77
6a	9497	274	255	270	265	-6.93	5.88	-1.85
6b	9597	288	280	310	250	-2.78	10.71	-19.35
6c	9697	290	270	310	310	-6.90	14.81	0.00
6d	9797	260	245	255	235	-5.77	4.08	-7.84
6e	11497	265	260	270	245	-1.89	3.85	-9.26
6f	11597	267	255	270	245	-4.49	5.88	-9.26
7a	9897	245	255	270	250	4.08	5.88	-7.41
7b	9997	255	270	280	270	5.88	3.70	-3.57
7c	10097	294	275	315	270	-6.46	14.55	-14.29
7d	10197	270	265	290	270	-1.85	9.43	-6.90
7e	11697	277	260	270	265	-6.14	3.85	-1.85
7f	11797	241	275	315	290	14.11	14.55	-7.94
	Mean:	267.55	262.27	277.42	259.03	*** -1.74	5.94	* -6.52
	± SE	± 3.23	± 2.82	± 3.28	± 3.04	± 1.06	± 1.10	± 0.79
n =		33	33	31	31	33	31	31

Shadowed areas: measurement was not done due to the death of the offspring

* :$p < 0.05$; ***:$p < 0.001$

Table 5

Litter characteristics
(litter size, weight, body mass)

Control (offspring of rats on normal diet)

	Litter size				Total litter weight (g)				Mean body mass of the offspring (g)			
	Postnatal days				Postnatal days				Postnatal days			
Box identification number	1	7	14	21	1	7	14	21	1	7	14	21
1a	13	13	13	13	88	175	300	475	6.77	13.46	23.08	36.54
1c	11	11	11	11	73	150	250	425	6.64	13.64	25.45	38.64
1d	12	12	12	12	84	151	280	425	7.00	12.58	23.33	35.42
1e	14	13	13	13	99	165	300	450	7.07	12.69	23.08	34.62
1f	14	13	13	13	88	160	300	445	6.29	12.31	23.08	34.23
Total No of offspring	64	62	62	62								
Mean:	12.80	12.40	12.40	12.40	86.40	160.10	292.00	444.00	6.75	11.94	23.60	35.89
± SE	± 0.58	± 0.40	± 0.40	± 0.40	± 4.18	± 4.64	± 4.90	± 9.27	± 0.14	± 0.26	± 0.47	± 0.79
No of litters	6	6	6	6								

Offspring of rats on iron deficient diet

	Litter size				Total litter weight (g)				Mean body mass of the offspring (g)			
	Postnatal days				Postnatal days				Postnatal days			
Box identification number	1	7	14	21	1	7	14	21	1	7	14	21
2a	13	13	13	13	73	120	215	245	5.62	9.23	16.54	18.85
2b	9	9	9	9	53	103	200	270	5.89	11.44	22.22	30.00
2c	13	2			74	9			5.69	4.50		
2d	15	14	14	14	83	140	250	300	5.53	10.00	17.86	21.43
2e	15	11	11	11	82	115	215	270	5.47	10.45	19.55	24.55
2f	12	12	12	12	80	110	225	300	6.67	9.17	18.75	25.00
3a	14	12	12	9	65	100	170	70	4.64	8.33	14.17	14.00
3b	14	12	12	12	80	100	210	260	5.71	8.33	17.50	21.67
3c	15	14	14	14	80	147	205	270	5.33	10.50	14.64	19.29
3d	12	12	12	12	70	140	200	295	5.83	11.67	16.67	24.58
3e	13	11	12	12	79	100	210	240	6.08	9.09	17.50	20.00
3f	12	12	12	11	72	130	210	225	6.00	10.83	17.50	20.45
4a	12	12	12	11	75	155	220	265	6.25	12.92	18.33	24.09
4b	12	5			55	19			4.58	3.80		
4d	14	14	13	13	64	123	190	250	4.57	8.79	14.62	19.23
4f	15	15	15	15	86	145	245	260	5.73	9.67	16.33	17.33
5a	12	12	12	12	75	165	240	310	6.25	13.75	20.00	25.83
5b	14	7	7		72	57	90		5.14	8.14	12.86	
5c	15	14	14	14	81	135	200	250	5.40	9.64	14.29	17.86
5d	15	15	15	15	80	150	220	260	5.33	10.00	14.67	17.33
5e	4				30				5.00			
5f	11	10	10	10	67	104	190	240	6.09	10.40	19.00	24.00
6a	12	12	12	10	69	129	190	190	5.75	10.75	15.83	19.00
6b	16	14	13	13	87	110	185	215	5.44	7.86	14.23	16.54
6c	12	11	10	8	92	94	170	170	5.11	8.55	17.00	21.25
6d	11	11	11	11	65	133	190	270	5.91	12.09	17.27	24.55
6e	14	14	14	14	83	140	245	295	5.93	10.00	17.50	21.07
6f	9	9	9	8	53	96	195	230	5.67	10.67	21.67	28.75
7a	14	12	12		70	103	165	60	5.00	8.58	13.75	12.00
7b	13	12	12		68	100	160		5.23	8.33	13.33	
7c	15	15	15	14	87	155	245	270	5.80	10.33	16.33	19.29
7d	13	11	11	11	65	130	210	245	5.00	11.82	19.09	22.27
7e	15	15	15	15	84	140	225	300	5.60	9.33	15.00	20.00
7f	12	11	3	3	74	97	65	70	6.17	8.82	21.67	23.33
Total No of offspring	463	385	368	327								
Mean:	13.03	11.67	11.97	11.28	72.38	114.97	198.39	237.76	5.57	9.63	16.96	21.16
± SE	± 0.42	± 0.50	± 0.45	± 0.57	± 2.31	± 6.09	± 7.23	± 12.49	± 0.09	± 0.35	± 0.45	± 0.74
No of litters	34	33	31	29								

The died litters are represented by shadowed areas.

Table 6

The effect of iron deficiency on the postnatal development of rat pups

	No of litters	Offspring died during lactation (%)		Perinatal index % *		Survival index % **	
	n	Mean	± SE	Mean	± SE	Mean	± SE
Control	6	19.05	7.1	81.00	16.26	83.33	16.67
Iron deficient	35	29.41	6.42	85.37	4.32	73.63	6.85

*: Perinatal Index = $\frac{\text{No of surviving offspring on day 5}}{\text{No of newborns}} \times 100$

**: Survival index = $\frac{\text{No of surviving offspring on day 21}}{\text{No of surviving offspring on day 5}} \times 100$

Body mass and relative body mass gain of offspring during the treatment period

Offspring on normal diet

Identification number of boxes	Individual marking of the offspring	Body mass (g) Treatment Day 1	Day 7	Day 14	Day 21	Relative body mass gain (%) Treatment Days 1 - 7	Days 7 - 14	Days 14 - 21
7a	if-jm3-jh1	42	66	110		57.14	66.67	
7a	if-jm3-jh2	48	60			25.00		
7a	if-jm3-jh3	41	74			80.49		
7a	if-jm3-jh4	47	72			53.19		
7a	if-jm3-jh5	35	61			74.29		
7a	if-jm3-bh1	37	64	115		72.97	79.69	
7a	if-jm3-bh2	42	72	120		71.43	66.67	
7a	if-jm3-bh3	41	60	96		46.34	60.00	
7a	if-jm3-bh4	36	66			83.33		
7a	if-jm3-bh5	36	62	108		72.22	74.19	
7b	if-jm4-jh1	36	45	82	125	25.00	82.22	52.44
7b	if-jm4-jh2	38	47	96	150	23.68	104.26	56.25
7b	if-jm4-jh3	45	54	98	140	20.00	81.48	42.86
7b	if-jm4-jh4	49	60	110	170	22.45	83.33	54.55
7b	if-jm4-jh5	36	45	90	135	25.00	100.00	50.00
7b	if-jm4-bh1	38	46	95	155	21.05	106.52	63.16
7b	if-jm4-bh2	47	57	106	165	21.28	85.96	55.66
7b	if-jm4-bh3	29	35	76	115	20.69	117.14	51.32
7b	if-jm4-bh4	40	46	95	155	15.00	106.52	63.16
7b	if-jm4-bh5	52	61	115	180	17.31	88.52	56.52
7b	if-jm4-bh3	42	85	130	163	102.38	52.94	25.38
7c	if-bm1-jh1	42	85	130	163	102.38	52.94	25.38
7c	if-bm1-jh2	46	87	140	170	89.13	60.92	21.43
7c	if-bm1-jh3	32	65	105		103.13	61.54	
7c	if-bm1-jh4	47	83	132	162	76.60	59.04	22.73
7c	if-bm1-jh5	40	82	130	167	105.00	58.54	28.46
7c	if-bm1-bh1	34	65	110	147	91.18	69.23	33.64
7c	if-bm1-bh2	44	85	135	165	93.18	58.82	22.22
7c	if-bm1-bh3	35	70	110		100.00	57.14	
7c	if-bm1-bh4	45	79	112		75.56	41.77	
7c	if-bm1-bh5	35	74	130	166	111.43	75.68	27.69
7d	if-bm2-jh1	40	75	*130		87.50	73.33	
7d	if-bm2-jh2	46	73			58.70		
7d	if-bm2-jh3	45	77	124		71.11	61.04	
7d	if-bm2-jh4	39	70	120		79.49	71.43	
7d	if-bm2-jh5	41	76	129		85.37	69.74	
7d	if-bm2-bh1	40	71	114		77.50	60.56	
7d	if-bm2-bh2	38	66	108		73.68	63.64	
7d	if-bm2-bh3	49	82			67.35		
7d	if-bm2-bh4	49	82	130		67.35	58.54	
7d	if-bm2-bh5	43	79	129		83.72	63.29	
	mean:	40.74	66.26	112.03	154.71	62.22	74.98	42.79
	± SE	± 0.90	± 2.09	± 3.07	± 4.23	± 5.30	± 3.41	± 3.75
	n =	40	40	33	17	40	33	17

Relative body mass gain: the differences of body masses measured on days 1 and 7, 7 and 14, 14 and 21, respectively, expressed as percentages of body masses measured on days 1, 7 and 14, respectively.

Mean: arrithmetic mean

± SE = ± Standard Error

Empty boxes: animals used for clinical laboratory examinations

Table 7b

Offspring on iron deficient diet

Identification number of boxes	Individual marking of the offspring	Body mass (g) Treatment Day 1	Day 7	Day 14	Day 21	Relative body mass gain (%) Treatment Days 1 - 7	Days 7 - 14	Days 14 - 21
6a	bm3-4-jh1	27	33			22.22		
6a	bm3-4-jh2	26	38			46.15		
6a	bm3-4-jh3	20	26	35	37	30.00	34.62	5.71
6a	bm3-4-jh4	21	29			38.10		
6a	bm3-4-jh5	21	33			57.14		
6a	bm3-4-bh1	15	18	30	24	20.00	66.67	-20.00
6a	bm3-4-bh2	20	25	38	31	25.00	52.00	-18.42
6a	bm3-4-bh3	26	39			50.00		
6a	bm3-4-bh4	19	26	40	46	36.84	53.85	15.00
6a	bm3-4-bh5	20	27			35.00		
6b	jf-jh1	26	37	54		42.31	45.95	
6b	jf-jh2	17	25	35	35	47.06	40.00	0.00
6b	jf-jh3	21	28	37		33.33	32.14	
6b	jf-jh4	22	31	45		40.91	45.16	
6b	jf-jh5	19	27	36		42.11	33.33	
6b	jf-bh1	20	30	47	47	50.00	56.67	0.00
6b	jf-bh2	22	28	37		27.27	32.14	
6b	jf-bh3	20	26	35	34	30.00	34.62	-2.86
6b	jf-bh4	26	33	50		26.92	51.52	
6b	jf-bh5	21	31	44		47.62	41.94	
6c	jf-jm1-jh1	26	39	65	55	50.00	66.67	-15.38
6c	jf-jm1-jh2	27	43	82	66	59.26	90.70	-19.51
6c	jf-jm1-jh3	25	33	64		32.00	93.94	
6c	jf-jm1-jh4	28	40	75		42.86	87.50	
6c	jf-jm1-jh5	22	33	40	29	50.00	21.21	-27.50
6c	jf-jm1-bh1	23	32	50		39.13	56.25	
6c	jf-jm1-bh2	22	34	72		54.55	111.76	
6c	jf-jm1-bh3	17	23	34		35.29	47.83	
6c	jf-jm1-bh4	19	31	60	52	63.16	93.55	-13.33
6c	jf-jm1-bh5	26	45	88	71	73.08	95.56	-19.32
6d	jf-jm2-jh1	22	26	48		18.18	84.62	
6d	jf-jm2-jh2	26	42			61.54		
6d	jf-jm2-jh3	21	29	55		38.10	89.66	
6d	jf-jm2-jh4	26	36	57	52	38.46	58.33	-8.77
6d	jf-jm2-jh5	24	30	67	55	25.00	123.33	-17.91
6d	jf-jm2-bh1	27	44	70		62.96	59.09	
6d	jf-jm2-bh2	28	36			28.57		
6d	jf-jm2-bh3	18	26	38		44.44	46.15	
6d	jf-jm2-bh4	21	32	64	53	52.38	100.00	-17.19
6d	jf-jm2-bh5	26	38	70	62	46.15	84.21	-11.43
	mean:	22.37	32.05	50.71	45.29	40.82	62.28	-10.68
	± SE	± 0.56	± 1.04	± 2.99	± 3.78	± 2.22	± 5.13	± 3.22
	n =	40	40	32	16	40	32	16

Relative body mass gain: the differences of body masses measured on days 1 and 7, 7 and 14, 14 and 21, respectively, expressed as percentages of body masses measured on days 1, 7 and 14, respectively.
Mean: arrithmetic mean
± SE = ± Standard Error
Empty boxes: animals used for clinical laboratory examinations

Table 7c

Offspring on iron deficient diet, treated with Aktiferrin

Identification number of boxes	Individual marking of the offspring	Body mass (g) Treatment Day 1	Day 7	Day 14	Day 21	Relative body mass gain (%) Treatment Days 1 - 7	Days 7 - 14	Days 14 - 21
5a	bm1-3-jh1	24	40	76		66.67	90.00	
5a	bm1-3-jh2	30	53	90	131	76.67	69.81	45.56
5a	bm1-3-jh3	30	48	86		60.00	79.17	
5a	bm1-3-jh4	28	51	87		82.14	70.59	
5a	bm1-3-jh5	26	45	82	117	73.08	82.22	42.68
5a	bm1-3-bh1	22	40	78		81.82	95.00	
5a	bm1-3-bh2	18	33	65	105	83.33	96.97	61.54
5a	bm1-3-bh3	24	43	85	122	79.17	97.67	43.53
5a	bm1-3-bh4	24	42	80	119	75.00	90.48	48.75
5a	bm1-3-bh5	25	42	82	118	68.00	95.24	43.90
5b	bm1-4-jh1	25						
5b	bm1-4-jh2	20	34			70.00		
5b	bm1-4-jh3	25	42			68.00		
5b	bm1-4-jh4	18	29			61.11		
5b	bm1-4-jh5	19	32			68.42		
5b	bm1-4-bh1	20	33	78	109	65.00	136.36	39.74
5b	bm1-4-bh2	24	39	75		62.50	92.31	
5b	bm1-4-bh3	20	35			75.00		
5b	bm1-4-bh4	20	31			55.00		
5b	bm1-4-bh5	19	39	72	120	105.26	84.62	66.67
5c	bm2-3-jh1	25	48			92.00		
5c	bm2-3-jh2	18	36	72		100.00	100.00	
5c	bm2-3-jh3	20						
5c	bm2-3-jh4	28	54	95	133	92.86	75.93	40.00
5c	bm2-3-jh5	19						
5c	bm2-3-bh1	17	33			94.12		
5c	bm2-3-bh2	18	38	75	108	111.11	97.37	44.00
5c	bm2-3-bh3	19	36	70		89.47	94.44	
5c	bm2-3-bh4	17	30			76.47		
5c	bm2-3-bh5	15	29	55	90	93.33	89.66	63.64
5d	bm2-4-jh1	18	37	70	101	105.56	89.19	44.29
5d	bm2-4-jh2	17	32	64	100	88.24	100.00	56.25
5d	bm2-4-jh3	20	37	71		85.00	91.89	
5d	bm2-4-jh4	18	36	70		100.00	94.44	
5d	bm2-4-jh5	18	36	66	97	100.00	83.33	46.97
5d	bm2-4-bh1	24	46	85		91.67	84.78	
5d	bm2-4-bh2	25	42			68.00		
5d	bm2-4-bh3	20						
5d	bm2-4-bh4	33						
5d	bm2-4-bh5	25	49	93	126	96.00	89.80	35.48
	mean:	21.37	38.53	75.83	112.14	81.39	91.16	48.20
	± SE	± 0.68	± 1.16	± 1.95	± 3.42	± 2.66	± 2.75	± 2.43
	n =	40	35	25	15	35	25	15

Relative body mass gain: the differences of body masses measured on days 1 and 7, 7 and 14, 14 and 21, respectively, expressed as percentages of body masses measured on days 1, 7 and 14, respectively.

Mean: arithmetic mean

± SE = ± Standard Error

Empty boxes: animals used for clinical laboratory examinations

Table 7c

Offspring on iron deficient diet, treated with Aktiferrin

Identification number of boxes	Individual marking of the offspring	Body mass (g) Treatment Day 1	Day 7	Day 14	Day 21	Relative body mass gain (%) Treatment Days 1 - 7	Days 7 - 14	Days 14 - 21
5a	bm1-3-jh1	24	40	76		66.67	90.00	
5a	bm1-3-jh2	30	53	90	131	76.67	69.81	45.56
5a	bm1-3-jh3	30	48	86		60.00	79.17	
5a	bm1-3-jh4	28	51	87		82.14	70.59	
5a	bm1-3-jh5	26	45	82	117	73.08	82.22	42.68
5a	bm1-3-bh1	22	40	78		81.82	95.00	
5a	bm1-3-bh2	18	33	65	105	83.33	96.97	61.54
5a	bm1-3-bh3	24	43	85	122	79.17	97.67	43.53
5a	bm1-3-bh4	24	42	80	119	75.00	90.48	48.75
5a	bm1-3-bh5	25	42	82	118	68.00	95.24	43.90
5b	bm1-4-jh1	25						
5b	bm1-4-jh2	20	34			70.00		
5b	bm1-4-jh3	25	42			68.00		
5b	bm1-4-jh4	18	29			61.11		
5b	bm1-4-jh5	19	32			68.42		
5b	bm1-4-bh1	20	33	78	109	65.00	136.36	39.74
5b	bm1-4-bh2	24	39	75		62.50	92.31	
5b	bm1-4-bh3	20	35			75.00		
5b	bm1-4-bh4	20	31			55.00		
5b	bm1-4-bh5	19	39	72	120	105.26	84.62	66.67
5c	bm2-3-jh1	25	48			92.00		
5c	bm2-3-jh2	18	36	72		100.00	100.00	
5c	bm2-3-jh3	20						
5c	bm2-3-jh4	28	54	95	133	92.86	75.93	40.00
5c	bm2-3-jh5	19						
5c	bm2-3-bh1	17	33			94.12		
5c	bm2-3-bh2	18	38	75	108	111.11	97.37	44.00
5c	bm2-3-bh3	19	36	70		89.47	94.44	
5c	bm2-3-bh4	17	30			76.47		
5c	bm2-3-bh5	15	29	55	90	93.33	89.66	63.64
5d	bm2-4-jh1	18	37	70	101	105.56	89.19	44.29
5d	bm2-4-jh2	17	32	64	100	88.24	100.00	56.25
5d	bm2-4-jh3	20	37	71		85.00	91.89	
5d	bm2-4-jh4	18	36	70		100.00	94.44	
5d	bm2-4-jh5	18	36	66	97	100.00	83.33	46.97
5d	bm2-4-bh1	24	46	85		91.67	84.78	
5d	bm2-4-bh2	25	42			68.00		
5d	bm2-4-bh3	20						
5d	bm2-4-bh4	33						
5d	bm2-4-bh5	25	49	93	126	96.00	89.80	35.48
	mean:	21.37	38.53	75.83	112.14	81.39	91.16	48.20
	± SE	± 0.68	± 1.16	± 1.95	± 3.42	± 2.66	± 2.75	± 2.43
	n =	40	35	25	15	35	25	15

Relative body mass gain: the differences of body masses measured on days 1 and 7, 7 and 14, 14 and 21, respectively, expressed as percentages of body masses measured on days 1, 7 and 14, respectively.
Mean: arithmetic mean
± SE = ± Standard Error
Empty boxes: animals used for clinical laboratory examinations

Table 7f

Offspring on iron deficient diet, treated with Granulatum II

Identification number of boxes	Individual marking of the offspring	Body mass (g) Treatment Day 1	Day 7	Day 14	Day 21	Relative body mass gain (%) Treatment Days 1 - 7	Days 7 - 14	Days 14 - 21
2a	jm4-jh1	21						
2a	jm4-jh2	20	32			60.00		
2a	jm3-jh3	25	40			60.00		
2a	jm4-jh4	29	44	74		51.72	68.18	
2a	jm4-jh5	22	27			22.73		
2a	jm4-bh1	23	33			43.48		
2a	jm4-bh2	26						
2a	jm4-bh3	24	37			54.17		
2a	jm4-bh4	27	45	76		66.67	68.89	
2a	jm4-bh5	28	39			39.29		
2b	bm1-jh1	28	44	70	122	57.14	59.09	74.29
2b	bm1-jh2	25	42	68		68.00	61.90	
2b	bm1jh3	27	42	71	115	55.56	69.05	61.97
2b	bm1-jh4	24	34	59		41.67	73.53	
2b	bm1-jh5	20	27	47	92	35.00	74.07	95.74
2b	bm1-bh1	30	48			60.00		
2b	bm1-bh2	20	32	51		60.00	59.38	
2b	bm1-bm3	22	39			77.27		
2b	bm1-bh4	24	39	63	109	62.50	61.54	73.02
2b	bm1-bh5	20						
2c	bm2-jh1	20	32			60.00		
2c	bm2-jh2	25						
2c	bm2-jh3	22	38	75	107	72.73	97.37	42.67
2c	bm2-jh4	21	36	65		71.43	80.56	
2c	bm2-jh5	24						
2c	bm2-bh1	21	32	62	96	52.38	93.75	54.84
2c	bm2-bh2	20						
2c	bm2-bh3	22	36			63.64		
2c	bm2-bh4	16	28			75.00		
2c	bm2-bh5	22	40	74		81.82	85.00	
2d	bm3-jh1	22	43	78	98	95.45	81.40	25.64
2d	bm3-jh2	17	33	64	100	94.12	93.94	56.25
2d	bm3-jh3	23	42	75		82.61	78.57	
2d	bm3-jh4	20	31	52	78	55.00	67.74	50.00
2d	bm3-jh5	21						
2d	bm3-bh1	21	37	68	95	76.19	83.78	39.71
2d	bm3-bh2	19	35	64	88	84.21	82.86	37.50
2d	bm3-bh3	20	27	49		35.00	81.48	
2d	bm3-bh4	21	37	65	100	76.19	75.68	53.85
2d	bm3-bh5	21	43	77		104.76	79.07	
	mean:	22.89	36.97	66.12	101.83	61.41	74.94	55.46
	± SE	± 0.55	± 1.04	± 2.30	± 4.37	± 3.16	± 2.98	± 6.74
	n =	40	33	22	12	33	22	12

Relative body mass gain: the differences of body masses measured on days 1 and 7, 7 and 14, 14 and 21, respectively, expressed as percentages of body masses measured on days 1, 7 and 14, respectively.
Mean: arrithmetic mean
± SE = ± Standard Error
Empty boxes: animals used for clinical laboratory examinations

Table 7g

Offspring on iron deficient diet, treated with DHS

Identification number of boxes	Individual marking of the offspring	Body mass (g) Treatment Day 1	Day 7	Day 14	Day 21	Relative body mass gain (%) Treatment Days 1 - 7	Days 7 - 14	Days 14 - 21
1a	jh1	31						
1a	jh2	32	57	103		78.13	80.70	
1a	jh3	30	51	93		70.00	82.35	
1a	jh4	24	45	82		87.50	82.22	
1a	jh5	22	32			45.45		
1a	bh1	19	35	69	120	84.21	97.14	73.91
1a	bh2	18	30			66.67		
1a	bh3	20	36	70		80.00	94.44	
1a	bh4	18	29			61.11		
1a	bh5	20						
1b	jm1-jh1	17	35	64	115	105.88	82.86	79.69
1b	jm1-jh2	19	31	59	103	63.16	90.32	74.58
1b	jm1-jh3	20	35	65	109	75.00	85.71	67.69
1b	jm1-jh4	19	35	67	103	84.21	91.43	53.73
1b	jm1-jh5	18						
1b	jm1-bh1	15	26			73.33		
1b	jm1-bh2	20	33	62		65.00	87.88	
1b	jm1-bh3	20	33	64	95	65.00	93.94	48.44
1b	jm1-bm4-bh	17	30			76.47		
1b	jm1-bh5	18	31			72.22		
1c	jm2-jh1	21						
1c	jm2-jh2	30	58			93.33		
1c	jm2-jh3	17	34	63		100.00	85.29	
1c	jm2-jh4	19	38	67	99	100.00	76.32	47.76
1c	jm2-jh5	26	48	80		84.62	66.67	
1c	jm2-bh1	18	37	64		105.56	72.97	
1c	jm2-bh2	21	45	76	104	114.29	68.89	36.84
1c	jm2-bh3	22	46	76	111	109.09	65.22	46.05
1c	jm2-bh4	19						
1c	jm2-bh5	23	42	71	105	82.61	69.05	47.89
1d	jm3-jh1	23	35	69	98	52.17	97.14	42.03
1d	jm3-jh2	19	30			57.89		
1d	jm3-jh3	20	36			80.00		
1d	jm3-jh4	21	36	63	90	71.43	75.00	42.86
1d	jm3-jh5	27	48	83		77.78	72.92	
1d	jm3-bh1	19	33	63	101	73.68	90.91	60.32
1d	jm3-bh2	19	36	62	96	89.47	72.22	54.84
1d	jm3-bh3	24	42	78		75.00	85.71	
1d	jm3-bh4	22	23			4.55		
1d	jm3-bh5	18	32	57	96	77.78	78.13	68.42
	mean:	21.23	37.74	71.98	104.33	79.40	81.83	56.34
	± SE	± 0.72	± 1.47	± 2.42	± 2.45	± 3.12	± 2.22	± 4.25
	n =	40	35	25	15	35	25	15

Relative body mass gain: the differences of body masses measured on days 1 and 7, 7 and 14, 14 and 21, respectively, expressed as percentages of body masses measured on days 1, 7 and 14, respectively.

Mean: arithmetic mean

± SE = ± Standard Error

Empty boxes: animals used for clinical laboratory examinations

Table 9

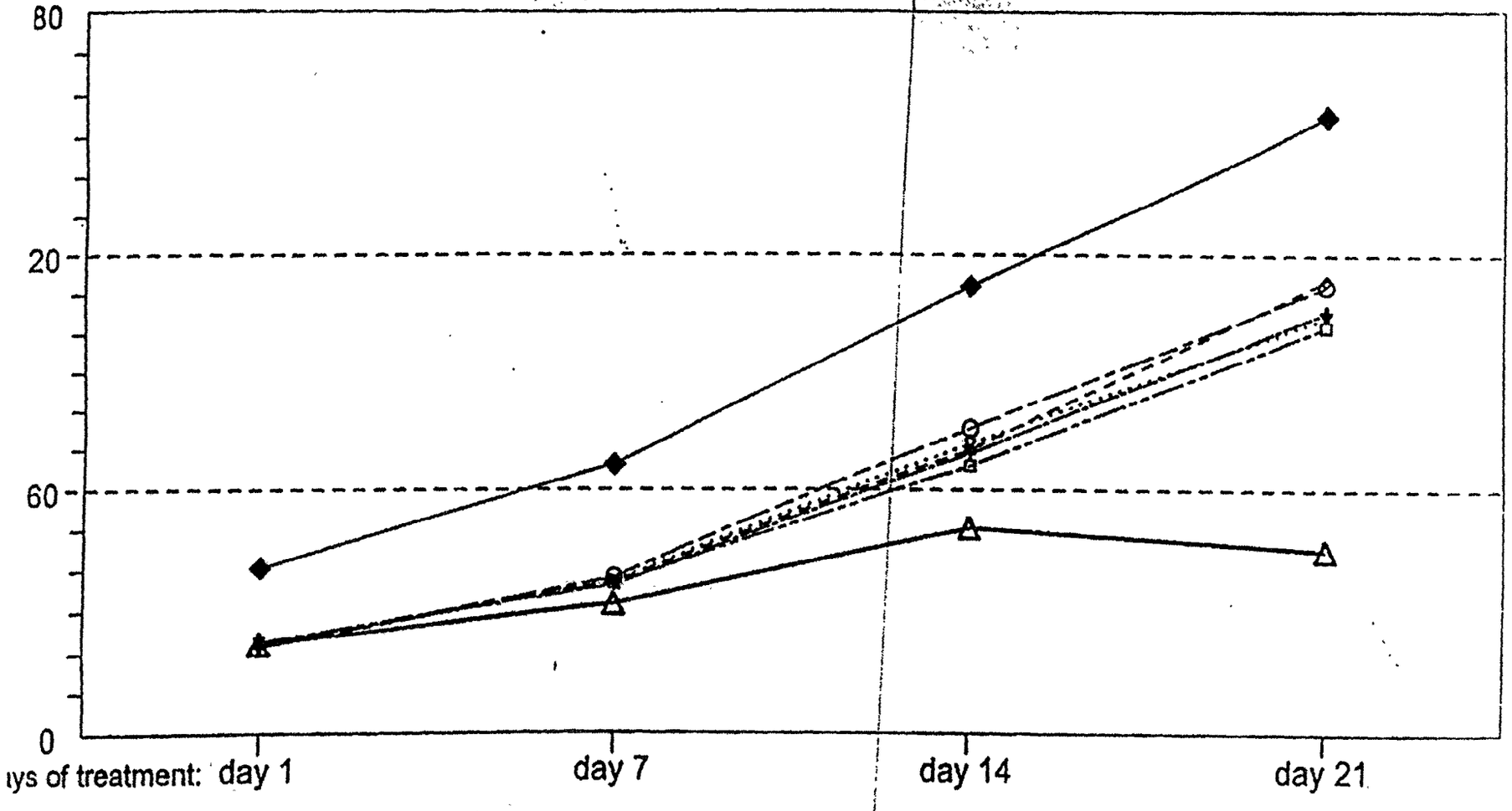
hanges in clinical laboratory parameters of iron deficient rat pups during iron repletion by different humic acid preparation

Values measured on day 14 of treatment

upe 10	RBC T/l		Hk g/100 ml		Htk %		MCV fl		Plt G/l		ZP µmol/mol hem		Fe µmol/l		TIBC µmol/l		Sat %		WBC G/l		MCH pg		MCHC g/100 ml	
	mean	± SE	mean	± SE	mean	± SE	mean	± SE	mean	± SE	mean	± SE	mean	± SE	mean	± SE	mean	± SE	mean	± SE	mean	± SE	mean	± SE
Control	5.09	± 0.10	11.09	± 0.11	33.67	± 0.50	65.60	± 0.82	687.70	± 16.35	67.80	± 1.86	77.92	± 1.41	83.46	± 2.67	94.09	± 2.94	4.07	± 0.24	22.15	± 0.41	33.27	± 0.47
Untreated	2.89	± 0.15	3.76	± 0.34	11.80	± 1.16	39.60	± 1.71	789.50	± 74.43	213.80	± 14.89	8.95	± 2.56	177.50	± 27.41	6.13	± 2.04	1.40	± 0.15	13.07	± 0.55	32.63	± 0.95
Hkb ferrin	* △△△ 4.80	± 0.06	△△△ 11.21	± 0.13	△△△ 34.52	± 0.59	** △△△ 71.40	± 1.37	631.60	± 34.12	** △△△ 86.80	± 4.71	*** 18.86	± 4.86	△△ 86.67	± 6.69	*** △ 23.38	± 8.12	*** △ 2.40	± 0.34	* △△△ 23.49	± 0.35	32.77	± 0.26
DHS	*** △△△ 4.46	± 0.08	△△△ 10.69	± 0.20	△△△ 32.20	± 0.57	*** △△△ 71.70	± 0.70	683.50	± 30.64	*** △△△ 88.20	± 2.97	*** △△△ 30.36	± 2.82	* △△ 70.9[illegible]	± 3.72	*** △△△ 43.85	± 4.80	* △△△ 3.05	± 0.29	*** △△△ 24.19	± 0.32	33.50	± 0.48
Dried Preparation	** △△△ 4.71	± 0.08	** △△△ 9.38	± 0.48	* △△△ 30.04	± 1.30	△△△ 63.30	± 2.20	708.60	± 40.84	*** △△△ 134.10	± 12.13	*** △△ 26.99	± 5.39	△△ 89.68	± 4.50	*** △ 32.71	± 6.96	** △△△ 3.31	± 0.29	* △△△ 20.04	± 0.86	31.38	± 0.33
Grasa III.	△△△ 4.80	± 0.15	*** △△△ 8.29	± 0.54	** △△△ 27.82	± 1.48	*** △△△ 57.40	± 1.83	684.80	± 43.32	*** △ 159.40	± 15.04	*** 14.59	± 3.70	* △ 109.74	± 10.45	*** 15.49	± 4.48	△△△ 3.33	± 0.37	*** △△△ 17.42	± 0.70	29.89	± 0.45
Gram II.	△△△ 4.82	± 0.09	** △△△ 9.25	± 0.57	* △△△ 29.70	± 1.70	△△△ 60.90	± 2.69	710.80	± 43.85	*** △△△ 129.80	± 12.48	*** △△ 27.70	± 5.60	117.64	± 24.13	29.37	± 6.13	△△△ 3.86	± 0.44	* △△△ 19.27	± 0.93	31.47	± 0.64

*: $p < 0.05$ compared to controls on day 14 △ : $p < 0.05$ compared to the untreated group on day 14
**: $p < 0.01$ - " - △△: $p < 0.01$ - " -
***: $p < 0.001$ - " - △△△: $p < 0.001$ - " -

Body mass changes of the offspring during the treatment period



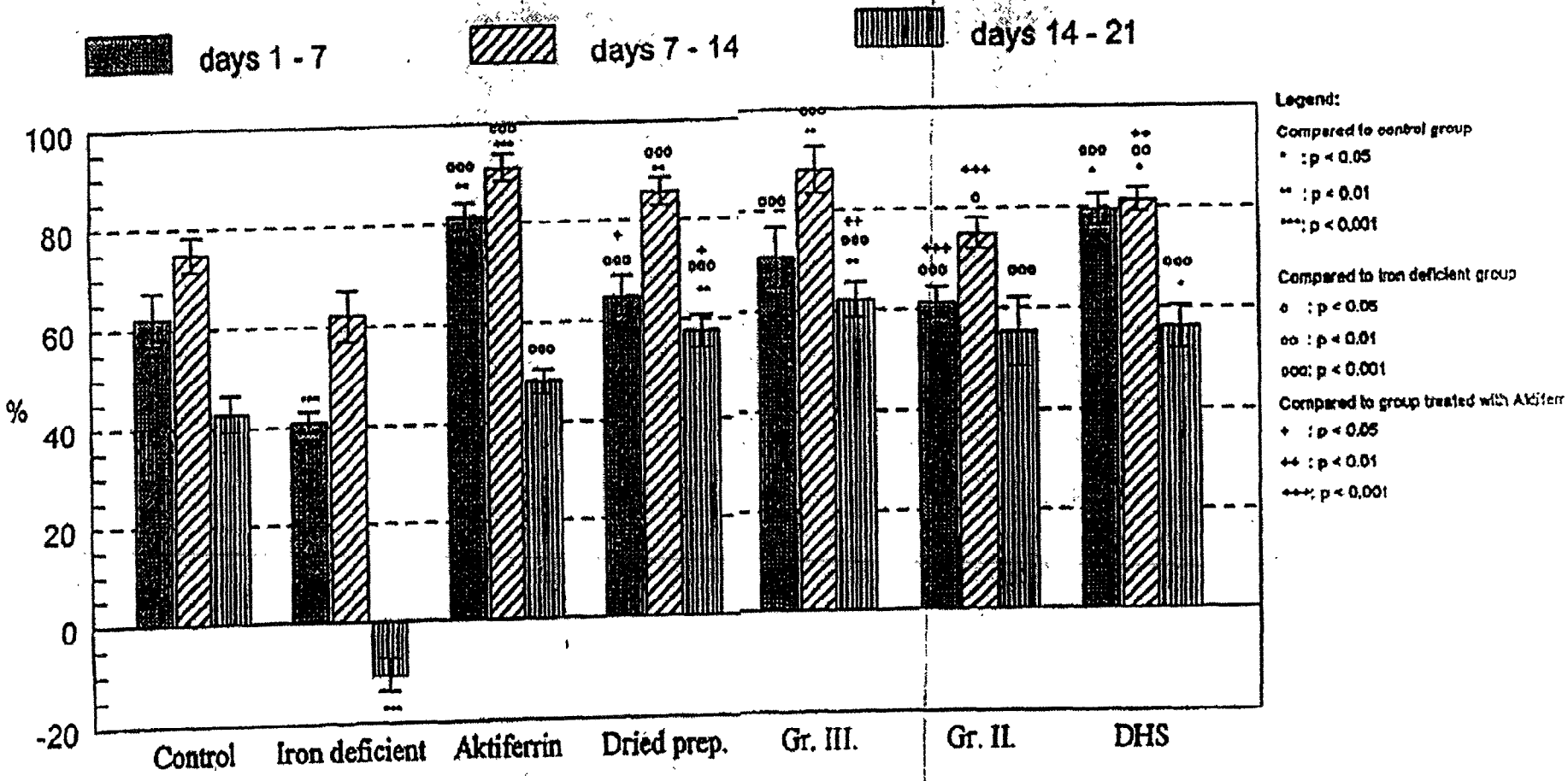
Control — Iron deficient — Aktiferrin — Dried preparation

Gr. III. — Gr. II. — DHS

The individual points represent the mean body mass values

Figure 2

Relative body mass gain of the offspring during the treatment period



Columns represent the arithmetic mean and ± SE values

Figure 3

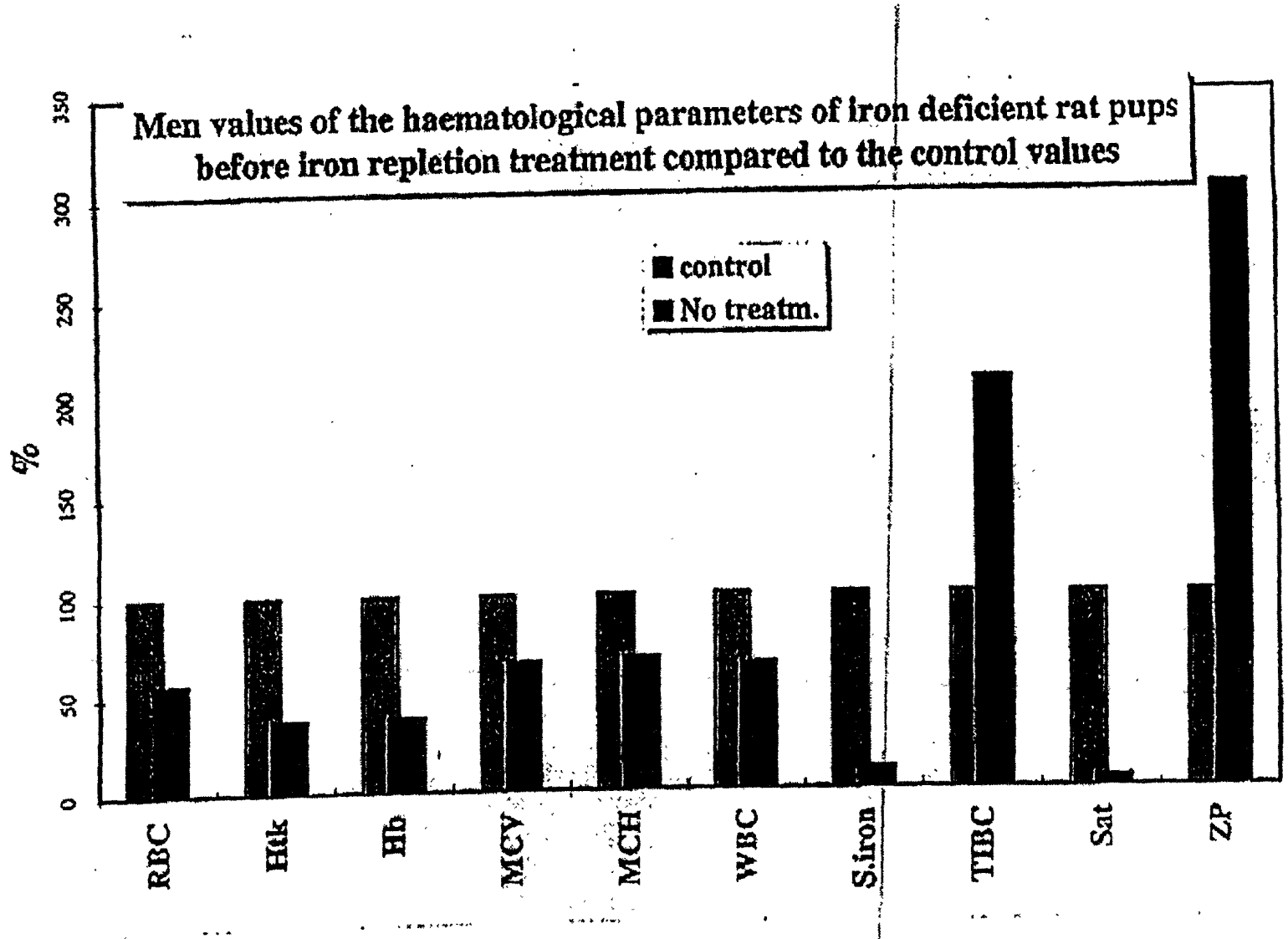

Men values of the haematological parameters of iron deficient rat pups before iron repletion treatment compared to the control values
control
No treatm.
%
0
50
100
150
200
250
300
350
RBC
Htk
Hb
MCV
MCH
WBC
S.iron
TIBC
Sat
ZP

Fig. 4

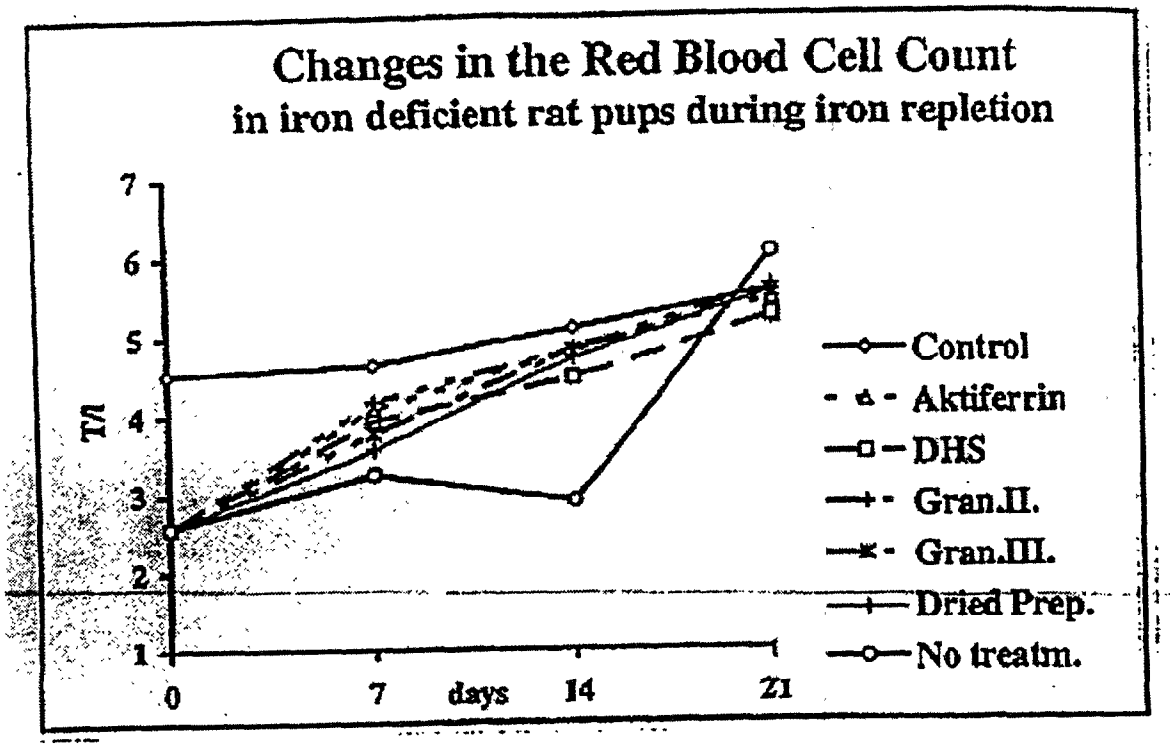

Changes in the Red Blood Cell Count
in iron deficient rat pups during iron repletion
T/l
7
6
5
4
3
2
1
0
7
days
14
21
Control
Aktiferrin
DHS
Gran.II.
Gran.III.
Dried Prep.
No treatm.

Fig. 5

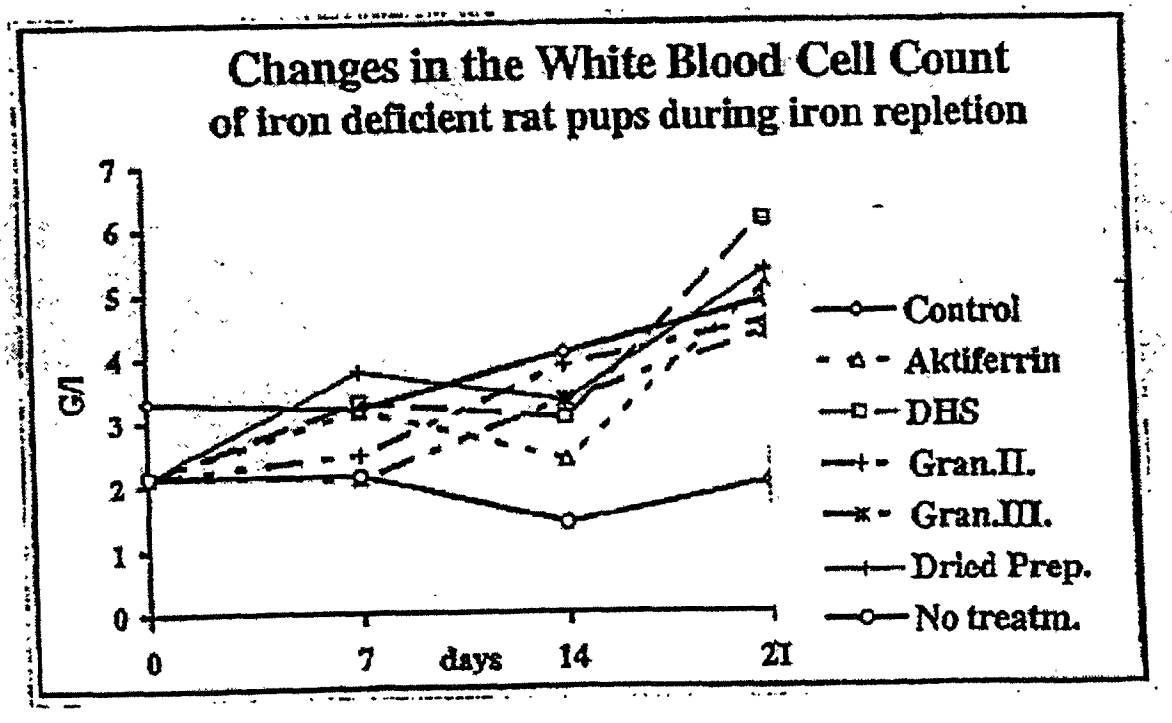

Changes in the White Blood Cell Count
of iron deficient rat pups during iron repletion
G/l
7
6
5
4
3
2
1
0
0
7
days
14
21
Control
Aktiferrin
DHS
Gran.II.
Gran.III.
Dried Prep.
No treatm.

Fig. 6

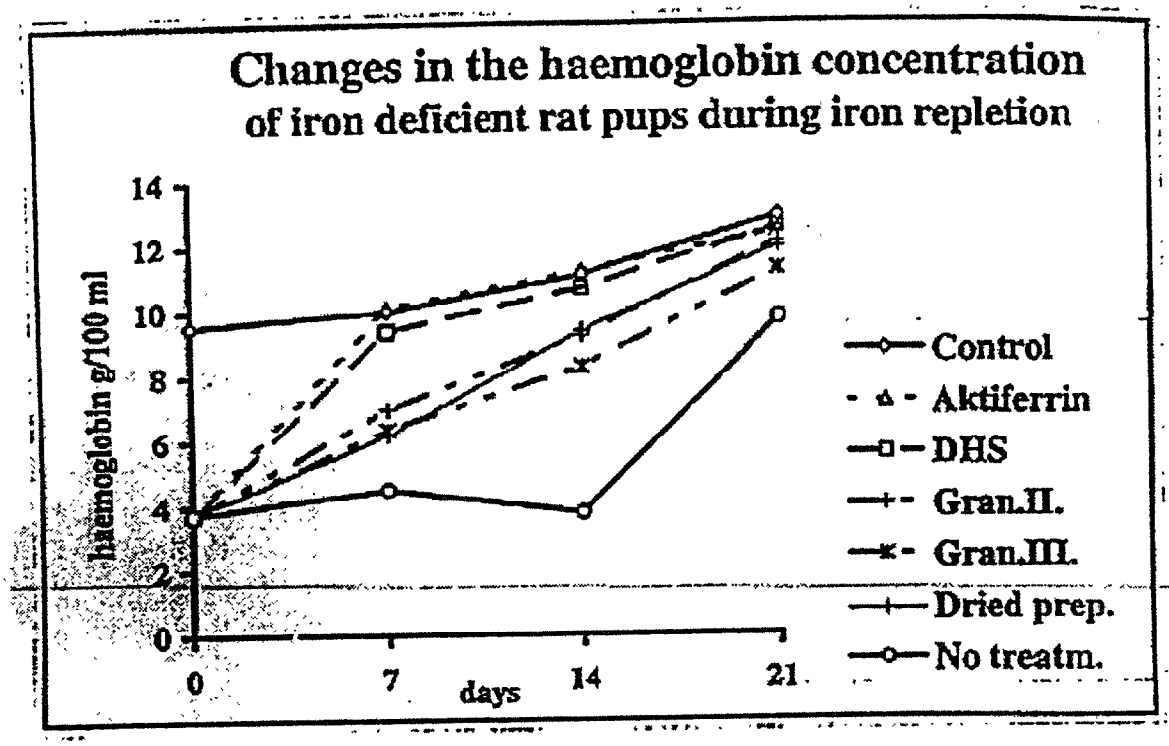

Changes in the haemoglobin concentration
of iron deficient rat pups during iron repletion
haemoglobin g/100 ml
14
12
10
8
6
4
2
0
0
7
14
21
days
Control
Aktiferrin
DHS
Gran.II.
Gran.III.
Dried prep.
No treatm.

Fig. 7

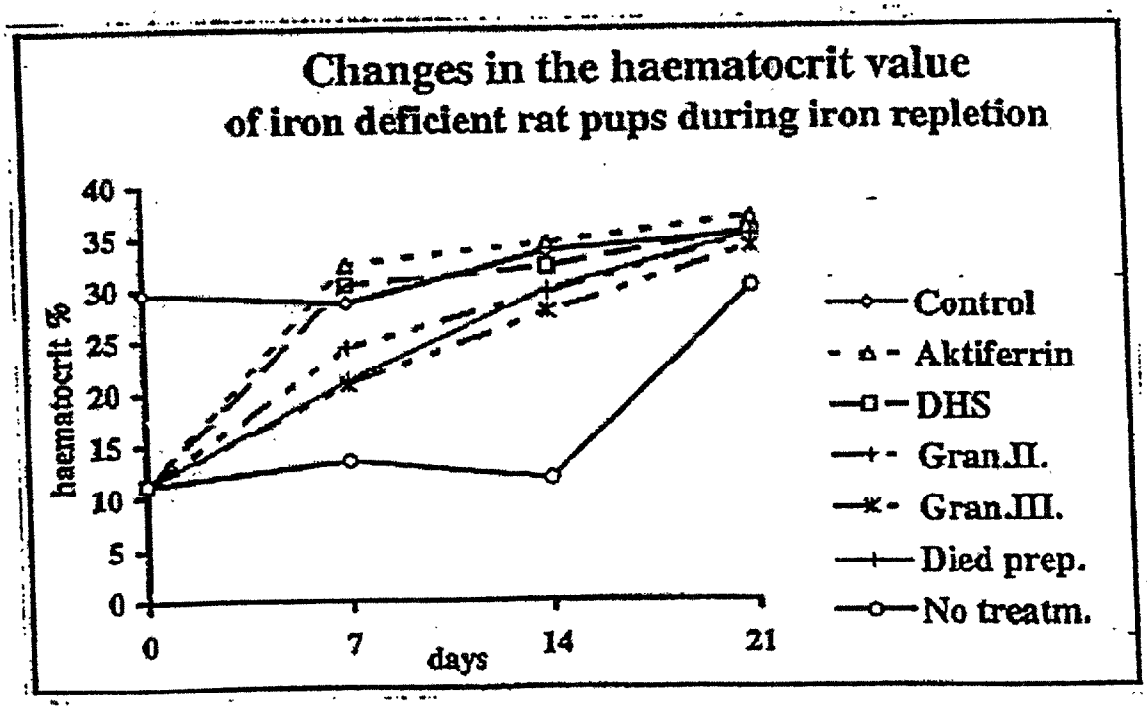

Changes in the haematocrit value
of iron deficient rat pups during iron repletion
haematocrit %
40
35
30
25
20
15
10
5
0
0
7
14
21
days
Control
Aktiferrin
DHS
Gran.II.
Gran.III.
Died prep.
No treatm.

Fig. 8

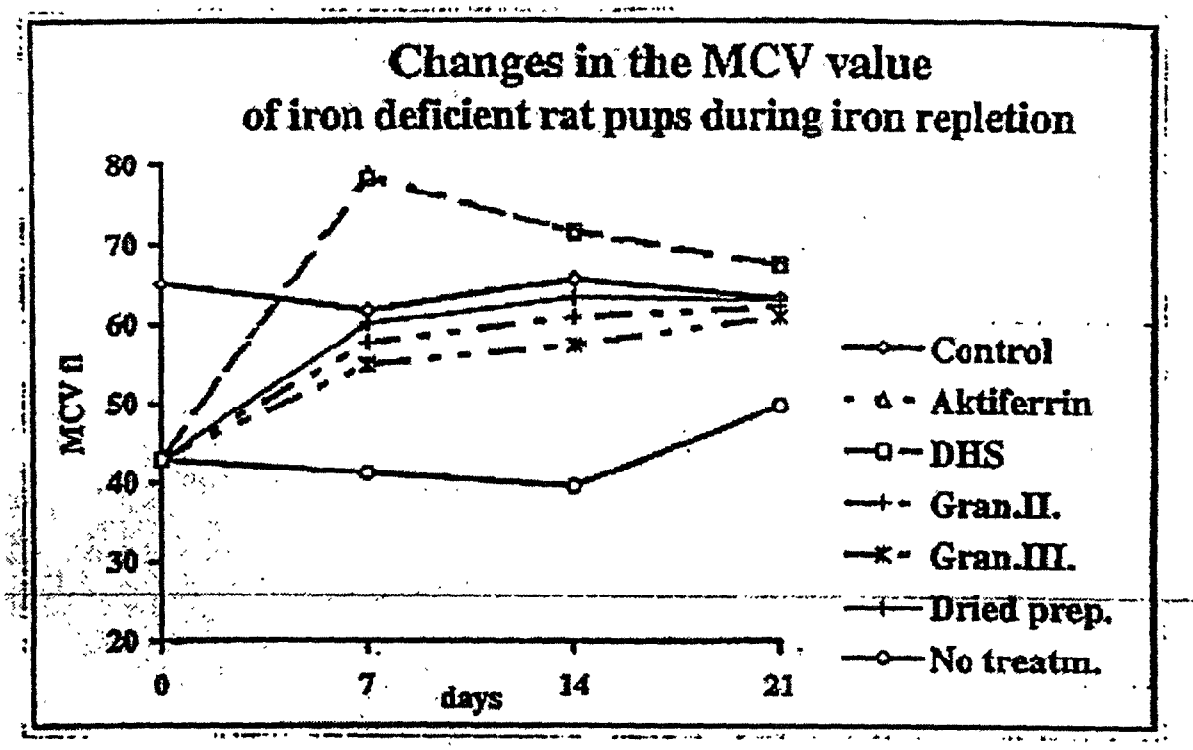

Changes in the MCV value
of iron deficient rat pups during iron repletion
MCV fl
80
70
60
50
40
30
20
0
7
14
21
days
Control
Aktiferrin
DHS
Gran.II.
Gran.III.
Dried prep.
No treatm.

Fig. 9

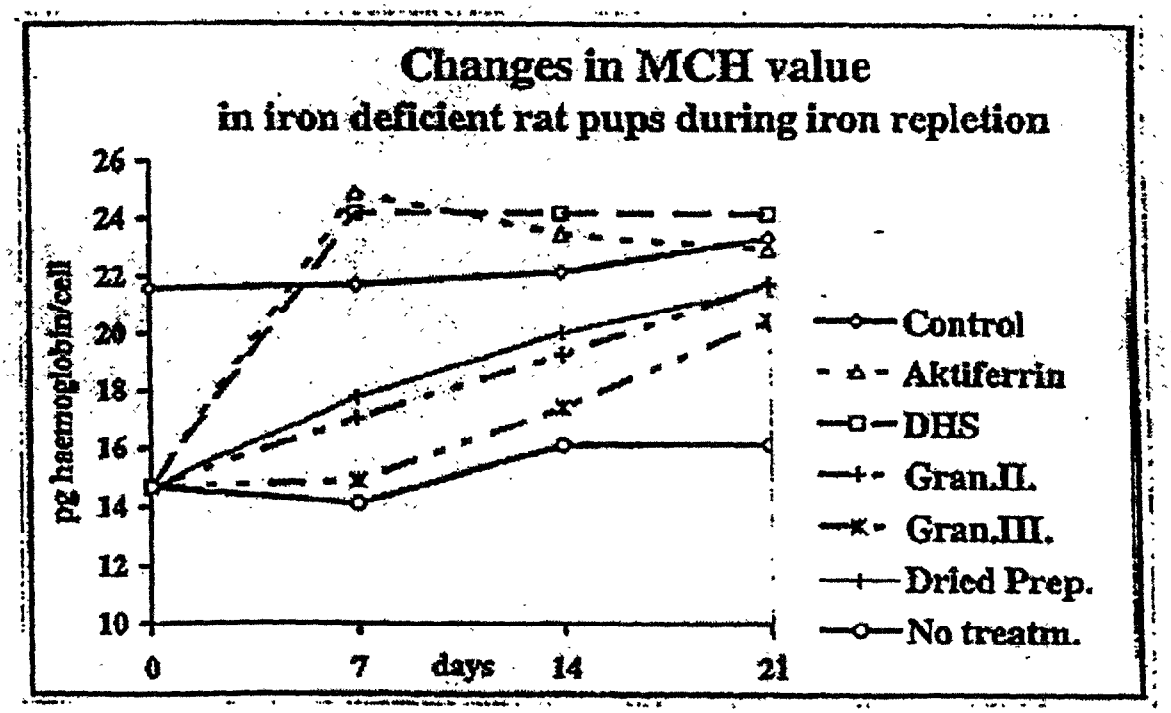

Changes in MCH value
in iron deficient rat pups during iron repletion
pg haemoglobin/cell
26
24
22
20
18
16
14
12
10
0
7
14
21
days
Control
Aktiferrin
DHS
Gran.II.
Gran.III.
Dried Prep.
No treatm.

Fig. 10

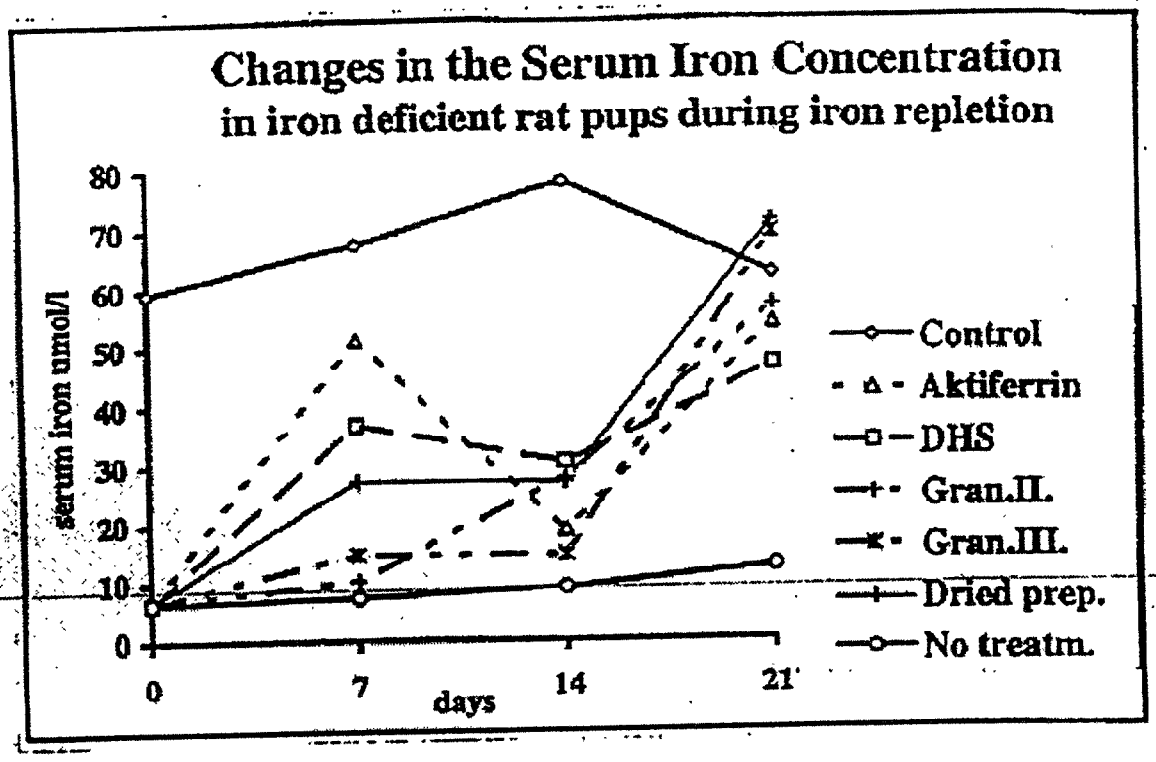

Changes in the Serum Iron Concentration
in iron deficient rat pups during iron repletion
serum iron umol/l
80
70
60
50
40
30
20
10
0
0
7
14
21
days
Control
Aktiferrin
DHS
Gran.II.
Gran.III.
Dried prep.
No treatm.

Fig. 11

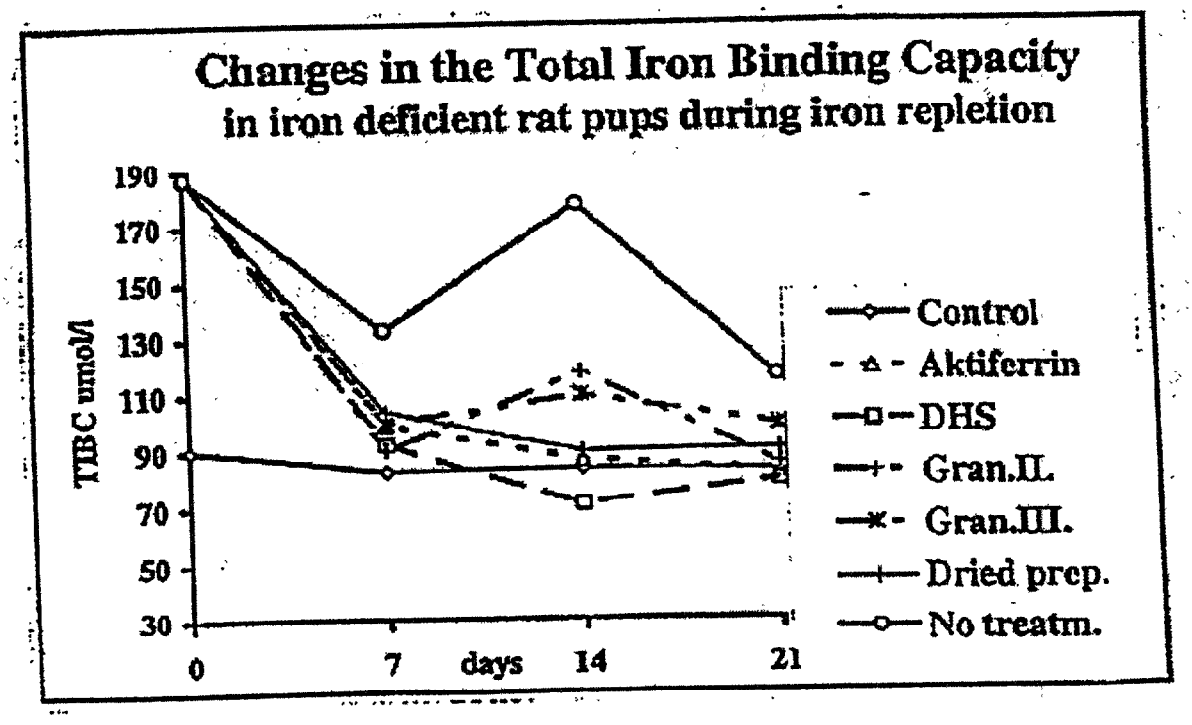

Changes in the Total Iron Binding Capacity
in iron deficient rat pups during iron repletion
TIBC umol/l
190
170
150
130
110
90
70
50
30
0
7
days
14
21
Control
Aktiferrin
DHS
Gran.II.
Gran.III.
Dried prep.
No treatm.

Fig. 12

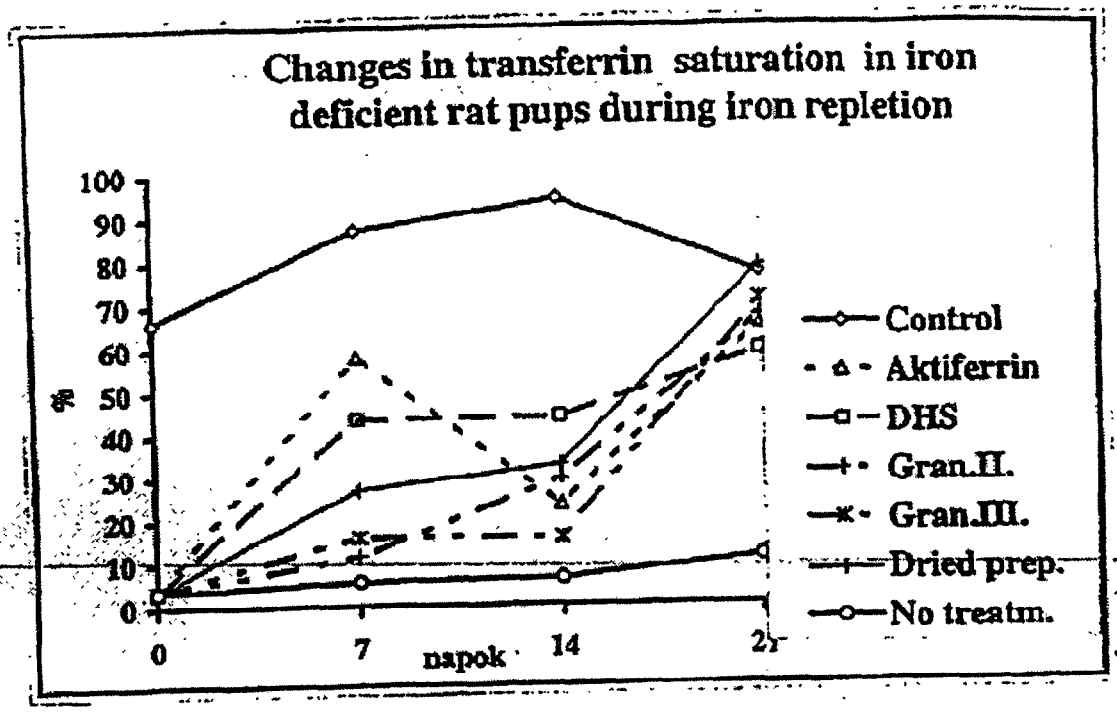

Changes in transferrin saturation in iron deficient rat pups during iron repletion
100
90
80
70
60
50
40
30
20
10
0
%
0
7
napok
14
21
Control
Aktiferrin
DHS
Gran.II.
Gran.III.
Dried prep.
No treatm.

Fig. 13

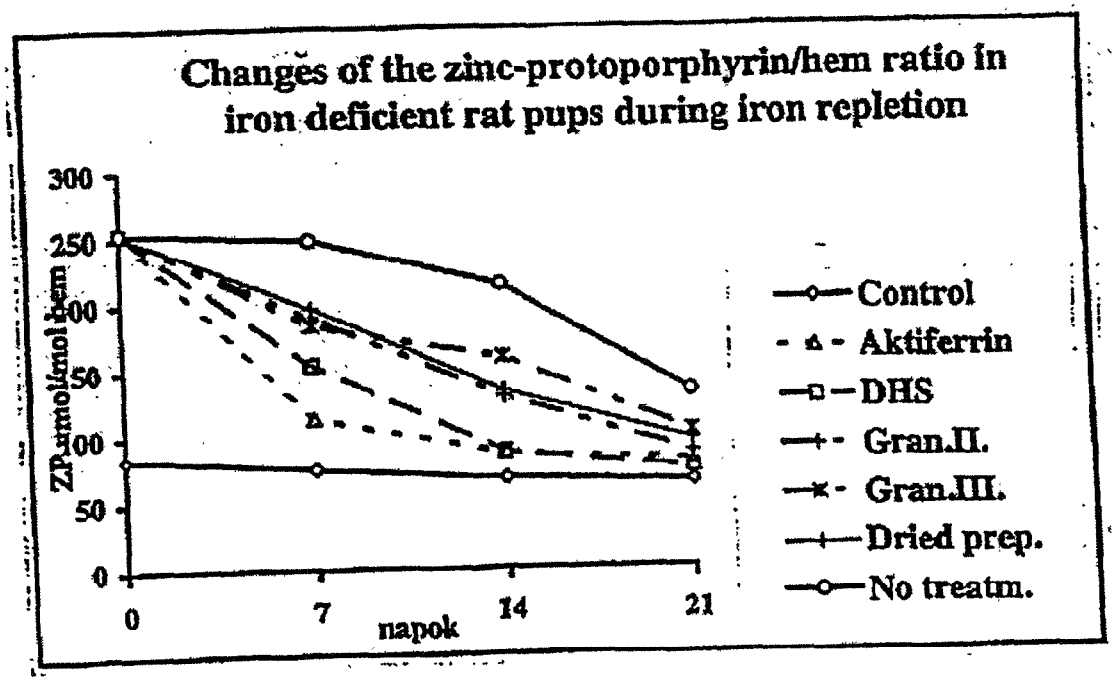

Changes of the zinc-protoporphyrin/hem ratio in iron deficient rat pups during iron repletion
300
250
200
150
100
50
0
ZP µmol/mol hem
0
7
napok
14
21
Control
Aktiferrin
DHS
Gran.II.
Gran.III.
Dried prep.
No treatm.

Fig. 14

Serum ASAT activity after 21 day iron repletion in iron deficient rat pups

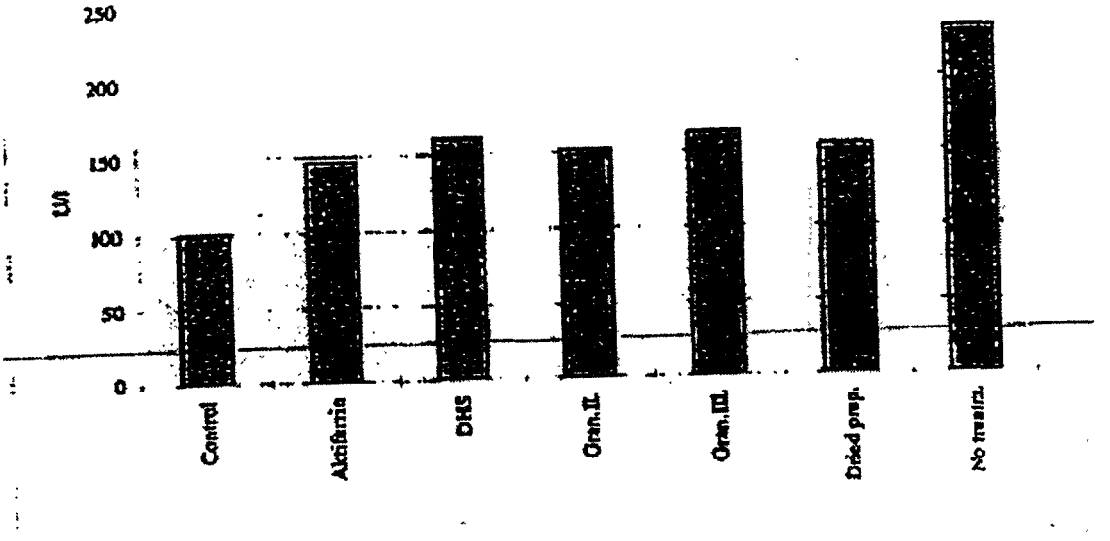


Fig. 15

Serum triglyceride concetration after 21 day iron repletion in iron deficient rat pups

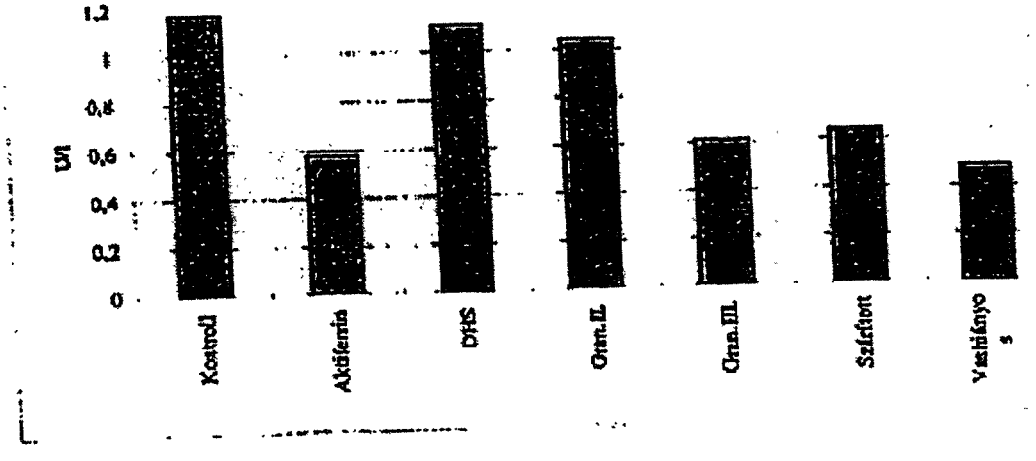


Fig. 16