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Rat
Protein deficiency
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The Erythrogram of Protein-Energy Deficient Male Wistar Rats

U. Oltersdorf and Irmgard Bitsch

Institut für Ernährungswissenschaft I, Justus Liebig Universität Giessen, Federal Republic of Germany

Summary: The erythrogram, the acid induced lysis of erythrocytes, was checked on male Wistar rats initially receiving restricted amounts of diets with a protein content of 3% and 25%, respectively. The rehabilitation process after ad libitum feeding of the high protein diet to all animals was followed up. Protein deficiency reduced slightly, but energy deficiency remarkable the resistance of the erythrocytes to hydrochloric acid. After two weeks of rehabilitaiton only slight modifications of the erythrogram could be observed compared with the erythrograms of the deficient animals; after 11 weeks the erythrograms of the initially different treated rats were similar.

Introduction

The erythrogram describes the hydrochloric acid induced hemolysis of erythrocytes. It gives an indication of the distribution of the stability, and thus age, of an erythrocyte population [7]. The erythrogram depends on many factors like species, age, blood composition or toxines [7]. During investigations of the influence of protein deficiency on the action of hemolytic acting drugs, we observed that erythrocytes of protein depleted rats showed in the erythrogram a higher resistance compared with those of the control group, which received the same restricted amount of feed but with a normal protein level. These results will be published elsewhere. It seemed worthwhile to check this observation. The main intention was to prove whether the erythrogram could be used as an indicator for protein-

energy deficiencies. For this purpose rats were brought into deficiency by feeding them restricted amounts of diets containing different amounts of casein and afterwards all animals were rehabilitated.

Materials and Methods

Inbred male Wistar rats (Meyer-Arend, D – 4902 Bad Salzuflen), 100–120 g, were matched into two groups of 20 each. In the first period all rats received daily the same restricted amount of iso-energetic, semi-synthetic diets, which differed only in the protein and carbohydrate content (Tab. 1).

Tab. 1: Composition of experimental diets

	High protein (HP) diet	Low protein (LP) diet
Alkal. sol. casein ..... (g)	250,0	31,7
Methionine ..... (g)	2,0	0,3
Saccharose ..... (g)	550,0	770,0
Basic components* ..... (g)	198,0	198,0

* 84,0 g rice starch, 62,5 g lard, 7,5 g oil, 40,0 g salt mix (USP XIII)
1,5 g vitamin mix (4)
100 mg retinyl acetate, 5,5 mg calciferol, 114,5 mg α-tocopheryl acetate, 172 g choline chloride

The amount of diet was adjusted to a level which allowed all rats to ingest the food completely within 24 hours (equal feeding) [6], being between 7.5 and 5.5 g/rat/day. This feeding regime was valid for 11 weeks, another 11 weeks all rats received ad libitum the HP diet in order to rehabilitate the rats. The rats were maintained individually in wirebottomed cages in air-conditioned rooms (24° C, 50 % humidity). All rats had free allowance to water.

Beside weight (twice a week) and food consumption (daily) further investigations were carried out according to the following pattern:

DI : after 5 weeks of equal feeding
DII : after 9 weeks of equal feeding
RI : after 13 weeks or 2 weeks of rehabilitation
RII : after 22 weeks or 11 weeks of rehabilitation.

Blood was collected from the tail vene for the determinations of hemoglobin (Hb) as cyanmethemoglobin [5a], hematocrit (Hc) (Hawksley Microfuge), red blood cell count (RBC) (Celloscope 401, Linson Inst., Stockholm), total serum protein by Biuret-reagent [5b], and electrophoretic separation of serum protein fractions on cellulose-acetate-membran (Vogel, D – 6300 Giessen).

The method of Schmidt and Dehnen [8] for the erythrogram was slightly modified: 10 µl blood were pipetted direct from the tail vene (Marburg® pipettes, Eppendorf, Hamburg) into 2,5 ml 0,9 % NaCl-solution. The suspension was brought to 25° C in a thermostated cuvetteholder and afterwards 1,0 ml 0,01 N HCl (25° C) was added. The hemolysis was recorded by the change of extinction at 623 nm by an lin-log recorder (2 cm/min). The determination should be carried out within 3 hours after blood collection. The extinction (E)-time (t) curve was evaluated manually for

t_m – the time of maximal hemolysis, by differentation: maximum of dE/dt

t_f – the end of the hemolysis, taking minimum E + 2 % of total difference between E at the start and minimum E. This procedure had to be applied, because the hemolysis approaches the end assymptotically, thus it was impossible to define exact absolute t_f.

The data were statistically evaluated by Student's t-test.

Tab. 2a: Statistical analysis of biochemical parameters in blood of rats *

	HP DII	HP RI	HP RII	LP DI	LP DII	LP RI	LP RII
HP DI	RBC	Hb, Hc, tp, a, a/g	a, a/g	Hb, Hc, RBC, tp, a	Hb, Hc, tp, a, a/g	Hb, Hc, RBC, a, a/g	a, a/g
HP DII		RBC, a, a/g	RBC, a, a/g	Hb, Hc, RBC, tp, a	Hb, Hc, RBC, tp, a, a/g	Hb, RBC, a, a/g	RBC, a, a/g
HP RI			Hb	Hb, Hc, RBC, tp, a/g	Hb, Hc, tp, a	RBC	RBC
HP RII				Hb, Hc, RBC, tp, a/g	Hb, Hc, tp, a	Hb	
LP DI					Hc, RBC, a, a/g	Hb, Hc, tp, a/g	Hb, Hc, RBC, tp, a/g
LP DII						Hb, Hc, tp, a, a/g	Hb, Hc, tp, a
LP RI							Hb

* Only the statistical significant differences ($P < 0.05$) between the values of two groups are noted; e. g. comparing the values for rats of the groups HP DI and HP DII, only those of the RBC's were found to be significant different (for explanation of the abbrevations refer to Tab. 2).

Results and Discussion

The growth curve (Fig. 1) and the biochemical parameters (Tab. 2) are showing that the feeding regime brought all rats to protein and energy deficiency states, respectively. Their situation became worse after 11 week on the restricted diet, especially LP rats. In the LP group died 3 rats compared with one in the HP group. Thereafter the feeding pattern for all rats were switched to ad libitum feeding of the HP diet.

LP rats showed the known signs of protein deficiency [1, 2, 3, 9]: anorexia, weight losses, low values for Hb, Hc, RBC and serum proteins. Their values are significant more depressed compared with the energy deficient HP rats. Energy deficiency in man and animals causes only slight reduction of hematologic parameters and serum proteins, sometimes these values are normal or even elevated, *e. g.* in severe cases, due to

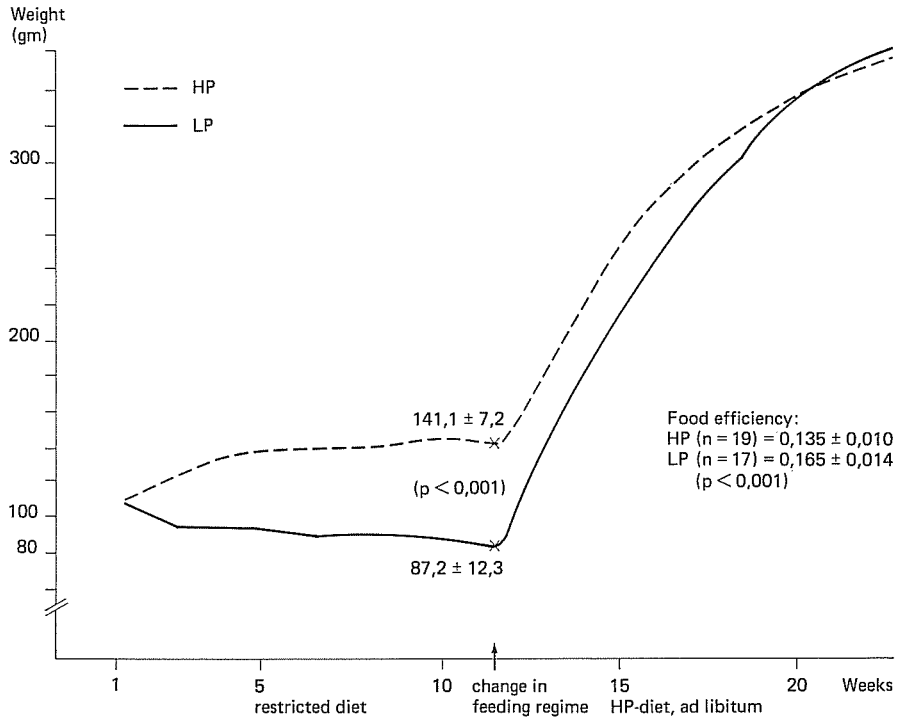


Fig. 1: Growth curve of rats

Tab. 2: Biochemical parameters in blood of rats

	HP * (n = 19)				LP * (n = 17)			
	DI *	DII *	RI *	RII *	DI *	DII *	RI *	RII *
Hemoglobin (Hb) (g/100 ml)	15.3 ± 1.2	14.8 ± 1.4	14.1 ± 0.9	15.6 ± 1.0	11.9 ± 1,12	11,4 ± 2,4	13,1 ± 1,9	15,2 ± 1,1
Hematocrit (Hc) (%) ..	48.3 ± 2 3	45.8 ± 3.7	43.8 ± 3.1	45.7 ± 2.3	38.5 ± 4.0	32.6 ± 3.5	43.0 ± 4.5	45.4 ± 2.2
Red Blood Cell Count (RBC) (Mill./mm³)	7.56 ± 1.13	9.04 ± 0.95	7.58 ± 0.87	7.21 ± 1.03	5.85 ± 1.14	6 89 ± 0.89	6.51 ± 0.88	6.87 ± 0.32
Total Protein (g/100 ml) (tp)	7.2 ± 0,5	7.0 ± 0.6	6.6 ± 0.7	6.8 ± 0.3	5.4 ± 0.4	5.0 ± 0.9	7.0 ± 0.6	6.8 ± 0.6
Albumin (a) (g/100 ml)	3.9 ± 0.3	3.6 ± 0.3	3.0 ± 0.6	3.1 ± 0.2	2.8 ± 0.4	2.0 ± 0.6	3.2 ± 0.4	3.1 ± 0.4
$\frac{\text{Albumin}}{\text{Globulin}}$ = ratio (a/g) .	1.16 ± 0.20	1.09 ± 0.16	0.83 ± 0.18	0.86 ± 0 09	1 10 ± 0.18	0.72 ± 0.22	0.87 ± 0.15	0.82 ± 0.11

* For explanation refer to text, section: methods and materials.

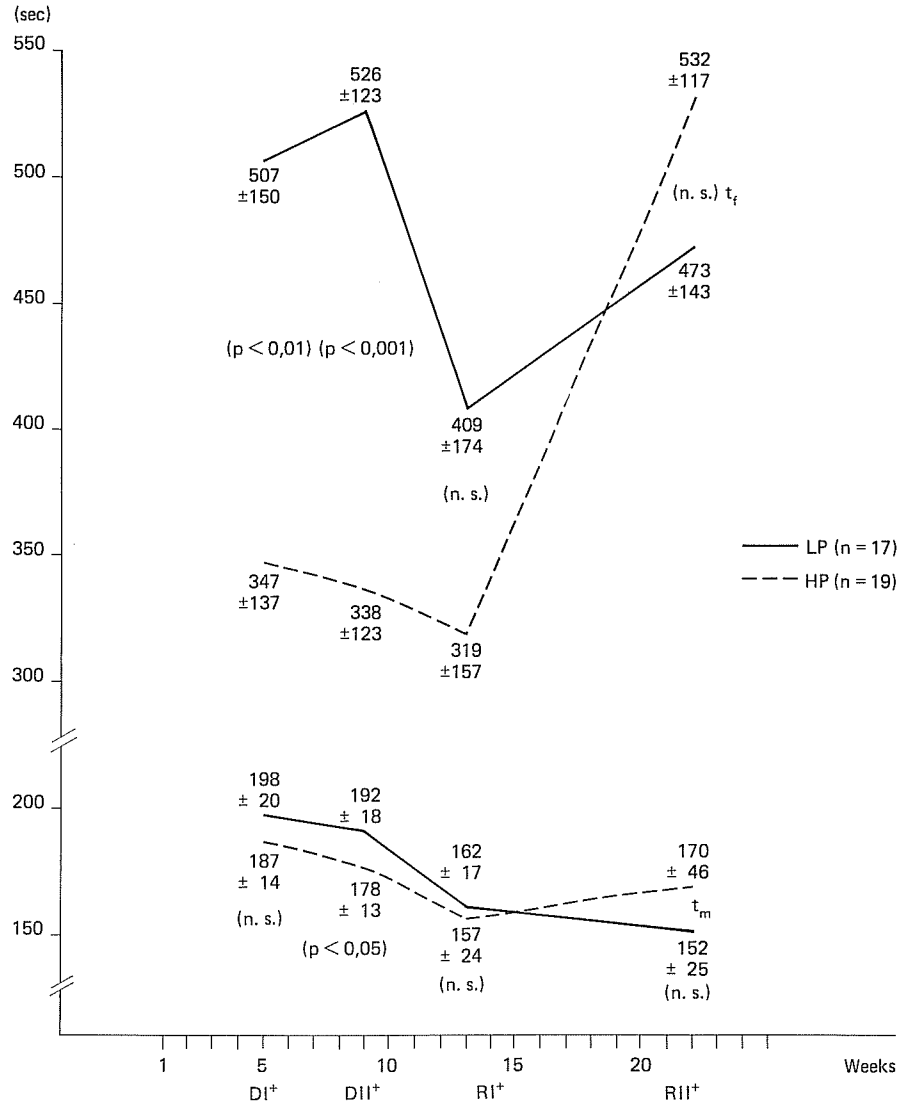
dehydration. During rehabilitation an initial fall of the mentioned parameters can be observed [1, 2, 3, 9].

During the second, the rehabilitation phase of the experiment, all rats of both groups, still named LP and HP, respectively, showed immediate response. They gained weight and both groups had at the end the same average weight. LP rats gained more weight, had a higher food intake and a better food efficiency than HP rats. The rehabilitation could be proved by the change in the biochemical parameters, too. The values for LP rats increased significantly. The former energy deficient HP rats showed an initial depression (Tab. 2, RI), which can occur at the beginning of rehabilitation of energy deficiency, as already mentioned above.

The erythrogram, results are summarized in Fig. 2 and Tab. 3, showed, that the erythrocytes from protein deficient LP rats were more resistant to the hydrochloric acid attack than erythrocytes from energy deficient HP rats. This observations is consistent with our former observation. The difference is considerable for t_f ($p < 0{,}001$) smaller for t_m ($p < 0{,}05$) (Fig. 2), and thus highly significant for the t_f/t_m-ratio (Tab. 3), too ($p < 0{,}001$) despite of rather high individual variation of the erythrogram. Rehabilitation of the rats had not an immediate effect on the erythrogram as seen for the other parameters. After two weeks of rehabilitation (RI) a difference between LP and HP rats still consists. There were 11 weeks needed till the erythrogram of the initially different treated rats were comparable.

It is surprising that protein deficiency has obviously less effect on the stability of erythrocytes than the energy deficiency or the restricted intake of a balanced diet, respectively. The deviation from the normal erythrogram is much less in LP rats compared with HP rats. The final values of the erythrogram (RII) are representing the normal picture of an erythrogram for rats [7]. A shift to shorter time of hemolysis means reduced stability of erythrocytes. Older and less resistant erythrocytes are lysed earlier than young and more resistant ones, congruently the erythrogram of older rats is recorded in an earlier time interval than those of infantile ones [7].

Our data do not allow to give an explanation for the observed differences of the erythrograms. We will not try to investigate for it immediately, but rather to evaluate the erythrogram for further nutritional disor-



(+ for explanation refer to text; section: materials and methods)

Fig. 2: The erythrogram of rats

Tab. 3: The erythrogram of rats: Ratio between t_f and t_m (t_f/t_m) *

	DI	DII	RI	RII
HP (n = 19)	1,84 ± 0,67	1,89 ± 0,61	2,00 ± 0,86	3,39 ± 1,09
LP (n = 17)	2,53 ± 0,61	2,73± 0,57	2,49 ± 0,97	3,23 ± 1,15
	($p < 0,01$)	$p < 0,001$	n. s.	n. s.

* For explanation of abbreviations refer to text, section: materials and methods.

ders and its validity for other species, especially for malnourished children. Since the erythrogram is a simple, quick and cheap method, which requires only minute amounts of blood (10 µl), it seems for us worthwhile to check it as a tool for the assessment of the nutritional status. Our reported results show – at least, for the applied conditions and for rats – the erythrogram can differentiate between protein and energy deficiency. The erythrogram is especially sensitive to energy deficiency, just opposite to the common biochemical indicators of protein-energy deficiency.

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Dr. U. Oltersdorf, Institut für Ernährungswissenschaft I, Justus-Liebig-Universität, D – 6300 Giessen, Wilhelmstrasse 20