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Interactions between Malnutrition and Primaquine Studied on Wistar Rats I. Protein Deficiency

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Summary: The action of primaquine on male Wistar rats depleted on protein (3 % casein) and equal-fed control (25 % casein) was investigated. The following parameters indicated a slightly increased primaquine toxicity in protein deficiency: depression of weight and food intake; reduced increments of organ weights; lowered hematologic values, especially hemoglobin; increased fragility of erythrocytes to hydrochloric acid, β-globulinemia, reduced glutathione content and pyruvate-lactate-ratio of blood. No difference could be observed regarding other serum protein fractions, methemoglobinemia and the activities of glucose-6-phosphate-dehydrogenase and glutathione-reductase of erythrocytes.

1. Introduction

Primaquine and other aminoquinoline derivates are currently used for the prophylaxis and treatment of malaria. They are known to cause hemolytic anemia in sensitive individuals at the prophylactic and therapeutic dosage and in normale people at a ten-times higher concentration [23]. Sensitive individuals in this respect are those whose erythrocytes are characterised by hereditary defects of enzymes involved in the reduction

of glutathione, its synthesis or in its role of detoxifying hydrogenperoxide [4]. Particulary G-6-PD deficiency is common, which probably affects more than 100 million people [26]. The severity of the hemolysis in G-6-PD-deficient subjects depends on the level of the enzyme in the red cell, its biochemical characteristics and on the blood level of the hemolytic agent. In the case of primaquine the most active hemolytic principles are those catabolites of the drug which possess high redox properties. They are characterised by a quinoline-quinone structure and affect essential components of the red cell metabolism and membrane structure by oxidative denaturation.

The activity of erythrocyte G-6-PD and the stability of red cells against various hemolytic agents are often reduced in protein deficiency [1, 27]. From this, one could propose a higher sensitivity of malnourished people against the hemolytic action of primaquine and similar drugs. On the other hand protein deficiency may diminish the catabolism of primaquine in the liver. This would result in lower blood levels of derivates with high redox properties. That would mean, the hemolytic action of primaquine would be lower in protein deficiency.

Several studies brought evidence that malnutrition alters the action and metabolism of different nonnutrients [10, 14, 22, 24, 25]. The aim of our present investigation was to check, whether protein-malnutrition

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Abbreviations:

G-6-PD	= Glucose-6-phosphate dehydrogenase, D-glucose-6-phosphate: NADP oxidoreductase (E. C. 1.1.1.49)
GSH	= glutathione
GSH-reductase	= $NAD(P)H_2$: glutathione oxidoreductase (E. C. 1.6.4.2)
NAD (NADH)	= nicotinamide-adenine-dinucleotide (reduced form)
NADP (NADPH)	= nicotinamide-adenine-dinucleotide phosphate (reduced form)
Hb	= hemoglobin
Hc	= hematocrit, packed cell volume
Met-Hb	= methemoglobin
MCH	= mean cell hemoglobin
MCHC	= mean cell hemoglobin content
MCV	= mean cell volume
HP	= rats receiving high protein food (25 % casein)
LP	= rats receiving low protein food (3 % casein)
HP_c, LP_c	= rats of the control groups
HP_p, LP_p	= rats of the primaquine treated groups

influences the action of primaquine. This seems to us not only of interest from a theoretical view, but one has to remember that in many of the less developed areas of the world, people are suffering from protein-energy malnutrition, the antimalarias are used, too, often for a long period of time, as part of preventive health measurements. Besides primaquine several other drugs, such as nitrofurantoin or certain sulfonamides, are causing hemolytic reactions in G-6-PD-deficient peoples [4].

2. *Materials and Methods*

Inbred male Wistar rats (Meyer-Arend, D-4902 Bad Salzuflen), 80–100 g, received, after an initial feeding period of one week with Altromin-oatmeal-chow, isoenergetic, semi-synthetic diets, which differed only in protein and carbohydrate content (Tab. 1).

Tab. 1: Composition of the experimental diets

	HP [1]	LP [1]
Alkal.-sol. Casein (g)	250.0	31.7
Methionine (g)	2.0	0.3
Saccharose (g)	550.0	770.0
Basic components [2] (g)	198.0	198.0

[1] See: Abbreviations

[2] 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 [16]
100 mg retinyl acetate, 5.5 mg calciferol, 114.5 mg α-tocopheryl acetate, 1.72 g choline chloride

Rats of all groups received the same restricted amount of diet. The amount of diet was adjusted to a level which allowed all rats to ingest the food completely (equal feeding) [19] being 7 to 9 g/day/rat. All rats had free allowance to water. Rats were maintained in individual wirebottomed cages in an air-conditioned room (24° C, 50% humidity).

After 4 weeks on the experimental diets each group was subdivided and the primaquine administration started. Rats in the different subgroups received on 5 days per week (in the morning before getting new feed) via stomach-tube an aqueous solution (5 ml/kg bodyweight) of phosphate-citric acid buffer, pH 2.9 (control) or 30 mg prima-

quine [1]/kg bodyweight. After one week of drug administration every day one rat out of each group was killed by bleeding after anesthesia by diethylether. Rats received thus between 6 to 15 (average 10.5) doses.

Recorded was food intake (daily), bodyweight (twice per week) and weights of liver, spleen, kidney and heart (at autopsy). Blood was collected from the tail vene (between 3rd and 7th day before, 2nd and 5th day after the begin of drug administration and immediately prior to killing) for the determinations of Hb as cyanmethemoglobin [18b], Hc (Hawksley Microfuge), erythrocyte count (Celloscope 401, Linson Inst., Stockholm), erythrogram (recording of the hydrochloric acid induced hemolysis of an erythrocyte suspension in 0.9 % NaCl-solution) [17, 21], total serum protein by Biuret-reagent [18a] and electrophoretic separation of serum protein fractions on cellulose-acetate-membrane (Vogel, D-6300 Giessen). During killing heparinized blood was collected by bleeding for the determinations of Met-Hb [18c], glutathione [3], pyruvate (Böhringer test combination) and lactate (Böhringer test combination) in whole blood; and GSH-reductase [8] and G-6-PD (Böhringer, biochemical information) in erythrocytes.

All determinations were done at the day of blood collection. The data were statistically evaluated by Student's t-test.

3. Results

From altogether 44 rats five were killed during catheterization with the stomach tube and five died due to primaquine – four from LP_p and one from HP_p. Four of the last ones died within the first week of primaquine application, the otherone afterwards. It was observed that some rats were especially susceptible to primaquine; they showed severe reactions. Others tolerated rather well. Generally the reactions were more severe in the beginning of primaquine application and weakened after few days.

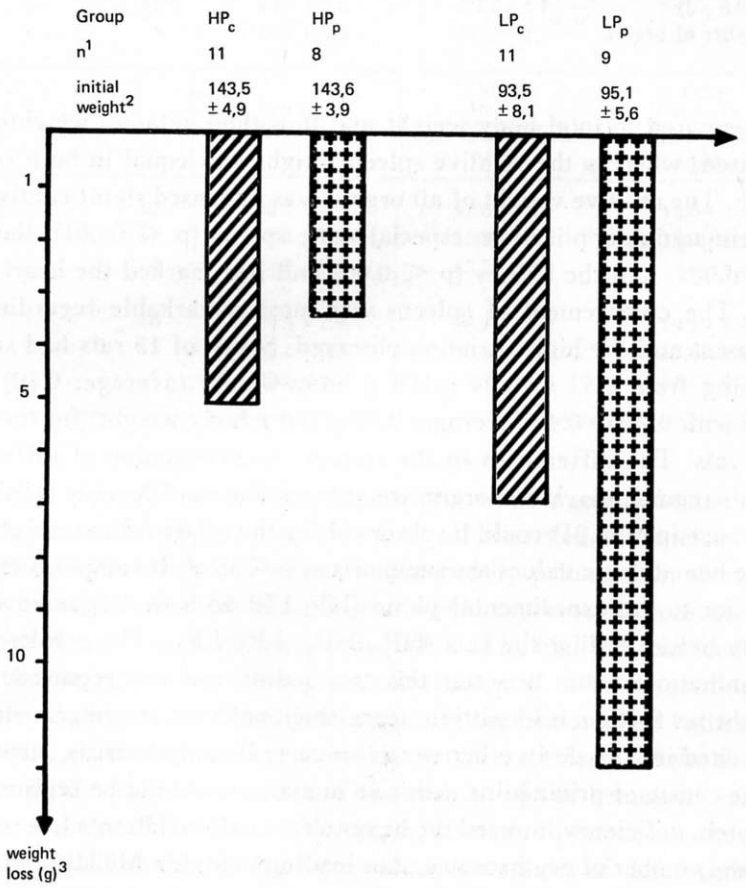
The food intake was according to the outline of the experiment equal in all rats before primaquine was administered (7.3 g/rat/day), afterwards the offered amount of feed had to be reduced further (6.0 g/rat/day). HP rats took the whole food always, whereas the LP rats responded to the stress by loss of apetite, even the control animals (LP_c). But the LP_c-rats recovered after one week (intake from 5.8 back to 6.0 g/rat/day), the primaquine receiving LP_p-rats had a constantly depressed intake 5.1 g/rat/day).

The weight development (Fig. 1) reflected the different protein content of the feed. The stress of the manipulations during the experiment

[1] Given as an aqueos solution of primaquine-diphosphate, which was kindly donated by Bayer, Leverkusen.

caused weight losses in all groups. The losses in the LP-groups were significantly ($p < 0.001$) higher than in the HP groups. Primaquine led only in LP-rats to further weight losses.

The relative wet weights of liver, spleen, kidney and heart are shown in Tab. 2. Protein deficiency led to weight losses, the tissue losses from different organs were varying. Liver, heart and kidney were protected relatively more than spleen. Than weight of the former ones were less depres-



1 number of rats
2 weight of rats one day before the experiment started
3 for difference the weight at the day of killing was taken

Fig. 1: Bodyweight changes of rats during the experimental period

Tab. 2: Relative wet weights of liver, spleen, kidney and heart of rats

Group	n [1]	Liver	Spleen	Kidney	Heart
		(g/100 g body weight)			
HP_c	11	3.27 ± 0.35	0.25 ± 0.05	0.88 ± 0.22	0.35 ± 0.03
HP_p	8	3.88 ± 0.42	0.45 ± 0.15	0.95 ± 0 12	0.37 ± 0.02
LP_c	11	4.80 ± 0.72	0.27 ± 0.04	0 96 ± 0.25	0.44 ± 0.05
LP_p	7	4.66 ± 0.48	0.59 ± 0.25	1.10 ± 0.08	0.46 ± 0.06

[1] Number of organs

sed compared to total body weight and thus their relative weights were increased; whereas the relative spleen weight was equal in both control groups. The relative weight of all organs was increased significantly after the primaquine application; especially the spleen ($p < 0.001$), the liver ($p < 0.005$) and the kidney ($p < 0.05$) and less marked the heart ($p < 0.05$). The enlargement of spleens was most remarkable regarding the increment and the high variation observed. Seven of 15 rats had spleens weighing from 0.53 to 0.94 g/100 g body weight (average: 0.70) compared with 0.28 to 0.46 (average: 0.36 g/100 g body weight) for the other eight rats. The differences in the respond to primaquine of differently fed rats regarding relative organ weights were minor; the only significant difference ($p < 0.01$) could be observed for the relative liver weight.

The hematologic values are summarized in Tab. 3. It compares the values prior to the experimental phase (HP, LP) with those gained immediately before killing the rats (HP_c, HP_p; LP_c, LP_p). The results of the determinations done between this two points are not reported. They brought no further information, since their values were intermediate to those cited in Tab. 3. In other words an early hemolytic crisis, mentioned for the course of primaquine action in humans, could not be registered.

Protein deficiency lowered the hematologic values. Hb was less reduced than the number of erythrocytes, thus leading to higher MCHC and MCH in LP compared with HP. The volume of the erythrocytes (MCV) was not altered by protein deficiency.

The hematologic values were showing again the stress of the rats, which was caused by the experiment. The values of the control groups

Tab. 3: Hematologic values of rats before and after the experimental period

Groups	n	Hb (g/100 ml)	Hc (%)	Erythrocyte count (millions/mm³)
HP	16	16.4 ± 1.5	50 6 ± 2.9	8.87 ± 0.93
HP_c	11	16.0 ± 1.3	48.1 ± 3.2	8.77 ± 1.28
HP_p	8	14.0 ± 2.1	41.8 ± 1.4	7.70 ± 1.29
LP	16	15.2 ± 1.1	44.6 ± 3 8	7.96 ± 0.73
LP_c	11	12.5 ± 1.2	39.7 ± 3.7	7.16 ± 0.95
LP_p	5	10.7 ± 0.8	36.0 ± 4.3	7.58 ± 1.61

		MCHC (Hb/Hc) × 100	MCV $\frac{Hc \times 10}{\text{erythrocyte count}}$ (μ^3)	MCH $\frac{Hb \times 10}{\text{erythrocyte count}}$ ($\gamma\gamma$)
HP	16	32.5 ± 2.0	57.6 ± 5.6	18.6 ± 1.6
HP_c	11	33.3 ± 1.9	55.9 ± 8.8	18.5 ± 2.5
HP_p	8	33.6 ± 6.2	55.7 ± 11.8	18.6 ± 4.4
LP	16	34.3 ± 2.9	56.3 ± 5 0	19.2 ± 1.5
LP_c	11	31.5 ± 1.7	56.0 ± 6.2	17.6 ± 2.2
LP_p	5	29.6 ± 2.5	53.6 ± 2.2	14.8 ± 2.4

(HP_c, LP_c) became lower during the experiment, especially in LP_c. Primaquine did cause further depression of all observed parameters. The hemoglobin molecule was stronger influenced by primaquine, shown by more reduced Hb and MCH, than the whole erythrocyte, indicated by less reduced erythrocyte count, Hc and MCV. The high protein feed protected the rat better than the low protein feed ($p < 0.005$) regarding the influence of general stress and primaquine on the hematologic values.

From the erythrograms two main data sets were extracted: the time of maximum rate (t_m) and the time of the end (t_f) of the hemolysis. Tab. 4 shows their values before and after the experimental period. For the same reason as stated at the hematologic values the results of determinations done in the intermediate phase are not reported. Erythrocytes from LP-rats were more resistant to the hemolysis. The hemolysis lasted in LP rats 353 seconds compared with 183 seconds in HP rats ($p < 0.002$), and the maximum rate was earlier in HP than in LP ($p < 0.001$).

Tab. 4: Erythrogram: time of maximum rate (t_m) and of end (t_f) of hemolysis of an erythrocyte suspension in 0.9 % NaCl-solution induced by 0.01 N HCl, of rats, measured before (HP, LP) and after ($HP_{c,\,p}$, $LP_{c,\,p}$) the experimental period.

Groups	n	t_m (sec)	t_f (sec)
HP	17	119 ± 17	183 ± 104
HP_c	11	159 ± 50	235 ± 66
HP_p	8	132 ± 29	172 ± 76
LP	18	126 ± 12	353 ± 136
LP_c	11	141 ± 17	306 ± 148
LP_p	7	126 ± 28	273 ± 204

The general stress of the experiment caused in HP_c a prolongation of the hemolysis (higher t_m and t_f), whereas in LP_c only the maximum rate shifted to a later time, but the duration of the hemolysis was shortened.

The action of primaquine caused in both groups (HP_p, LP_p) an increased fragility of the erythrocytes, expressed in earlier time for maximum rate and end of hemolysis. There was no significant difference in the respond to primaquine regarding the different protein availability of the rats; only a tendency to a better resistance at higher protein levels was observed.

Serum proteins, summarized in Tab. 5, were a good indicator of the protein nutriment. The values for total protein, albumin, γ-globulin and thus the albumin-globulin-ratio were lowest in LP-rats. The α- and β-globulin fractions were independent from the protein content of the feed.

Tab. 5: Serum protein levels in rats prior to the experimental phase

Groups	n	Total Protein	Albumin	α_1-Globulin	α_2-Globulin	β-Globulin	γ-Globulin	$\frac{\text{Albumin}}{\text{Globulin}}$ Ratio
		(in g/100 ml serum)						
HP ..	18	6.41	3.03	0.76	0.72	1.14	0.71	0.94
		± 0.50	± 0.31	± 0.12	± 0.19	± 0.21	± 0.18	± 0.16
LP ..	17	5 09	2.27	0.71	0.62	1.04	0.44	0.81
		± 0.34	± 0.32	± 0.13	± 0.10	± 0.13	± 0.12	± 0.15

During the experiment neither stress nor primaquine did alter any serum protein fraction, except β-globulin, which was showing higher levels especially due to primaquine. Comparing the different groups LP_p had the higher β-globulin level than HP_p (n. s.) (Tab. 5a).

Tab. 5a: β-globulin fraction in serum of rats after the experimental phase

Groups	n	β-Globulin in g/100 ml	Total Protein %
HP_c	7	1.19 ± 0.22	18.4
HP_p	6	1.45 ± 0.21	22.1
LP_c	8	1.22 ± 0.16	21.9
LP_p	4	1.36 ± 0.22	26.8

Methemoglobinemia was caused by primaquine (Tab. 6), but protein deficiency did not lead to higher Met-Hb values despite of the observation of raised Met-Hb levels in protein deficiency itself ($LP_c > HP_c$).

G-6-PD, the key enzyme of the pentose-phosphate-shunt, in erythrocytes was not influenced by protein level in the diet and by primaquine (Tab. 6).

Tab. 6: Results of determinations in blood and erythrocytes of rats after the experimental phase

Groups	n [1]	Met-Hb [2]	G-6-PD [3]	GSH [4]	GSH reductase [5]	Pyruvate [6] / Lactate
HP_c	11	1.3 ± 0.5	50.0 ± 5.3	35.4 ± 7.6	108 ± 32	4.3 ± 1.5
HP_p	8	4.0 ± 1.6	49.2 ± 3.7	41.7 ± 5.8	155 ± 49	5.9 ± 2.3
LP_c	10	2.3 ± 1.2	53.3 ± 6.1	24.4 ± 10.7	130 ± 38	3.7 ± 1.4
LP_p	7	5.0 ± 3.1	54.3 ± 11.6	24.4 ± 6.1	170 ± 63	3.0 ± 0.8

[1] Number of samples
[2] In % of Hb
[3] In µMol/min., 1 litre of erythrocyte suspension (Hc = 35 %), 25° C
[4] In mg/100 ml blood
[5] In µMol/min., 1 litre of erythrocyte suspension (Hc = 35 %), 35° C
[6] Ratio of pyruvate and lactate in mg/100 ml blood each, times 100

GSH, a protector of sulfhydryl-groups of the erythrocyte enzymes against oxidative agents, was significantly reduced in LP rats ($p < 0.02$). Administration of primaquine did show in elevated GSH levels ($p < 0.01$) in HP rats, whereas protein deficiency inhibited the elevation of GSH.

The GSH-reductase activity in erythrocytes was slightly increased in protein deficiency (n. s.) (Tab. 6). The GSH-reductase activity was elevated by primaquine, but due to the high variations in its activities the differences were not significant.

The pyruvate-lactate-ratio in blood, an index of the redox-status, was lowered due to protein deficiency (n. s.). Primaquine caused in increase ($p < 0.002$) of that ratio in HP-rats, but not in those of LP-groups (Tab. 6). Pyruvate levels were more increased by pyrimaquine in HP-rats than in LP ones; lactate levels were not altered by primaquine in HP, but elevated in LP rats.

Last not least it should be mentioned that we did run parallel experiments with a reduced methionine intake (same diets as in the here reported study but without methionine supplementation) and/or with a lower primaquine application (10 mg/kg body weight). Both manipulations did not show any appreciable effect. Reduced methionine intake had no influence on the action of primaquine. The lowered primaquine dose was well tolerated by the rats; the observed parameters did show only small changes in the directions reported here for the high primaquine dosage.

4. Discussion

The aim of the reported study was to investigate on rats the possible influence of different protein supply on the action of primaquine and to get a model for possible interactions between malnutrition and drug action. It was not intended to study the mechanisms of changes in detail but rather the general action of primaquine with some emphasis on its hemolytic properties.

The "primaquine-sensitive" subject is showing hemolytic anemia symptoms after 3 to 4 days of primaquine administration. The course of this adverse reaction to primaquine is a self-limiting one. The signs of

hemolytic anemia cease during a continued application of the same dose of primaquine after 10 to 40 days. The main mechanism behind this pattern is the fact that older erythrocytes are more susceptible to primaquine than the younger ones. Subsequently the erythrocyte population becomes younger and thus more resistant. The erythropoiesis is stimulated, too [23].

Primaquine is catabolized mainly in the liver, involving 5-hydroxylating and N-dealkylating reactions. The catabolites are excreted as glucuronate and sulfate esters. Some catabolites are quinoline-quinone-derivates having redox properties and are thought to be the active metabolites of primaquine regarding its antimalarial, methemoglobinemia-inducing and hemolytic activities [7].

Beside the above mentioned responses of an organism to primaquine – which is leading to a younger erythrocyte population and correspondingly to higher enzyme activities of the erythrocytes – there are alterations in metabolism of the erythrocytes in order to counteract the oxidating properties of the primaquine catabolites. Those catabolites are able to oxidize the intracellular and membrane sulfhydryl groups of the erythrocytes, *e. g. GSH.* An increased ratio of oxidized to reduced GSH however increases the rate of the pentose-phosphate-shunt and thereby the activity of its key enzyme G-6-PD [11]. In those cases, where the intracellular mechanisms for reduction of disulfides are abnormal – *e. g.* in people with congenital G-6-PD-deficiency – the erythrocytes are very sensitive to primaquine [2, 6, 7, 26].

Protein deficiency itself causes retarded growth, reduced serum proteins [13], anemia, increased sensitivity of the red cells against hemolytic agents [1, 27] and reduced activities of the hepatic microsomal enzymes of drug metabolism [25]. There is a wide range for speculations: on the one hand the action of primaquine could be more severe in protein deficiency because of a reduced protection against the oxidative stress; but on the other hand the action of primaquine could be weaker in protein deficiency since less catabolites of a high redox-potential should be produced.

The degree of protein deficiency in our study was a severe one and caused loss of apetite; to exclude any other difference except the intended different intake of protein, all rats received the same restricted amount of feed. High primaquine doses were chosen in order to observe

a hemolytic effect. The dose of 30 mg/kg bodyweight is approximately 100 to 150 times higher than those used in human malaria therapy.

The protein deficiency was manifested by many parameters – the growth, hematologic values and serum proteins. The actions of primaquine were obviously, the rats have shown weight losses; increased weights of liver, spleen and kidney; and reduced hematologic values. Beside the reaction of the rats to primaquine, there was to note a general stress for the rats originating from the procedures of the experimental phase, as further food restriction, blood losses and regular applications with the stomach tube. This general effect of the experiment onto the rats has to be considered in the interpretation of the results, making the data less sensitive regarding the specific primaquine action.

The general parameters observed showed protein-depleted rats had more severe reactions due to primaquine than rats fed an adequate protein diet. Rats of the LP-groups showed more depressed food intake and higher weight losses than those of HP-groups. Although the death rate was too small to draw definitive conclusions, it points to the same direction: 4 rats died in LP-groups compared with one in HP-groups. The general reaction of rats was comparable with the picture of a self-limiting, compensating process, which characterizes primaquine. Within the first week of primaquine administration the rats appeared more stressed, than later on. In accordance with this is the observation, that four rats died within the first week of drug application, whereas only one the other week. But it has to be pointed out, that this mentioned primaquine feature was not proved by the biochemical data. The hemolytic data were not lowest within the first days of primaquine application, as one should expect from the known reaction on humans; they were lowest at the end of the experiment.

Rats receiving primaquine had increased weights of organs, especially of those involved in detoxification – liver, kidney and spleen. Since the increase in weight was an absolutely one, the main reason for it, should be more directly due to primaquine. It is well known, that in most cases the liver is enlarged after drug administration, and very often this is accompanied by an accelerated drug metabolism [15]. Whether this holds true in this experiment cannot be judged from the collected data. It is interesting to note, LP-rats did not show increased liver weight, and one

could speculate they are not able to respond to primaquine by an increased turnover of it due to protein deficiency.

The considerable increase in spleen weight after primaquine administration has to be stressed. The spleen is absorbing from the blood stream "oxidized erythrocytes", which are produced by the action of primaquine [6]. A high variance of the reaction of rats was observed, it leads to the suggestion that there could be different susceptibility to primaquine within the rat strain we used. There were too few rats to conclude a heterogenity, but the uneven distribution of spleen weights was remarkable. From all rats receiving high doses of primaquine, 7 had very large spleens, averaging 0.70 g/100 g body weight, compared with the remaining eight which had in average 0.36 g/100 g body weight. Strengthened is this suggestion by similar variations observed, *e. g.* it was seen that some rats appeared strongly weakened by primaquine and others tolerated the same dose quite well. Comparing data of those rats having large spleens with those having moderately increased spleens, a stronger action of primaquine at the former ones is obvious, to mention only one example: Met-Hb is there 6.5 % compared with 3.8 %. Comparing the different protein intake, there was no significant difference in the action of primaquine regarding organ weights, except those for liver mentioned already.

The action of primaquine could be observed on the hematologic data, despite an marked influence of the general experimental stress on the rats. Both stresses diminished the hematologic values. Hb was more lowered by primaquine than the erythrocyte number and size. It seems primaquine reacts primarily onto Hb, most probably by oxidative processes. The low protein nutritional status had a negative effect in this respect, Hb of rats of the LP-groups was more depressed than in rats of the HP-groups.

The erythrogram showed that erythrocytes from protein-depleted rats were more resistant to hemolysis induced by hydrochloric acid than those collected from rats on high-protein feed. This observation we could prove on subsequent studies [17].

The erythrogram showed further that the general stress of the experiment led to increased resistance of the erythrocytes. The time for maximum rate of the hemolysis was prolonged in the HP- and LP-groups. The hemolysis ended later, but only in the HP-groups. A bigger portion

of erythrocytes is more resistant or younger when the maximum rate of hemolysis occurs later. The duration of the hemolysis depends on the most resistant erythrocytes. Both parameters together can be used as an indicator for the distribution within the erythrocyte population [20]. The observed increase in the resistance due to the experimental stress could be originate from increased erythropoiesis stimulated by blood losses.

Primaquine caused a decrease in the resistance of the erythrocytes – maximum rate and the end of the hemolysis were shortened according to the erythrogram. The erythrocyte populations became more homogenous. Our observations correlate with other reports that primaquine increases osmotic and mechanical fragility of erythrocytes [7]. High protein intake seemed to have a positive influence regarding the stability of erythrocytes under the condition of the erythrogram.

Primaquine did cause an increase of the β-globulin fraction in serum, which was slightly higher in protein depleted rats than those receiving high-protein diet. It could indicate hepato- and/or nephrotoxic actions of primaquine.

The catabolites of primaquine have oxidant properties, causing direct or via H_2O_2 methemoglobinemia [7, 9], which was observed in this experiment, too. Availability of protein had no influence on degree of methemoglobinemia. The Met-Hb-reductase system does not seem to be reduced to a critical low level by the protein deficiency having had in this experiment.

It is well known that the red cells are protecting themselves against oxidative damage, as *e. g.* caused by primaquine metabolites, by a system which contains GSH in its reduced form [2, 4, 6, 26]. Other parts of this system are the enzymes G-6-PD and GSH-reductase. Therefore we measured the activities of both enzymes and the concentration of GSH. Primaquine caused an increase of the GSH-reductase activity, which was independent from the nutritional status of the rats; but it did not alter the G-6-PD-activity, as one would have been expected. The GSH levels were elevated only in rats of the HP-groups after primaquine application. Higher GSH levels are protecting erythrocytes against primaquine. This corresponds well with the observed hematologic data and with other reports. It is known that primaquine leads initially to reduced levels of GSH, but later on to increased levels. The compensating mechanism is mainly

due to generally higher enzyme activities of a younger erythrocyte population emerging from the action of primaquine [5]. From our data, it is not possible to conclude, whether the altered activities of enzymes and concentrations of metabolites are due to younger erythrocytes or due to metabolic adaptations or due to both.

The pyruvate-lactate ratio was used as an indicator of the redox-status of blood. Primaquine causes a greater need for NADH, *e. g.* due to higher turnover of the Met-Hb-reductase [12], but NADH is needed for the reduction of pyruvate to lactate, too. Both reactions do compete with the result, that primaquine administration leads to elevated pyruvate levels and higher pyruvate-lactate ratios [6]. This study could confirm this, but only for rats of the HP-groups.

Comparing all results of the experiment, one can conclude that protein depleted rats did show more adverse reactions to primaquine than their mates fed with the high protein diet. Best indicators for this conclusion were the general reaction of rats, the body weight losses, depression of food intake and lowered hematologic values. The comperatively small negative effect of the reduced protein intake on primaquine actions may have an explanation in the possible two opposite influences of protein deficiency. On the one hand the availability of the toxic catabolites of primaquine could be reduced due to reduced drug metabolism of liver in protein deficiency, but on the other hand the toxic actions of the catabolites, especially to the erythrocytes, could be enhanced. Despite the known huge variance of drug action and metabolism between different species and the known difficulties to transfer results from one species to another one, the experiment gave a hint about the relevance of protein deficiency for human beings in relation to adverse primaquine reactions. Comparing the severeness of protein deficiency, the rather high dosage and the relatively small differences observed on rats with the normally occuring situations amongst human populations, the following statement seems to be valid: Primaquine in normal prophylactic and therapeutic doses will have no severe side effects to persons without an inborn genetic defect, but with moderate protein undernutrition.

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