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Interactions between Malnutrition and Primaquine studied on Wistar Rats

II. Complex Vitamin B Deficiency and Thiamine Deficiency

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Summary: The action of primaquine was investigated on male Wistar rats depleted on the vitamin B complex (approximately 50 % of their requirement for optimal growth), on thiamine (approximately 50 % of their requirement for optimal growth), and pair-fed control animals. There was only a marginal increase in adverse primaquine reactions in the malnourished, especially in the thiamine deficient rats.

1. Introduction

The commonly used antimalaria drug primaquine can produce hemolytic anemia, especially in G-6-PDH deficient traits [18]. In the forgoing report [12] it was shown that protein deficiency slightly increased the primaquine toxicity. Several studies brought evidence that deficiencies of B-vitamins altered the activity of the hepatic drug-metabolizing enzymes [3] and thus the actions of nonnutrients [13]. The most active hemolytic principles of primaquine are its catabolites which are characterized by a quinoline-quinone redox structure. In the catabolism of primaquine riboflavin and niacin could be involved because of their requirements for the flavoprotein cytochrome P 450 reductase and for the pyridine nucleotides, respectively. Thiamine and other B vitamins, are of general importance for many metabolic liver functions.

Some B vitamins are involved in the erythropoesis and are necessary for the integrity and stability of the erythrocytes [19, 20].

Deficiencies of B vitamins could have different implications considering their effects on the primaquine action. Probably the concentrations of the active

primaquine catabolites could be reduced, due to the decreased activity of the hepatic mixed functional oxidases in vitamin B deficiencies [9, 13]. So the primaquine toxicity would be reduced. On the other hand the synthesis and the stability of erythrocytes could be diminished in vitamin B deficiencies, and would lead to an increased susceptability to primaquine, as indicated by the fact, that thiamine deficiency reduces the activity of G-6-PDH [17]. Still there are further interactions possible between primaquine and vitamin B's only to mention: altered absorption and distribution, and changed reactions with the receptors of primaquine and/or its catabolites.

There is studied only one of the many possible interactions between primaquine and the different B vitamins. This investigation showed almost no influence of a riboflavin deficiency on the hemolytic action of primaquine [2].

It was the aim of the here reported study to investigate on rats the overall effects of a complex vitamin B deficiency and of a thiamine deficiency on the actions of primaquine.

2. *Material 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 the content of some B vitamins and of thiamine, respectively (Tab. 1). The feed with the complex vitamin B deficiency (CD) was lacking on some further vitamins, which are regarded as non-essential for rats because they are produced by the intestinal microflora of rats. The intake of the B vitamins and of thiamine by rats given the deficient diets can be considered as approximately 50 % of the requirement for optimal growth. The rats were pair-fed and had free allowance to water. Rats were maintained in individual wirebottomed cages in an airconditioned room (24° C, 50 % humidity).

After 10 weeks feeding the deficient diets the growth of rats ceased significantly. The rats of the three different fed groups were each subdivided into two groups of ten. Every rat of the control groups (C_c, CD_c, TD_c) received on 5 subsequent 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, and the rats of the primaquine groups (C_p, CD_p, TD_p) received in the same manner 30 mg primaquine[1]/kg bodyweight. After 8 days of drug administration every week two rats out of each group were killed by bleeding after anesthesia by diethylether. Rats received thus between 8 to 36 (average 22) doses.

Abbreviations:

C = rats receiving the control food
CD = rats receiving a food which is deficient on vitamins of the B-complex
TD = rats receiving a food which is deficient on thiamine
C_c, CD_c, TD_c = rats treated as control
C_p, CD_p, TD_p = rats of the primaquine treated groups
G-6-PDH = Glucose-6-phosphate dehydrogenase, D-glucose-6-phosphate: NADP oxidoreductase (E. C. 1. 1. 1. 49)
GSH = glutathione

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

Hb = hemoglobin
Hc = hematocrit, packed cell volume
MCHC = mean cell hemoglobin content
Met-Hb = methemoglobin
NADP (NADPH) = nicotinamide-adenine-dinucleotide phosphate (reduced form)

Recorded was food intake (daily), body weight (twice per week) and weights of liver and heart (at autopsy). Blood was collected from tail vene (between 3rd and 7th day before, 2nd and 7 th day after the begin of drug administration and immediately prior to killing) for the determinations of Hb as cyanmethemoglobin [14a], Hc (Hawksley Microfuge) and the erythrogram (recording of the hydrochloric acid induced hemolysis of an erythrocyte suspension in 0.9 % NaCl-solution) [11, 15]. During killing heparinized blood was collected by bleeding for

Tab. 1: Composition of the experimental diets [1]

	C [2]	CD [2]	TD [2]
Vitamin-free casein	250.0	250.0	250.0
Methionine (g)	2.0	2.0	2.0
Saccharose (g)	550.0	550.0	550.0
Rice starch (g)	84.0	84.0	84.0
Lard (g)	62.5	62.5	62.5
Oil (g)	7.5	7.5	7.5
Retinyl acetate (g)	0.1	0.1	0.1
Calciferol (mg)	5.5	5.5	5.5
α-Tocopherol acetate (mg)	114.5	114.5	114.5
Choline chloride (g)	1.7	1.7	1.7
Salt mix USP XIII (g)	40.0	40.0	40.0
Thiamine (mg)	22.0	1.0	1.0
Riboflavin (mg)	25.0	1.5	25.0
Pyridoxine (mg)	25.0	1.5	25.0
Calcium pantothenate (mg)	62.5	5.0	62.5
myo-Inositol (mg)	112.5	20.0	112.5
Cyanocobalamin (μg)	30.0	4.0	30.0
p-Aminobenzoic acid (mg)	112.5	0	112.5
Niacin (mg)	100.0	0	100.0
Biotin (mg)	0.5	0	0 5
Folic acid (mg)	2.0	0	2.0
Ascorbic acid (g)	1.0	0	1.0
Menadione (mg)	50.0	0	50.0

[1] Diets are according [5] and [10].
[2] See: Abbreviations.

the determinations of Met-Hb [14b] and glutathione [1], G-6-PDH (Boehringer, biochemical information) in whole blood, and for transketolase (E. C. 2.2. 1.1.) [16, modified by Hoffmann-La Roche] in the erythrocytes.

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

3. Results

As reported already in our earlier study [12], it was observed that some rats were especially susceptible to primaquine, others tolerated rather well. Generally the reactions were more severe in the beginning of primaquine application and weakened after few days. Tab. 2 shows the number of the died animals, all of them except one was dying within the first week of primaquine administration.

Tab. 2: Number of rats dying in the different groups (n = 10 in each group)

	Control	Primaquine
C[1]	0	2
CD[1]	0	3
TD[1]	1	4

[1] See: Abbreviations.

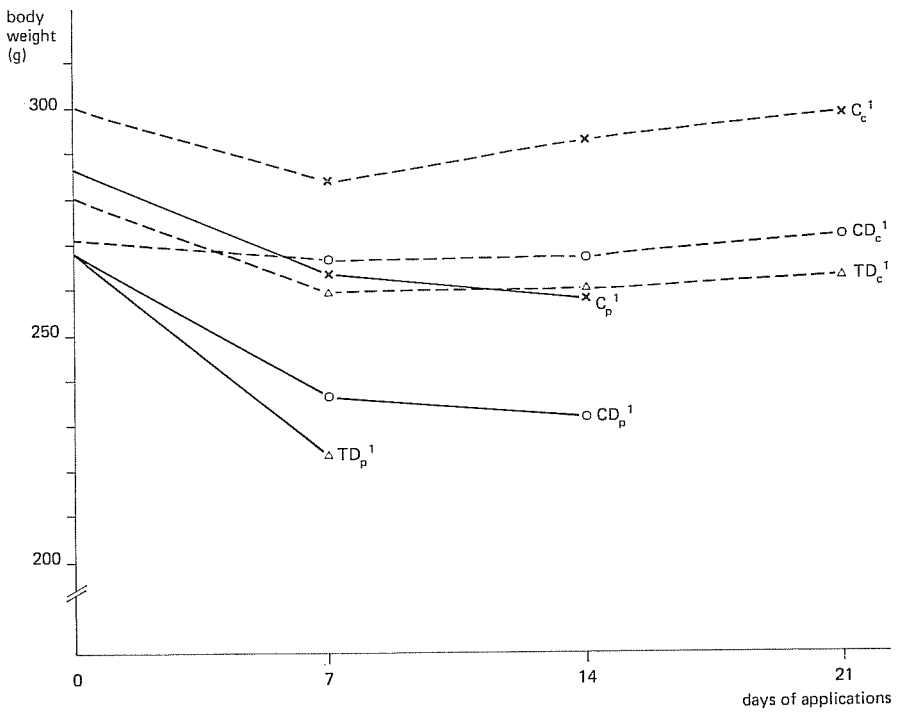

Fig. 1: Body weight of rats after application of primaquine (———) or buffer solution (– – – –) (n = 10 at the beginning, if lower then n = 5 no result is recorded). [1] See: Abbreviations.

Tab. 3: Activity of the erythrocyte transketolase (μMol) sedoheptulose-7-phosphate/min/litre erythrocyte suspension of Hc = 35 %, 35° C) (n = 10 in each group)

	Control	Primaquine
C[1]	126 ± 27	107 ± 45
CD[1]	13 ± 5	26 ± 10
TD[1]	21 ± 6	21 ± 4

[1] See: Abbreviations.

Prior to the drug administration the food intake was between 11 to 12 g/rat/day, afterwards it was reduced, especially within the first week, being 8 to 10 g/rat/day. But whereas the rats of the control group (C_c) did recover to the formerly common intake, the primaquine treated rats lost continously on appetite (2–6 g/rat/day).

The deficiency of B vitamins was leading to a depressed growth compared to the control (C_c), despite of the pair-feeding. The weight difference (Fig. 1, day 0) at the beginning of the primaquine administration was statistically significant ($p < 0{,}05$). Further the degree of thiamine deficiency can be judged by the reduced activities of erythrocyte transketolase (Tab. 3). The weight development during the experimental phase is shown in Fig. 1. As observed with protein-deficient rats [12] the general stress of manipulations of the rats during the experiment caused weight losses in rats of all groups. But the control rats receiving buffer recovered, whereas the rats receiving primaquine lost on weight continously ($p < 0{,}05$). Comparing the different weight losses in the different fed rats, only the losses of rats with specific thiamine deficiency (TD_p) were significantly greater than those of C_p- and CD_p-rats ($p < 0{,}05$).

The relative wet weights of liver and heart are shown in Tab. 4. There is no difference which is statistically significant. It seems that primaquine caused an enlargement of the liver in control rats (C_p) and those with complex deficiency on B vitamins (CD_p), but not in isolated thiamine deficiency (TD_p).

The hematologic values are summarized in Fig. 2. It compares the values prior to the experimental period with those gained immediately before killing

Tab. 4: Relative wet weights of liver and heart of rats (g/100 g body weight) (n = 10 in each group)

	C_c[1]	CD_c[1]	TD_c[1]	C_p[1]	CD_c[1]	TD_p[1]
Liver	3.18 ± 0.79	3.62 ± 0.74	3.64 ± 0.77	3.84 ± 0.56	4.32 ± 0.55	3.53 ± 0.41
Heart	0.30 ± 0.03	0.31 ± 0.09	0.36 ± 0.12	0.33 ± 0.04	0.40 ± 0.08	0.34 ± 0.11

[1] See: Abbreviations.

the rats. The results of the determinations done between those two points are not reported. They brought no further information, since their values were intermediate to those cited in Fig. 2. As observed with protein deficient rats [12] as well on vitamin B deficient animals no signs of an early hemolytic crisis could be registered.

Vitamin B deficiencies had no significant influence on the hematologic values. Primaquine had significant negative effects on the erythrocytes, as seen in the reduced values, but there was no significant difference regarding the response of the different fed rats, only a slight tendency that rats with isolated thiamine deficiency (TD_p) tolerated primaquine better than their mates (C_p, CD_p).

Primaquine did cause methemoglobinemia (Tab. 5), but the degree of it was independent of the vitamin B nutriture of the rats.

The activity of G-6-PDH, which produces reductive NADPH, in erythrocytes, and the level of GSH in blood were independent of the vitamin B intake of rats (Tab. 5). Primaquine application caused an increase, but this reaction was independent of B vitamins, except that in isolated thiamine deficiency the GSH level was reduced after primaquine administration (TD_p).

Tab. 5: Results of determinations in blood and erythrocytes of rats after the experimental phase (n = 10 in each group)

	C_e[1]	CD_e[1]	TD_e[1]	C_p[1]	CD_p[1]	TD_p[1]
Met-Hb[2] ...	1.2 ± 0.4	1.2 ± 0.5	1.5 ± 0.5	8.6 ± 7.8	5.2 ± 2.4	4.4 ± 1.7
GSH[3]	26.4 ± 3.9	25.6 ± 3.0	28.8 ± 4.0	34.1 ± 7.9	29.2 ± 9.8	23.9 ± 3.1
G-6-PDH[4] .	52.4 ± 9.1	47.5 ± 5.8	49.7 ± 7.1	64.8 ± 5.5	56.4 ± 5.1	59.3 ± 2.3

[1] See: Abbreviations.
[2] In % of Hb.
[3] In mg/100 ml blood.
[4] In µMol/min/l Litre of erythrocyte suspension (Hc = 35 %), 25° C.

4. Discussion

The aim of the reported studies was to investigate more the overall reactions of primaquine onto malnourished rats, than the effect of nutrient deficiency onto specific reaction of the drug with particular sites of an organism. Several features of the discussion of the results of this study will be very similar to the former one related to the primaquine actions onto protein-depleted rats [12].

Primaquine can cause hemolysis, and stimulates thus the erythropoesis. The older erythrocytes are more susceptible to primaquine than younger ones. Subsequently the erythrocyte population becomes younger and more resistant [18]. Primaquine is catabolized mainly in the liver. The main reactions are hydro-

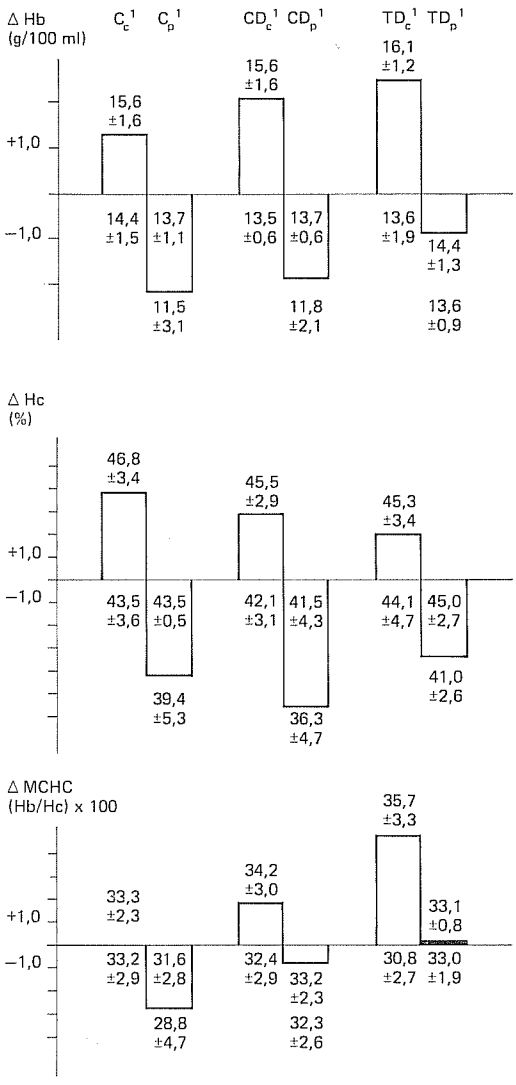

Fig. 2: Hematologic values of the rats before and at the end of the experimental period (n = 10, for each group).

xylation in position 5, demethylation and conjugation with glucuronic acid and with sulfate. The quinone catabolites are the hemolytic active principles due to their oxidative properties [4]. Those catabolites are able to oxidize sulfhydryl containing compounds, e. g. GSH, the stability of the erythrocyte membrane decreases, the redoxstatus is changed, and thereby the activity of G-6-PDH becomes increased [6].

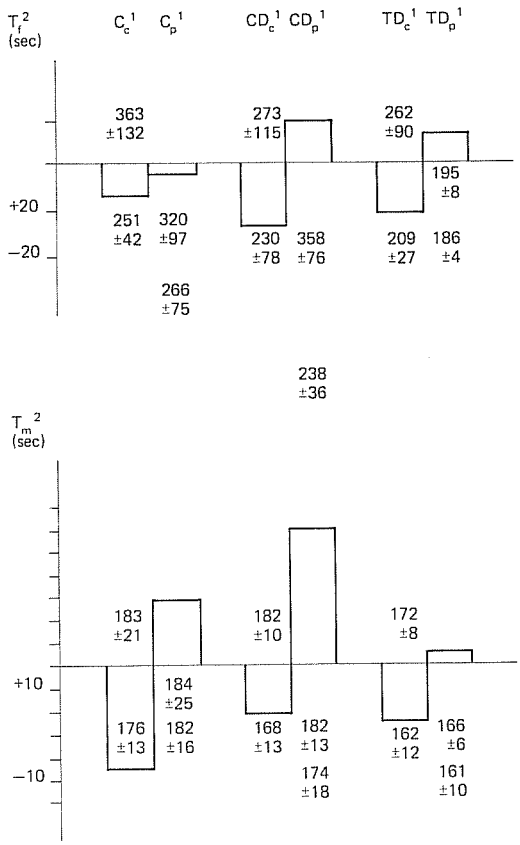

Fig. 2 (continued)

[1] See: Abbreviations.

[2] Erythrogram: time of the maximum rate (T_m) and of the end (T_f) of the hemolysis of an erythrocyte suspension in 0.9 % NaCl-solution induced by 0.01 N HCl.

In deficiencies of the B-vitamins the activity of several hepatic drug metabolizing enzymes is reduced, like those of barbital hydroxylase or glucuronyl transferases [3, 9]. Many B vitamins are involved in the erythropoesis and in the metabolism and stabilization of erythrocytes [19, 20].

Many hypothesis on possible results of the interactions between primaquine and vitamin B deficient rats could be postulated. A reduced drug metabolism leading to a lowered catabolite concentration could cause a better tolerance of primaquine. In nutrient deficiency the organism would be more susceptible to the actions of the drug. Both effects could be balance each other.

The effects of two different vitamin B deficiencies were investigated and compared. A complex deficiency of several B-vitamins (CD), which is more comparable to natural human situations, and a specific thiamine deficiency

(TD). Since it was known that riboflavin deficiency did not alter the primaquine action [2], the experiments with CD should reveal whether there could be an effect with other B vitamins. The results with the general, but balanced CD should be further compared with the isolated, unbalanced TD.

The deficiencies of the B vitamins generated in the experiments were manifested by anorexia and depressed growth and the low activities of the erythrocyte transketolase showed the degree of the thiamine deficiency. The effects of primaquine could be produced, but only with rather high doses, which were close to lethal ones. Primaquine weakened the rats generally, caused a lower food intake und lowered the blood values. Again the known pattern of the primaquine action on humans, a self-limiting, compensating process could not be observed, except in the general appearance of rats and the death rates. In the first week of primaquine application the rats appeared severely stressed, they were very apathetic, several died in this period. Afterwards the rats recovered, they became more alert, despite of the same treatment. There was to note again the general stress of the experimental manipulations on the rats: the regular applications with the stomach tube and the blood losses. When comparing the effects of primaquine on the different nourished rats, there was to observe only a slightly negativ effect of the vitamin B deficiencies, surprisingly more by isolated TD than by the general CD. 4 rats from the TD, 3 rats from the CD and 2 rats from the control group died after primaquine application. The weight losses due to primaquine were the same in C and CD, but the thiamine deficient TD rats lost significantly more weight. It should be noted, that control rats were not normal grown rats due to the pair-feeding arrangement. They had the same low energy intake than the anoxeric vitamin B deficient animals. The food intake of the rats was reduced by primaquine to the same extent in C and CD, and again more in TD. The weights of liver and heart did increase slightly due to primaquine, with the exception of the TD animals.

The general experimental stress seems to stimulate the erythropoesis, the hematologic values of all control rats (C_c, CD_c, TD_c) were increased during the experimental phase compared with the values at the begin of it. So in contrast to protein deficiency, which led to anemia [12], the experimental stress had no negative influence in vitamin B deficient animals. This finding is in accordance with old experiments of the effects of B vitamins on blood regeneration in rats [7, 8]. There it was shown that only the deficiencies of riboflavin and folic acid had some negative influence.

Primaquine did cause a decrease of all investigated hematologic parameters, but its effect was independent of the vitamin B availability. In contrast to the above mentioned other observations, there was a trend that in TD primaquine was slightly better tolerated than in CD. The methemoglobinemia caused by primaquine seemed to be independent of B vitamins. The indicators of a re-

ducing capacity, the activity of G-6-PDH and the level of GSH, were increased by primaquine as one could expect, but again there was no influence of vitamin B's.

The conclusion from all results is, that the toxic reactions of primaquine are almost independent of B vitamins. Only at the highest doses and in rather severe deficiency states a trend to more adverse reactions of primaquine could be registered. It is interesting to note, that a general – a balanced – deficiency of many B vitamins seemed to be better in comparison with the isolated – unbalanced – thiamine deficiency. One could speculate, whether such unbalanced artificial experimental diets indicate an interaction between the vitamin B's. Not only the amount of a B vitamin as such, but also its relation to the other vitamin B's could be of importance, as it is known for fatsoluble vitamins or minerals.

Considering the degree of vitamin B deficiencies occuring among the different human population groups, there will be no negative influence of them on the toxicity of primaquine if applicated in doses used as malaria prophylaxis and therapeutics.

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