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Combined effect of ascorbic acid deficiency and underfeeding on the hepatic carnitine level in guinea-pigs

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Summary. Liver carnitine level decreased from 249±16.1 nmoles/g (mean ±SEM) control value to 148±9.8 nmoles/g (59.4%) in ascorbic acid deficient guinea-pigs, while in the underfed ('pair-fed') group it decreased to 181±14.1 nmoles/g (72.6%). Underfeeding also resulted in lower ascorbic acid levels; the depression of carnitine in the underfed animals could be prevented by an overdose (200 mg daily) of ascorbic acid.

According to the current view, carnitine (L-3-hydroxy-4-N-trimethylaminobutyrate) in combination with the related enzymes operates to transfer activated fatty acids from the cytoplasm into the mitochondrial matrix[1]. The pathway of carnitine biosynthesis has been well explored in animal[2,3] and human[4] studies. The proposed scheme involves 2 hydroxylating steps. The hydroxylase enzymes are dependent on α-ketoglutarate and ascorbic acid (AA)[2,5]. The stoichiometry and exact mode of action of AA, unlike those of α-ketoglutarate, were not defined by these in vitro studies[2,5].

In earlier studies of the in vivo effect of AA deprivation was found a severe depression of carnitine levels in guinea-pigs (unpublished). Later, it turned out that the reduced food ingestion, a concomitant symptom in the AA deficient state, can itself evoke carnitine depletion in both male and female animals. We reported depressed carnitine levels in the liver, serum and muscles of underfed male guinea-pigs[6]. The present work is devoted to revealing the relationship between AA deficiency and partial starvation in decreasing carnitine levels. For this purpose we determined the AA levels as well as those of carnitine in the livers of AA deficient and of underfed guinea-pigs supplied with normal amounts or an overdose of AA.

Methods. Young male guinea-pigs were used, with 350 g initial and 446 g terminal average body weights. Growth in itself did not alter the hepatic carnitine level, as was shown by separate measurements. An AA deficient diet with a low carnitine content (17.2 nmoles/g) was prepared as described previously[6]. This diet allowed the same growth as the commercial chow containing 50.2 nmoles/g carnitine[6]. The animals were fed this diet, and all of them were given 10 mg AA s.c. on every 2nd day for 2 weeks before starting the experiment. Then they were divided into 6 groups, as shown in the table. Average food consumption of the ad libitum fed AA-receiving animals (groups 1 and 3) was 30–40 g a day, 224 g in the 1st week and 266 g in the 2nd week of the experiment. The food supply for the underfed animals (groups 2 and 4) was limited to what was consumed by the AA-deficient animals (groups 5 and 6). In other words, groups 2 and 4 were 'pair-fed'. Quantitatively, the average consumption of the animals in groups 2, 4, 5, 6 gradually decreased (or was limited) to 10 g/day, so that they consumed 200 g in the 1st week and 115 g in the 2nd. An overdose of AA was supplied for groups 3 and 4 partly s.c. (100 mg/day) and partly per os (about 100 mg/day) with their drinking-water. On the 15hh day of the experiment the animals were decapitated between 08.00 and

Carnitine and ascorbic acid (AA) levels in the liver of AA deficient and underfed guinea-pigs

Group number and No. of animals	Terminal b.wt (g)	AA supply (daily mg)	Feeding	Carnitine in 1 g wet liver (nmoles)	AA in 1 g wet liver (μg)
1, n=6	446±12.2	5, s.c.	Free**	249±16.1	146±14.1
2, n=6	347±5.6	5, s.c.	Limited	181±14.1[a]	89±9.24[a]
3, n=6	437±16.1	100, s.c. 100, per os	Free**	269±20.1	213±17.2
4, n=6	349±16.7	100, s.c. 100, per os	Limited	255±21.4	167±11.2[d]
5, n=6	302±20.1	No	Free**	148±9.8[b]	Traces
6*, n=5	350±18.1	No	Free**	195±10.2[c]	Traces

Animals (initially weighing 352±11.8 g) were fed an AA deficient diet under the conditions indicated until the 15th day, when they were sacrified. *Animals in group 6 were sacrified on the 8th day. Values are means±SEM. **Free access to food. Levels of significance: [a]$p<0.01$; [b]$p<0.001$; [c]$p<0.05$; all vs group 1; [d]$p<0.05$ vs group 3.

10.00 h, a portion of the liver was freeze-clamped and stored under liquid N_2 until processing. Total carnitine, the sum of free and esterified carnitine, was measured after alkaline hydrolysis, using a radiochemical method[7] with the following modification; thiol groups were trapped with 0.2 mM N-aethyl-maleinimide. Total AA, the sum of AA and dehydro-AA, was measured as described[8]. Authentic L-carnitine was a kind gift from Sigma-Tau (Rome).

Results and discussion. The table shows a marked decrease of liver carnitine in AA deficient guinea-pigs (groups 5 and 6), which is consistent with our early findings. On the other hand, a depletion of liver carnitine is also seen in the underfed ('pair-fed') animals receiving a normal AA dose (group 2). This finding confirms our previous report[6], although in that experiment[6] the food restriction was more severe (10 g/day) and as expected the decrease in liver carnitine was more pronounced. However, these results are in contrast with those[9] which did not find a decrease of liver carnitine either in 'pair-fed' or even in scorbutic guinea-pigs. That paper[9] reported a carnitine decrease only in the muscles of AA deficient guinea-pigs. The reason for this discrepancy is not yet clear.

The action of AA deficiency is easily conceivable on the basis of in vitro studies[2,5]. It is of importance that limited feeding can evoke a decrease of AA level in the liver (table, groups 2 and 4). This finding raises the possibility that underfeeding acts via depression of tissue AA level in lowering the carnitine level. Whether the AA depression seen in group 2 ($p < 0.01$) was sufficient in itself to lower the carnitine level is difficult to investigate. So this finding cannot exclude some other kind of mechanism operating in the underfed state. Yet the result that an overdose of AA prevented the effect of underfeeding in depressing the carnitine level (compare group 3 with group 4) gives support to the view that in the combined effect of AA deficiency and underfeeding the ascorbic acid deficiency is the primary factor.

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A further naphthoquinone derivative from the fungus *Hendersonula toruloidea*

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Summary. A further yellow naphthoquinone derivative was isolated as a metabolite from several human pathogenic strains of *Hendersonula toruloidea* Nattrass. It was identified as 2,7-dimethoxy-5-hydroxy-6-(1-hydroxyethyl)-1,4-naphthoquinone by means of physico-chemical methods.

In a previous paper[3] the isolation and identification of 2,7-dimethoxy-5-hydroxy-6-(1-acetoxyethyl)-1,4-naphthoquinone (compound A) and 2,7-dimethoxy-6-ethyl-5-hydroxy-1,4-naphthoquinone (compound B) from several human pathogenic strains of *Hendersonula toruloidea* Nattrass have been described. The compounds showed R_f-values of 0.31 and 0.47 on silica gel thin-layer plates using the solvent system toluene/acetone = 95:5 (v/v). During further investigations a yellow spot (R_f 0.19) of a minor metabolite (C) from some strains was detected.

The identification was undertaken with *H. toruloidea* CBS 137.77, since this strain produced compound C in sufficient quantities to warrant successful isolation. The strain was grown on malt agar in 225 petri dishes for 20 days at 24 °C. To avoid possible hydrolysis, the cultures were extracted with chloroform instead of ethyl acetate, which was used in previous experiments. The evaporated extract (496 mg) was chromatographed on a 30 × 3 cm silica gel/kieselguhr (2:1 w/w) column using toluene/acetone = 4:1 (v/v). The eluted yellow fraction was applied to several 2 mm silica gel thick-layer plates (Merck) and developed with toluene/acetone = 95:5 (v/v).

The yellow bands comprising compound C were scraped off and eluted with chloroform/methanol = 2:1 (v/v). For a final purification the fraction was chromatographed after concentration on 0.5 mm silica gel plates with the solvent system toluene/acetone = 4:1 (v/v). C was obtained as optically inactive orange needles by twice recrystallizing from toluene/light petroleum 60–80 °C (1:1, v/v). C showed an UV-visible spectrum similar to those of A and B, indicating the same chromophoric system.

High resolution MS of C gave the formula $C_{14}H_{14}O_6$. The MS- and UV-visible data indicated that C compared with B must have a hydroxyl group in the ethyl side-chain. Since the mass-spectrum showed the elimination of an aliphatic methyl it was concluded that the side-chain was the 1-hydroxyethyl group. Confirmation of this conclusion was obtained by examination of the ^{1}H-NMR-spectrum. Typical resonances were observed: a doublet ($-CH_3$) at 1.55 (δ in ppm, J = 6.75 Hz), a doublet (−OH) at 3.72 (J = 11.95 Hz), a double quartet (1H) at 5.30 (J = 11.95, 6.75 Hz) and a singlet (−OH) at 12.88. The signals at 3.72 and 5.30 pointed to a slow rate of exchange of the proton of