ACTA CHEMICA SCANDINAVICA 18 (1964) 2179–2181

Studies on the Effects of the Nutrition on Antioxidant Levels of the Body

II. Antioxidant Levels in Livers of Rats on a Necrogenic Diet

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A necrogenic diet containing *Torula* yeast as protein source produces an increase of fat-soluble and a decrease of water-soluble antioxidants in the livers in comparison with rats receiving normal colony diet. Tocopherol and selenium both protect against necrosis but do not restore normal antioxidant levels.

In our previous paper [1] studies on the level of antioxidants in blood, liver, and brain of chicks receiving an encephalomalacia-producing diet were reported. A decrease in the concentration of water-soluble antioxidants of the brain compared with animals supplemented with vitamin E was observed. Studies on the effects of the diet on the antioxidant level of livers of rats on a necrogenic diet in comparison with animals protected with selenium or tocopherol or receiving a normal colony diet are reported in this paper.

EXPERIMENTAL

Newly weaned female albino rats were divided into three groups and placed on the experimental diets. Group 1 was fed a necrogenic diet, group 2 received the same diet supplemented with D,L-α-tocopherol acetate, 0.2 mg/g, and group 3 the diet supplemented with selenium dioxide, 1.4 μg/g diet.

The necrogenic diet had the percentage composition: *Torula* yeast (Lake States Yeast Corporation, Rhinelander, Wisconsin), 40; salt-mixture,[2] 5; vitamin mixture,[2] 0.5; choline chloride, 0.2; and sucrose, 54.3. Vitamins A and D were given orally as an aqueous solution,[2] 0.1 ml twice a week, corresponding to vitamin A, 57 i.u., and vitamin D_3, 5.7 i.u. per rat daily.

The animals were inspected daily. The first case of fatal liver necrosis was observed after 20 days, and the animals were killed during the period varying from 20 to 28 days on the diets. The rats were weighed daily, and animals showing weaknes or sudden growth retardation were preferentially chosen. The animals were killed by chloroform, autopsied, and the livers carefully examined for the presence of necrotic areas. Eight out of 24 animals on the necrogenic diet died during the experiment and all disclosed necrotic livers. However, only the livers of the remaining 16 animals which were killed by chloroform

were used for antioxidant determinations; 12 of the 16 livers showed smaller or larger necrotic areas. Simultaneously with the animals on the necrogenic diet, the same number of animals from groups 2 and 3 were also killed and their livers examined after autopsy. No incidence of necrosis was observed in the two groups.

Samples of the livers of all groups were used for the determination of water-soluble antioxidants which was carried out as described in our previous paper. Other samples weighing about 500 mg were used for the determination of fat-soluble antioxidants, which was carried out in the same manner as was used for chicken brain in the previous paper.

RESULTS

The results are presented in Table 1. For comparison the normal content of the three groups of antioxidants found in livers of rats on normal colony stock diets is included in the table.

DISCUSSION

The results presented in Table 1 show a significant decrease of the water-soluble antioxidants of the livers of the animals reared on a necrogenic diet in comparison with those receiving the normal colony diet. The decrease was not prevented by selenium or tocopherol which both prevented any onset of necrosis.

Only the non-protein fraction was affected. The non-protein water-soluble antioxidants of the livers of the three experimental groups showed a manifest decline in comparison with livers from the normal colony group. When the antioxidant of the proteins is calculated, as the difference between total and non-protein activity, it is found to be unaffected by the diet.

The most prominent water-soluble antioxidants of the liver are glutathione and ascorbic acid.[3] The results agree well with earlier observations by Lindan and Work [4] on the decline of glutathione and ascorbic acid in livers of rats fed necrogenic diets. The decline is probably induced by the deficiency of sulphur-containing amino-acids in the diet.

The content of fat-soluble antioxidants of the livers of the animals on the necrogenic diet is several times higher than that of the control group receiving the normal colony diet. The content seems to increase further when the necrogenic diet is supplemented with tocopherol or, especially, selenium.

Table 1. Mean values with their standard errors for the antioxidant content of livers of rats given different diets.

Diet	Number of determinations	Water-soluble antioxidants μequiv./g dry matter		Fat-soluble antioxidants μequiv./g fresh weight
		Total	Non-protein	
Necrogenic diet	16	117 ± 4	19.3 ± 2.8	2.5 ± 0.1
do. do. + Se	14	105 ± 3	18.3 ± 1.1	3.7 ± 0.3
do. do. + vit. E	15	99 ± 3	17.6 ± 0.9	3.2 ± 0.3
Normal colony diet	14	131 ± 7	40.0 ± 1.4	0.57 ± 0.04

The most important fat-soluble substances in the liver reacting with diphenyl-picrylhydrazyl are the tocopherols and ubichromenols.[3] Diplock *et al.*[5] found a high content of ubichromenol in *Torula* yeast. As the necrogenic diet contains 40 % *Torula* yeast, the high content of fat-soluble antioxidants of the livers on the necrogenic diet probably indicate that dietary ubichromenol is deposited in the liver. The higher content of the livers of the tocopherol-supplemented group is probably due to the deposition of tocopherol in the livers, that of the selenium-supplemented group most likely indicates an especially high ubichromenol level which could be due to the ubichromenol-sparing effect of selenium observed by Edwin *et al.*[6]

The relationship dietary liver necrosis/peroxidation and antioxidants remains a puzzle. On one hand, the *fat-soluble* antioxidant, tocopherol, protects against necrosis, and it can be replaced by certain synthetic substances of antioxidant or redox character. On the other hand, large amounts of ubichromenol may occur in a necrogenic diet. Yet, ubichromenol is chemically rather closely related to tocopherol, reacts in the same way as a free-radical scavenger, is deposited in the liver, and has a delaying effect upon certain other symptoms of vitamin E-deficiency.

On one hand, a manifest decline in *water-soluble* antioxidants of the liver seems to be a constant feature in the development of the necrotic symptom. On the other hand, the substances which most effectively alleviate the symptoms do not influence the level of water-soluble antioxidants.

Thus, it is clearly established that no straight-forward relationship exists between the occurrence of the necrotic condition and the level of water-soluble or fat-soluble free-radical scavengers of the liver. If the mode of action of the protective substances is of antioxigenic nature, the activity must probably be performed in connection with enzyme systems, membranes or other anatomically or functionally localized seats.

Acknowledgement. This work was supported by a grant from *P. Carl Petersens Fond.*

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Received June 17, 1964.