

## Cerebral monoamine metabolism in guinea-pigs with ascorbic acid deficiency

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Guinea-pigs kept on a diet deficient in vitamin C showed, after 3 weeks, a marked decrease of ascorbic acid in brain and blood leucocytes as well as of the activity of alkaline phosphatase in blood plasma. Pair-fed animals did not exhibit these changes. The  $\alpha$ -methyl-*p*-tyrosine ( $\alpha$ MpT)-induced diminution of noradrenaline in the hypothalamus and the rest of the brain was attenuated in pair-fed animals, but restored in guinea-pigs deficient in ascorbic acid. The cerebral noradrenaline content (without administration of  $\alpha$ MpT) showed a decrease in both pair-fed and ascorbic acid deficient animals. The noradrenaline of the heart exhibited a similar tendency. The  $\alpha$ MpT-induced dopamine decrease in the striatum of ascorbic acid deficient animals was attenuated and the dopamine content (without  $\alpha$ MpT administration) decreased. Pair-fed animals showed a similar tendency. The striatal concentration of homovanillic acid (HVA) was diminished in both pair-fed and ascorbic acid deficient guinea-pigs. The cerebral content of 5-hydroxyindoleacetic acid showed a decrease in pair-fed as well as in ascorbic acid deficient animals. It is concluded that ascorbic acid deficiency enhances the turnover of brain noradrenaline, whereas under-nutrition without ascorbic acid deficiency (pair-feeding) diminishes the turnover of cerebral noradrenaline, 5-hydroxytryptamine and striatal dopamine.

Vitamins, e.g. of the B-complex and L-ascorbic acid, are known to act as cofactors in enzymatic reactions involved in the biosynthesis and metabolism of neurohumoral transmitters (aromatic monoamines,  $\gamma$ -aminobutyric acid etc.) in brain. An increase or a deficiency, e.g. in L-ascorbic acid, has been shown to cause alterations of neurotransmitters, especially of aromatic monoamines in brain and other organs (Thoa, Wurtman & Axelrod, 1966; Izquierdo, Jofré & Acevedo, 1968; Sears, 1969; Izquierdo, Jofré & Acevedo, 1972; Dashman, Horst & others, 1973; Ceaser, Hague & others, 1974; Jonsson, Lohmander & Sachs, 1974). However, in most of the deficiency experiments controls with pair-fed animals have not been carried out making it difficult to distinguish between the effects of vitamin deficiency and those of under-feeding.

In this presentation, the content and turnover of the endogenous cerebral noradrenaline, dopamine and 5-hydroxytryptamine (5-HT) in guinea-pigs with experimental ascorbic acid deficiency is compared with that of a pair-fed group as well as with animals with free access to a vitamin C containing diet.

### METHODS

Guinea-pigs (88) (Himalayan spotted outbred rotation), 240 g mean weight, and previously maintained on a commercial pellet diet (ALTROMIN-MS), were adapted to a special, powdered feed (Nutritional Biochemical Corp., Cleveland) (Hanck &

Weiser, 1973) enriched with all vitamins including ascorbic acid (2000 ppm). The animals were randomized and divided into 3 groups. After an adaptation time of 3 days, the ascorbic acid was omitted from the diet of group III ( $n = 32$ ). Group II ( $n = 28$ ) was pair-fed to group III (the animals received the quantity of feed consumed by their vitamin C deficient mates the day before); group I ( $n = 28$ ) obtained the diet *ad libitum*. At the beginning and the termination of the experiment, the ascorbic acid content of the diet was analysed according to Vuilleumier & Müller-Mulot (1965). The guinea-pigs were weighed twice each week.

On days 20, 21 and 22 after initiation of the diets, 1/3 of the animals per group were anaesthetized each day with halothane (Hoechst) and blood samples taken by heart puncture. The alkaline phosphatase activity of the plasma was measured according to Richterich (1965). On the following day the rectal temperature of the animals (submitted to heart puncture the day before) was taken by insertion of a flexible thermistor. The means  $\pm$  s.e. were:  $39.2 \pm 0.1^\circ$  ( $n = 30$ ) for group III,  $39.2 \pm 0.1^\circ$  ( $n = 28$ ) for group II and  $39.9 \pm 0.1^\circ$  ( $n = 28$ ) for group I. Some animals, which were maintained at  $30^\circ$  in order to keep the rectal temperature constant, received intraperitoneal injections of the tyrosine hydroxylase inhibitor L- $\alpha$ -methyl-*p*-tyrosine ( $\alpha$ MpT), 200 mg kg<sup>-1</sup> 4 h and 100 mg kg<sup>-1</sup> 1 h before being killed. All the animals were bled under light ether anaesthesia through a polyethylene cannula in the carotid artery. After separation of the leucocytes (Huleiger & Blazkovec, 1967), their grouped vitamin C content was determined according to Brubacher & Vuilleumier (1974). Thereafter the animals were decapitated and the hearts and brains removed. Each brain was put on an ice-chilled plate and separated into cerebellum, corpus striatum, hypothalamus and rest of brain. The cerebellum was used for the determination of the ascorbic acid content according to the method of Roe & Kuether (1943), modified by Vuilleumier, Probst & Brubacher (1967). Preliminary experiments have shown that the vitamin C concentration in the cerebellum of guinea-pigs maintained on an ascorbic acid free diet decreases in the same manner as that in other brain parts. In groups I and II the ascorbic acid analyses were performed on single cerebella, whereas in group III 6 cerebella were pooled for one determination.

Furthermore, the content of noradrenaline in the heart as well as of noradrenaline, dopamine, 5-HT, homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) in the brain was determined using spectrophotofluorimetric methods (Shellenberger & Gordon, 1971; Murphy, Robinson & Sharman, 1969; Giacalone & Valzelli, 1966). The following brain parts were used for these measurements: dopamine: pools of 2 striata and single halves of the rest of brain; noradrenaline: pools of 2 hypothalami and single halves of the rest of brain; HVA: single striata; 5-HIAA: single halves of the rest of brain. The average of the values obtained in the left and right halves of the rest of brain of one animal was taken as one experimental value.

The statistical analysis was performed by Student's two-tailed *t*-test.

## RESULTS

*Growth curves.* In the course of the experiment the animals kept on the diet deficient in ascorbic acid (group III) showed a decreasing feed consumption compared to the controls fed *ad lib.* (group I). Up to 15 days no statistically significant differences of the body weight between the 2 groups were found by regression analysis. Subsequently, the weight of the animals on the ascorbic acid deficient diet dropped considerably, reaching the initial values after three weeks. Pair-fed animals (group

II) showed a similar weight curve as the vitamin C deficient group, although the drop in the third week was less pronounced (Fig. 1).

*Ascorbic acid and alkaline phosphatase.* In the blood leucocytes of the guinea-pigs kept on a vitamin C deficient diet for three weeks, the content of ascorbic acid dropped to about 1% of that found in controls fed *ad lib.* Between the latter and the pair-fed

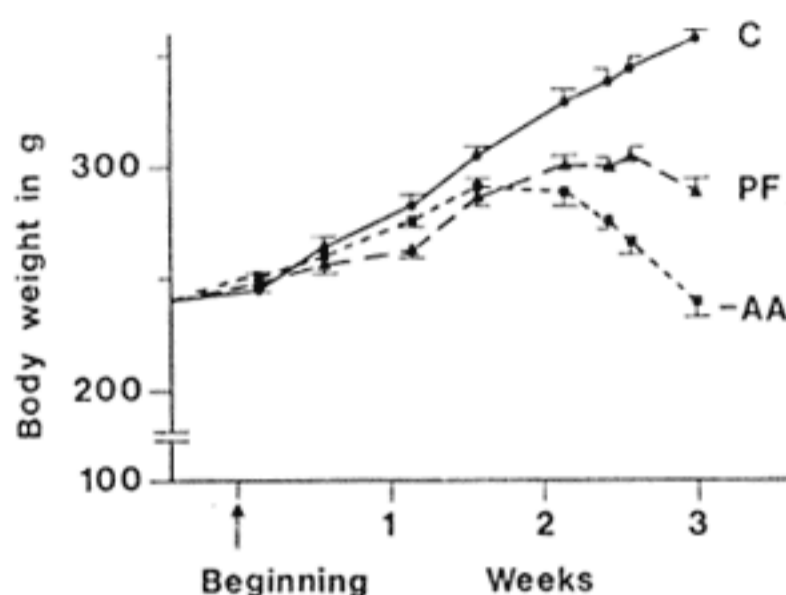


FIG. 1. Weight curves of guinea-pigs fed a vitamin C deficient diet for 3 weeks without or with addition of 2000 ppm ascorbic acid. The points are averages with s.e. ● controls fed *ad lib.* (C), ( $n = 28$ ); ▲ pair-fed (PF) ( $n = 28$ ); ■ fed an ascorbic acid deficient diet (AA) ( $n = 32$  initially, and 30 finally).

group no significant difference was observed. Furthermore, guinea-pigs with ascorbic acid deficiency showed a drop of the alkaline phosphatase in plasma to about 25% after three weeks compared to controls fed *ad lib.* and pair-fed animals (Table 1).

In the cerebellum of guinea-pigs kept on an ascorbic acid deficient diet for three weeks the content of this acid dropped to about 25% of that found in controls fed *ad lib.* The pair-fed animals did not show a significant decrease compared to the latter group (Table 1).

*Noradrenaline in brain.* In the hypothalamus of pair-fed animals the noradrenaline content showed a significant ( $P < 0.05$ ) decrease, whereas the  $\alpha$ MpT-induced diminution of the amine was less marked compared to controls fed *ad lib.* In the animals deficient in ascorbic acid the noradrenaline content also decreased in comparison to controls as well as to pair-fed animals. However, the  $\alpha$ MpT-induced diminution of the amine was enhanced compared with pair-fed animals, but not changed compared

Table 1. *Ascorbic acid content in leucocytes and cerebellum as well as activity of alkaline phosphatase in plasma of guinea-pigs after 3 weeks of feeding with the various diets.* The figures indicate averages with s.e., number of experiments in parentheses. The ascorbic acid content is indicated in  $\mu\text{g } 10^{-8}$  leucocytes and  $\mu\text{g g}^{-1}$  cerebellum respectively, the alkaline phosphatase in  $\mu\text{mol nitrophenol min}^{-1} \text{ litre}^{-1}$  plasma. \*4 determinations each from 6 pooled cerebella.

Diet	Ascorbic acid		Alk phosphatase
	Leucocytes	Cerebellum	
<i>Ad libitum</i>	$20.6 \pm 2.6$ (10)	$306.9 \pm 6.9$ (24)	$135.0 \pm 4.8$ (28)
Pair-fed	$17.0 \pm 2.4$ (8)	$285.3 \pm 13.3$ (24)	$127.4 \pm 5.0$ (28)
Ascorbic acid deficient	$0.3 \pm 0.1$ (10)	$77.6 \pm 4.2$ (4*)	$31.6 \pm 2.2$ (31)

with controls. The rest of the brain behaved similarly to the hypothalamus with the exception that the decrease of the noradrenaline content in animals with ascorbic acid deficiency was not significantly different from that in pair-fed guinea-pigs (Fig. 2).

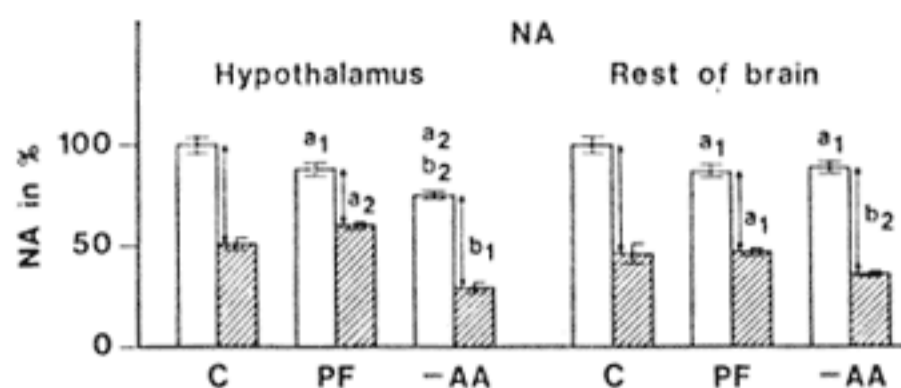


FIG. 2. Cerebral noradrenaline (NA) content in guinea-pigs 21–23 days after feeding with the various diets. C = controls fed *ad lib.*, PF = pair-fed, -AA = kept on a diet deficient in ascorbic acid. The L- $\alpha$ -methyl-*p*-tyrosine ( $\alpha$ MpT, 200 mg kg<sup>-1</sup> 4 h and 100 mg kg<sup>-1</sup> 1 h before death) was administered intraperitoneally. The values are indicated in % of controls fed *ad lib.* (=100%). The columns represent the mean with s.e. of 6 experiments with hypothalami or 12 experiments with rest of brain. Columns: open—NA content without  $\alpha$ MpT; hatched—NA content after  $\alpha$ MpT.  $a_1 = P < 0.05$ ;  $a_2 = P < 0.01$  versus controls fed *ad lib.*  $b_1 = P < 0.05$ ;  $b_2 = P < 0.01$  versus pair-fed animals. Absolute NA values of controls:  $1.94 \pm 0.08 \mu\text{g g}^{-1}$  for the hypothalamus;  $0.25 \pm 0.01 \mu\text{g g}^{-1}$  for the rest of brain.

**Noradrenaline in heart.** The only clear-cut change seen in the heart was a decrease of the noradrenaline content in animals with ascorbic acid deficiency. This diminution showed significance versus pair-fed animals if the amine was related to the organ weight and versus pair-fed as well as control animals if the amine per whole heart was indicated. The  $\alpha$ MpT-induced decrease in noradrenaline exhibited a similar tendency to that in the brain; the differences between the ascorbic acid deficient, pair-fed and control animals were not, however, significant (Fig. 3).

**Dopamine.** In the striatum of animals with ascorbic acid deficiency the dopamine content was diminished and the  $\alpha$ MpT-induced decrease in dopamine was less marked compared to controls. Similar differences existed when ascorbic acid deficient animals were compared with pair-fed guinea-pigs; the level of significance was not reached, however. The HVA content in both pair-fed and vitamin C deficient animals showed a decrease in comparison to controls; the diminution in the animals with ascorbic acid deficiency was, however, less marked than that in pair-fed guinea-pigs.

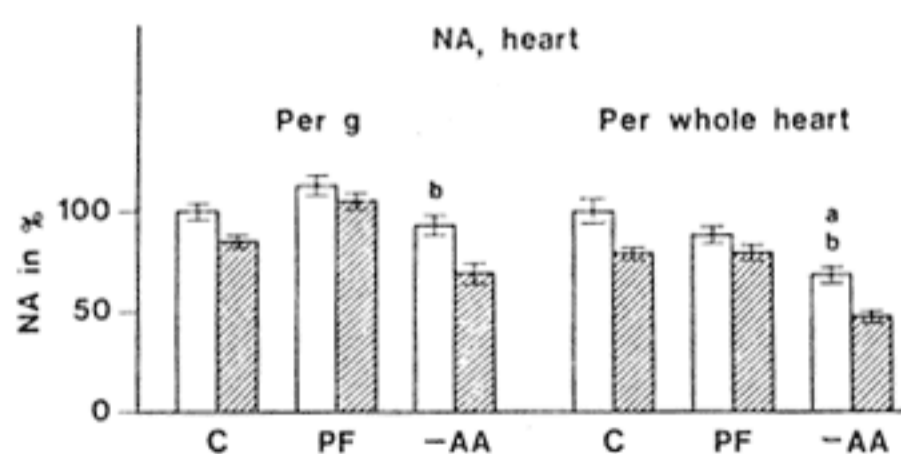


FIG. 3. Noradrenaline (NA) content of the hearts of guinea-pigs 21–23 days after feeding with the various diets. C = controls fed *ad lib.*; PF = pair-fed; -AA = kept on a diet deficient in ascorbic acid. The L- $\alpha$ -methyl-*p*-tyrosine ( $\alpha$ MpT; 200 mg kg<sup>-1</sup>, 4 h and 100 mg kg<sup>-1</sup>, 1 h before death) was administered intraperitoneally. Each column represents an average with s.e. of 10–12 experiments. The values are indicated in % of controls fed *ad lib.* (=100%). Columns: open—NA content without  $\alpha$ MpT; hatched—NA content after  $\alpha$ MpT. The absolute NA values (ng) were calculated either per gram tissue or per whole heart. a:  $P < 0.01$  versus controls fed *ad lib.*; b:  $P < 0.01$  versus pair-fed animals. Absolute NA values of controls  $2.44 \pm 0.1 \mu\text{g g}^{-1}$ ;  $2.53 \pm 0.13 \mu\text{g}$  per whole heart.

The rest of the brain exhibited a significant decrease of dopamine in the vitamin C deficient compared to the pair-fed group (Fig. 4).

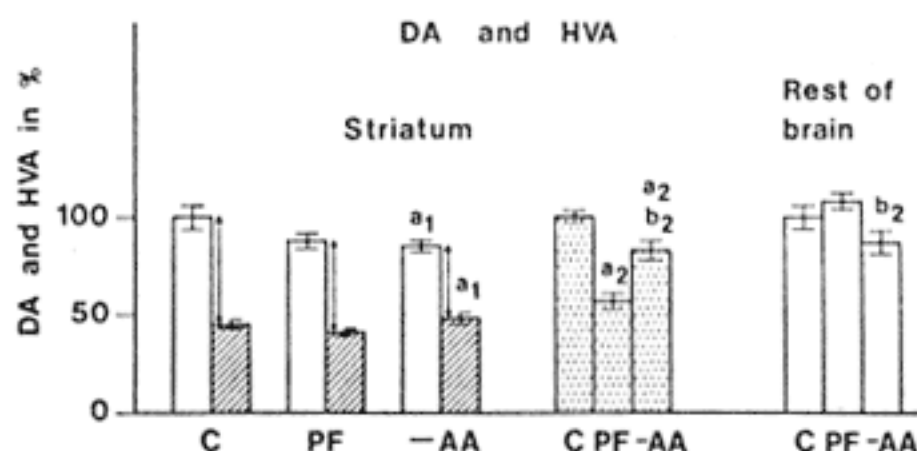


FIG. 4. Cerebral dopamine (DA) and homovanillic acid (HVA) content of guinea-pigs 21–23 days after feeding with the various diets. C = controls fed *ad lib.*; PF = pair-fed; -AA = kept on a diet deficient in ascorbic acid. The *L*- $\alpha$ -methyl-*p*-tyrosine ( $\alpha$ MpT; 200 mg kg<sup>-1</sup> 4 h and 100 mg kg<sup>-1</sup> 1 h before death) was administered intraperitoneally. The values are indicated in % of controls fed *ad lib.* (=100%). Columns: open—DA content without  $\alpha$ MpT, average, with s.e. of 6 (striata) and 12 (rest of brain) experiments; hatched—DA content after  $\alpha$ MpT average with s.e. of 11 experiments; dotted—HVA content without  $\alpha$ MpT, average with s.e. of 11 experiments. a<sub>1</sub> =  $P < 0.05$ ; a<sub>2</sub> =  $P < 0.01$  versus controls fed *ad lib.*; b<sub>1</sub> =  $P < 0.05$ ; b<sub>2</sub> =  $P < 0.01$  versus pair-fed animals. Absolute DA values of the controls: 6.96  $\pm$  0.39  $\mu$ g g<sup>-1</sup> for the striatum; 0.21  $\pm$  0.01  $\mu$ g g<sup>-1</sup> for the rest of brain. Absolute HVA value of the controls: 2.14  $\pm$  0.07  $\mu$ g g<sup>-1</sup>.

*5-Hydroxytryptamine.* No significant change of the cerebral 5-HT content occurred either in pair-fed or in ascorbic acid deficient animals. However, the concentration of 5-HIAA was significantly decreased in both these groups (compared to controls fed *ad lib.*), but no significant difference between pair-fed and vitamin C deficient animals was detected (Fig. 5).

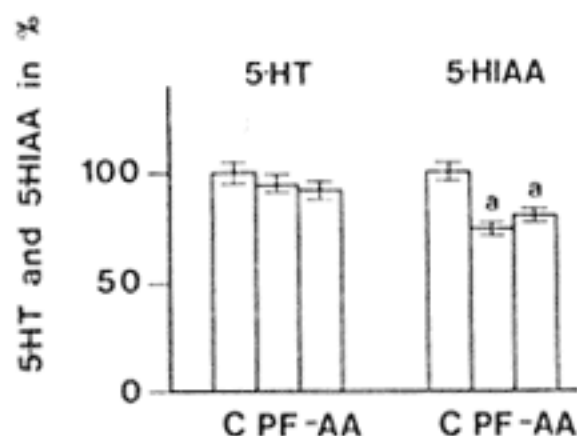


FIG. 5. Content of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in the rest of brain of guinea-pigs 21–23 days after feeding with the various diets. C = controls fed *ad lib.*; PF = pair-fed; -AA = kept on a diet deficient in ascorbic acid. Each column represents an average with s.e. of 12 experiments. The values are indicated in % of controls fed *ad lib.* (=100%). a:  $P < 0.01$  versus controls fed *ad lib.* Absolute values of controls: 5-HT 0.24  $\pm$  0.01  $\mu$ g g<sup>-1</sup>; 5-HIAA 0.21  $\pm$  0.01  $\mu$ g g<sup>-1</sup>.

#### DISCUSSION

The growth curves of the guinea-pigs indicate that the ascorbic acid deficient diet as well as the pair-feeding (which did not markedly affect the ascorbic acid in leucocytes and the alkaline phosphatase in plasma) led to a general state of undernutrition. In addition, the animals of group III showed a marked ascorbic acid deficiency as measured by their content of endogenous ascorbic acid and their activity of alkaline phosphatase which as previously shown (Hanck & Weiser, 1973) are highly correlated. Thereby, in agreement with earlier results (Hughes, Hurley & Jones, 1971), the ascorbic

acid of the brain was more resistant to the deficient diet than that of the blood leucocytes.

Nutritional deficiency without ascorbic acid depletion (as seen in pair-fed animals) attenuated the  $\alpha$ MpT-induced diminution of the noradrenaline content in the hypothalamus and the rest of the brain. This probably indicates a decreased noradrenaline turnover in pair-fed animals compared to controls fed *ad lib.* In guinea-pigs with ascorbic acid deficiency the  $\alpha$ MpT-induced diminution of noradrenaline was significantly more marked than in pair-fed animals indicating an enhancement of the turnover of the amine compared to the pair-fed group. This turnover of noradrenaline in ascorbic acid deficient animals did not, however, exceed that in controls fed *ad lib.*, since the  $\alpha$ MpT-induced decrease in noradrenaline was not different in either of these groups. Whether the slight decrease of the endogenous cerebral noradrenaline content in pair-fed animals and its enhancement in the hypothalamus by ascorbic acid deficiency was a consequence of the changes in noradrenaline turnover (new steady state levels) and/or of other factors remains to be elucidated. A decrease of noradrenaline uptake and storage in ascorbic acid deficient animals has been claimed by some (Thoa & others, 1966; Bhagat, West & Robinson, 1966), but denied by other authors (Jonsson & others, 1974).

In the heart, in contrast to the brain, the noradrenaline metabolism of pair-fed animals showed no significant change compared to controls fed *ad lib.* However, ascorbic acid deficiency seemed to influence the metabolism of noradrenaline similarly to that in the brain. In fact, in ascorbic acid deficient compared to pair-fed animals the cardiac content of the amine was lower especially when indicated per whole heart, agreeing with previous findings (Thoa & others, 1966). Furthermore, the  $\alpha$ MpT-induced decrease of the amine tended to be enhanced although this enhancement did not reach the level of significance. It has therefore to be considered that vitamin C deficiency also led to an acceleration of the cardiac turnover of the amine.

The present results regarding the turnover of noradrenaline are in principal agreement with previous ones. Thus, in guinea-pigs with ascorbic acid depletion the  $\alpha$ MpT-induced decrease of cerebral noradrenaline showed a tendency to be enhanced (Jonsson & others, 1974), and in similar experiments a shorter turnover time for the amine has been observed in the iris of the eye (Sears, 1969). However, the present findings indicate that an enhancement of the amine's turnover due to ascorbic acid deficiency may be masked by other nutritional factors since in pair-fed animals the  $\alpha$ MpT-induced decrease of cerebral noradrenaline was attenuated compared to controls. This complex interaction may give rise to discrepancies regarding the effect of ascorbic acid on cerebral monoamines unless the results of experiments with ascorbic acid deficiency are related to those with pair-fed animals.

The increase of the turnover of noradrenaline in ascorbic acid deficient guinea-pigs seems puzzling since ascorbic acid has been shown to be a cofactor of dopamine- $\beta$ -hydroxylase, the enzyme transforming dopamine into noradrenaline (Levin, Levenberg & Kaufman, 1960). However, it has to be considered that the ascorbic acid depletion of the brain was not complete and that the remaining vitamin C was sufficient for the normal function of that enzyme.

The cerebral dopamine metabolism of ascorbic acid deficient animals also showed changes when compared to controls fed *ad lib.*, i.e. an attenuation of the  $\alpha$ MpT-induced decrease in dopamine as well as a diminution of the endogenous content of HVA. These alterations indicate a decreased turnover of cerebral dopamine. The

slightly diminished content of endogenous dopamine in the striatum and the rest of the brain may be due to the decreased amine turnover although other factors, e.g. a diminished storage and/or uptake capacity for dopamine cannot be excluded. However, the reduced turnover of this amine is hardly to be attributed to the ascorbic acid deficiency alone, since the striatal HVA content was also decreased in pair-fed animals. Furthermore, in the latter animals the dopamine as well as the  $\alpha$ MpT-induced decrease of this amine in the striatum showed a tendency to be reduced. Therefore, other nutritional deficiencies than that of ascorbic acid were possibly involved in the decreased turnover of dopamine. The finding that in ascorbic acid deficient guinea-pigs the striatal HVA content was less markedly reduced than in pair-fed animals cannot be explained. It may be due to an impaired transport of the HVA from the brain into the blood as a consequence of the ascorbic acid deficiency.

The slight decrease in the cerebral 5-HIAA content without major changes of the 5-HT concentration in groups II and III indicates a diminished turnover of 5-HT. However, this change was probably not specifically connected with the lack of vitamin C since ascorbic acid deficient animals showed a similar 5-HIAA decrease as pair-fed guinea-pigs.

In conclusion, ascorbic acid deficiency probably leads to a specific enhancement of the cerebral and possibly the cardiac turnover of noradrenaline, whereas under-nutrition without ascorbic acid deficiency (pair-fed animals) seems to decrease the turnover of brain noradrenaline and 5-HT as well as of striatal dopamine.

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