

# Moderate Alcohol Consumption and Vitamin C Status in the Guinea-Pig and the Rat

Z. ZLOCH, E. GINTER<sup>1</sup>

*Institute of Hygiene, Faculty of Medicine, Charles University, Plzeň, Czech Republic and <sup>1</sup>Institute of Preventive and Clinical Medicine, Bratislava, Slovak Republic*

Received February 7, 1995

Accepted March 13, 1995

## Summary

The influence of regular moderate ethanol consumption on the status of vitamin C was followed in guinea-pigs and rats. In the guinea-pigs examined, 10-day consumption of ethanol (4.5 g per day and kg of body weight), administered in drinking water under a vitamin C-deficient diet, caused a greater decrease in the tissue concentrations and the body-pool of this vitamin than in the group without alcohol. In the rats, on the contrary, the daily consumption of ethanol (6 % vol) during 10 months resulted in an increase in the body stores of vitamin C, especially in the liver, adrenals, kidneys, and lungs. Moreover, the biosynthesis of ascorbate from D-glucuronolactone *in vitro* was more intensive (by 30 %) in the liver microsomes of alcoholized rats than in those of controls (without alcohol). These results indicate that the need of vitamin C during chronic consumption of moderate alcohol doses is enhanced. This is due to the participation of ascorbate in oxidoreducing processes connected with ethanol metabolism which leads to its irreversible destruction. In the rat, this loss is compensated by its enhanced biosynthesis, while in the guinea-pig it produces increased demands for its exogenous intake. If these are not satisfied, a partial vitamin C deficiency may occur, which potentiates the harmful effect of alcohol on the health status.

## Key words

Vitamin C biosynthesis and metabolism – Ethanol consumption

## Introduction

Ethanol plays an ambivalent role in the life style of man. It is a part of the dietary sources of energy and at the same time a drug harmful to human health. In the Czech Republic and in the Slovak Republic the alcohol consumption contributes significantly to the daily energy intake of adults (to 10–12 %). The systematic damage done to various human organs by alcohol is well-known. However, a less known aspect of alcohol abuse is the alteration of both metabolism and biological functions of essential nutrients, such as vitamins and minerals. Alcohol consumers often suffer from various types of malnutrition, that are caused by decreased resorption, deteriorated metabolic activation or disorders in deposition of vitamins A, B<sub>1</sub>, B<sub>6</sub>, E and folate (Hoyumpa 1986, Halsted and Heise 1987, Lieber 1990, Pirozhkov *et al.* 1992). Moreover, the results of experimental and clinical studies have shown serious changes of vitamin C metabolism in connection with

frequent alcohol consumption (Fazio *et al.* 1981, Hsu and Hsieh 1982, Susick *et al.* 1986, Seitz and Suter 1994). However, these findings do not appear consistent, and so far no precise explanation for the mechanism of such changes has been proved. Seeing that some social groups fail to have a sufficient dietary intake of vitamin C and often incline towards alcohol abuse, we have tried to resolve some relations between both of these nutritional aspects.

## Methods

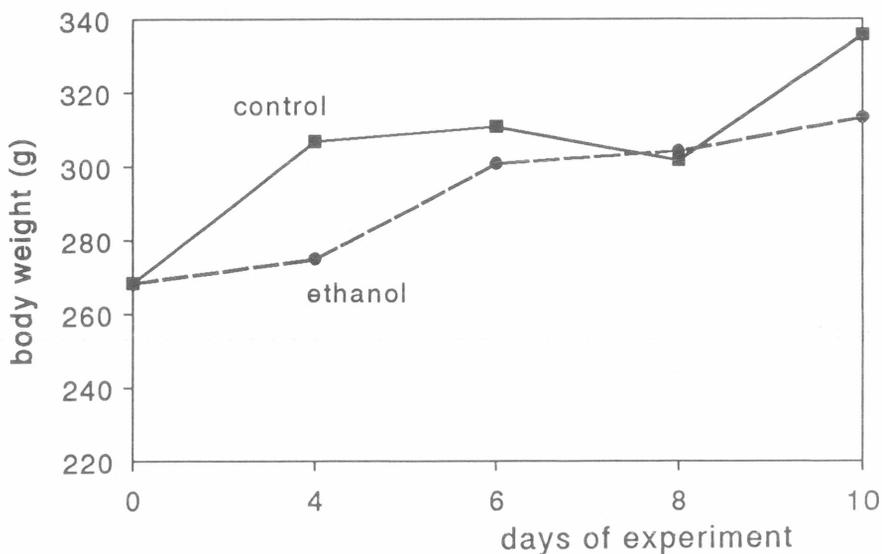
In our experiments we used, on the one hand, guinea-pigs that – similarly as man – do not synthesize vitamin C and depend entirely on its external supply, and, on the other hand, rats that synthesize vitamin C as is necessary. The experimental animals were males from the laboratory breeding VELAZ

company, Prague. The body weight of guinea-pigs was 290 g, that of rats 220 g.

In the course of 4 weeks, 21 guinea-pigs were adapted to a diet deficient in vitamin C (Ginter *et al.* 1968), vitamin C being added to their drinking water (0.2 g per liter). After this time, 7 animals were killed, and the remaining animals were transferred to the scorbutogenic diet (vitamin C was left out of the drinking water). Simultaneously, a half of them (seven animals) received a solution containing 7 ml of ethanol in 100 ml water for drinking. During the following 10 days, the consumption of food and water as well as the body weight were followed in both experimental (i.e. alcoholized) and control (without alcohol) groups. At the end of a 10-day period, all of the animals were killed, and the vitamin C concentration was assessed in 12 metabolically important organs and tissues (Roe and Kuether 1943). On the basis of the known weight

of the analyzed organs and tissues (Wagner and Manning 1976), their content of vitamin C was calculated.

In a second series of experiments, 20 rats were fed a standard diet for 10 months. One half of them (the experimental group) were given 6% ethanol in the drinking water. In the course of this period, the consumption of food and drinking fluid as well as the body weight were followed in both groups. After killing the animals, the vitamin C concentration was determined in metabolically relevant tissues (Roe and Kuether 1943). Liver microsomes were separated by ultracentrifugation (Ayaz *et al.* 1976) and the intensity of L-ascorbate biosynthesis from D-glucuronolactone was measured (Chatterjee 1970, Zloch and Ginter 1984). The results obtained from the experimental and control group were statistically evaluated by the paired t-test.



**Fig. 1**

The body weight changes in guinea-pigs during the experimental period in alcoholized and control groups. The values are expressed as arithmetic means. No statistically significant difference between the experimental and control groups was obtained.

## Results

Fig. 1 shows that a moderate decrease in food consumption occurred in the experimental guinea-pigs drinking the alcohol solution. This was manifested itself by lesser growth, but the difference between the experimental and the control group was not statistically significant. In the rats, the food and water consumption as well as their weight growth were the same in both the control and experimental groups. The average

intake of alcohol in the experimental groups was calculated as 4.25 g per day and kg of body weight in both the guinea-pigs and rats.

The vitamin C concentrations found in guinea-pig tissues are given in Table 1. During the 10-day cessation of vitamin C in the diet, its concentration in most of the tissues decreased by 50% (in the brain), and even as much as by 95% (in the liver, lungs, heart and adipose tissue).

**Table 1**

Vitamin C concentrations in the body tissues of guinea-pigs at the beginning of the experiment (0 day) and the vitamin C concentrations and total content in the tissues at the end of the experiment

| Tissue    | Vitamin C concentration on day 0 (mg/100 g) | Experimental group                 |                                 | Control group                      |                                 | P <   |
|-----------|---|------------------------------------|---------------------------------|------------------------------------|---------------------------------|-------|
|           |   | Vitamin C concentration (mg/100 g) | Total content of vitamin C (mg) | Vitamin C concentration (mg/100 g) | Total content of vitamin C (mg) |       |
| Blood     | 0.08±0.06                                   | 0.11±0.03                          | 0.021±0.006                     | 0.15±0.01                          | 0.033±0.002                     | -     |
| Liver     | 26.6±2.7                                    | 1.3±0.2                            | 0.143±0.022                     | 2.4±0.2                            | 0.294±0.024                     | 0.01  |
| Lung      | 36.3±1.5                                    | 1.8±0.3                            | 0.040±0.010                     | 3.8±0.5                            | 0.098±0.012                     | 0.01  |
| Heart     | 8.7±0.3                                     | 0.2±0.01                           | 0.001±0.001                     | 0.8±0.1                            | 0.006±0.001                     | 0.001 |
| Muscle    | 2.9±0.2                                     | 0.08±0.06                          | 0.102±0.077                     | 0.14±0.01                          | 0.206±0.061                     | -     |
| Testes    | 46.9±3.6                                    | 10.3±0.6                           | 0.184±0.011                     | 9.1±0.4                            | 0.188±0.028                     | -     |
| Intestine | 30.2±0.9                                    | 1.5±0.4                            | 0.202±0.054                     | 2.1±0.3                            | 0.330±0.047                     | 0.05  |
| Adrenals  | 171.1±13.9                                  | 9.5±1.9                            | 0.018±0.004                     | 15.8±2.7                           | 0.035±0.006                     | 0.05  |
| Brain     | 28.3±0.7                                    | 13.5±0.9                           | 0.304±0.021                     | 13.3±0.5                           | 0.350±0.013                     | -     |
| Fat       | 7.8±0.7                                     | 0.41±0.23                          | 0.093±0.052                     | 0.53±0.08                          | 0.138±0.021                     | -     |
| Skin      | 10.4±0.4                                    | 0.5±0.1                            | 0.280±0.056                     | 1.4±0.2                            | 0.894±0.057                     | 0.01  |
| Bone      | 13.6±1.2                                    | 1.4±0.2                            | 0.340±0.049                     | 1.6±0.2                            | 0.456±0.13                      | -     |
| Total     |   |                                    | 1.728±0.130                     |                                    | 3.027±0.317                     | 0.05  |

Data are means ± S.E.M. P = statistically significant differences between the experimental (alcoholized) and control group.

**Table 2**

Vitamin C concentrations in body tissues of the rats and L-ascorbic acid biosynthesis in liver microsomes

| Tissue   | Experimental group (mg/100 g) | Control group (mg/100 g) | P <  |
|--|-------------------------------|--------------------------|------|
| Blood  | 2.14±0.34                     | 1.72±0.23                | 0.05 |
| Liver  | 58.0±6.0                      | 50.5±7.5                 | 0.05 |
| Spleen   | 59.1±4.7                      | 46.7±6.6                 | 0.01 |
| Kidneys  | 15.9±1.7                      | 13.0±0.7                 | 0.05 |
| Lung   | 15.0±3.7                      | 13.4±2.3                 | -    |
| Brain  | 15.7±0.7                      | 15.0±0.03                | -    |
| Intestine                                      | 18.7±2.2                      | 19.6±6.0                 | -    |
| Adrenals                                       | 264±61                        | 212±34                   | 0.05 |
| L-ascorbic acid production (μmol/h/mg protein) | 0.202±0.054                   | 0.170±0.034              | 0.05 |

P - statistical significance of differences between the experimental (alcoholized) and control group

Changes in the tissue concentrations of vitamin C generally differ between the experimental and control groups. A statistically significant decrease in vitamin C concentrations prevails in the group of experimental (alcoholized) guinea-pigs. The total content of vitamin C, established by summing its partial contents in the individual organs, is also significantly lower in the experimental animals than in the controls (5.44 mg and 8.22 mg per kg of body weight, respectively). These values represent a substantial part of the body pool because the 12 analyzed tissues constitute approximately 90 % of the body mass of guinea-pig weighing 350 g.

Table 2 indicates that the vitamin C concentrations in rats influenced by ethanol ingestion are contrary to those exhibited by the guinea-pigs. In the most of rat tissues analyzed the content of vitamin C was significantly higher in alcoholized animals than that in the control group (without alcohol). There are several possible reasons for the enhanced ascorbate concentration in the tissues of alcohol intoxicated rats: a) its active transport into the tissues as well as its distribution therein, b) its diminished need, while the intensity of its biosynthesis remains unchanged, c) its enhanced biosynthesis (Horio *et al.* 1983, Hornig *et al.* 1984, Rose 1988).

In an independent study (Table 2), we ascertained that the rate of L-ascorbate biosynthesis in microsomes, isolated from the liver of experimental rats, is significantly higher (by 30 %) than in the controls. The increased ascorbate production manifests itself in higher levels in the blood as well as in the metabolically important organs, such as the liver, adrenals, kidneys and lungs.

## Discussion

The alcohol ingestion in guinea-pigs resulted in accelerated vitamin C catabolism, which was manifested – when compared with the control group – by a more rapid decrease in the body pool of this vitamin. Similar results, yet limited to the tissue concentrations of a lesser number of the organs examined, were also obtained by several other authors dealing in single experiments with guinea-pigs subjected either to acute or chronic ethanol intoxication (Yunice *et al.* 1984, Halsted and Heise 1987). Likewise, the study of human alcoholics has shown that, independently of the level of alcohol intake the concentrations of vitamin C in their leukocytes and liver are always lower. As realimentation with synthetic ascorbate proceeds, with concomitant alcohol consumption, it is much slower than in teetotallers. Our results demonstrate that vitamin C occurs ubiquitously in the organism of the guinea-pig, and it catabolizes at a different rate in various organs. Under the concomitant effect of regular moderate alcohol

doses, the rate of its catabolism is approximately higher by one third. The practical consequence of this finding is the fact that regular alcohol consumers require a relatively greater intake for vitamin C, and that they are facing enhanced risk of a latent deficiency of this vitamin unless this need is satisfied.

Vitamin C is an essential factor of enzyme-catalyzed hydroxylation processes. These constitute a type of oxido-reduction changes during which ascorbate decomposes irreversibly (Hoyumpa 1986, Gershoff 1993, Nagyová and Ginter 1994, Tatara and Ginter 1994). Besides, ascorbate is actively involved in the cytochrome P450 cycle, influencing positively its monooxygenase activity (Koop and Tierney 1990, Nagyová *et al.* 1993). One of the isoenzymes of this system (CYP IIE1) is induced by larger or more frequent doses of ethanol, and participates actively in its enzyme oxidation (the microsomal ethanol oxidizing system. Sinclair *et al.* 1991, Kukielka and Cederbaum 1992, Morimoto *et al.* 1993). The results of experimental work suggest that the rate of ethanol metabolism, as well as its toxicity, may partly be affected through enhanced doses of vitamin C (Yunice *et al.* 1984, Chen *et al.* 1990). Thus, during ethanol intoxication an increased need for vitamin C can occur, which is compensated in rats by increased ascorbate biosynthesis. This effect has already been demonstrated previously in rats intoxicated by various xenobiotics, e.g. DDT and aminopyrine (Horio *et al.* 1983). In guinea-pigs, which do not synthesize vitamin C, an enhanced need for this vitamin, connected with ethanol metabolism, leads to acceleration of the metabolic rate of ascorbate and consequently to a reduction of its tissue concentrations.

Ascorbate, as a very effective hydrosoluble antioxidant, inhibits among others the formation of free radicals and quenches reactive oxygen species (Henson *et al.* 1991, Niki 1991). The abuse of ethanol enhances the production of superoxide and hydroxyl radicals, both being very harmful in the intracellular medium and represents one of the modes of alcohol toxicity (Cederbaum 1989, McCay *et al.* 1992, Nordmann *et al.* 1992, Rashba-Step *et al.* 1993, Kukielka *et al.* 1994). Many experimental studies have proved intensive peroxidative changes of intracellular membrane lipids after alcohol intoxication in both rats and guinea-pigs. Under certain circumstances, ascorbate can be a preventive factor inhibiting these undesirable oxidative alterations (Susick *et al.* 1986, Wefers and Sies 1988, Staats and Colby 1989, Nordmann 1994). The action of ascorbate in this type of oxidative processes again leads to its irreversible destruction which, in the guinea-pig and in the man, manifests itself by decreasing their body reserves whereas, in the rat, it results in its increased biosynthesis *de novo*. If the body pool of vitamin C is not renewed by an adequate intake, a state of its latent

marginal deficiency can arise. Many biological activities of ascorbate then become limited, and together with the pathogenic effect of alcohol, this state may contribute to an overall threat to an individual's health.

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#### Reprint Requests

Doc. Ing. Z. Zloch, Institute of Hygiene, Faculty of Medicine, Charles University, Lidická 4, 301 66 Plzeň, Czech Republic.