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an Antioxidative Effect: A Feeding Test with Rats**

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Note

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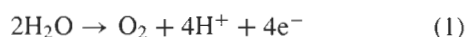
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A new type of electrolyzed hydrogen-saturated (EHS) water was produced using a water-electrolyzing device equipped with a special cation exchanger. Use of the EHS water for drinking in a feeding test with rats elicited an antioxidative effect. After intraperitoneal injection of 2,2-azobis-amidinopropane dihydrochloride, urinary secretion of 8-hydroxydeoxyguanosine and hepatic formation of peroxidized lipid were significantly lessened in rats which had received the EHS water for one week. These results suggest the possibility that this drinking water shows an effect in reduction of oxidative stress in the body.

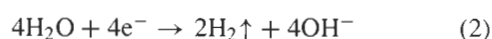
Key words: water; electrolyzed hydrogen-saturated water; oxidative stress; antioxidant

Westernization of the Japanese dietary style has increased the possibility of consuming processed foods with enhanced peroxidized lipids to form even more active-oxygen species in the body. Such endogenous and exogenous factors, responsible for oxidative stress, might eventually cause a number of lifestyle-related diseases and some abnormal modalities. Hence the importance of using certain antioxidant foods to reduce the risk has been emphasized globally in recent years. These foods include functional formulas with added polyphenols, carotenes, ascorbic acid, tocopherol, and so forth.

We have investigated and developed a new type of antioxidant water for drinking use. The water is characterized by hydrogen contained at the saturation level, which can be produced by electrolysis of dechlorinated tap water at the anode through



and simultaneously at the cathode through



where the resulting H₂-containing water on the cathode

side is alkalized by the OH⁻ produced. Our device, however, is equipped with a special perfluorosulfonic acid cation-exchanging Nafion[®] membrane (Du Pont, Tokyo) which facilitates movement of the produced H⁺ (1) into the cathode side because the ionic form of our membrane is H⁺. The water thus turns to neutrality (approximately pH 7.5). These two processes, electrolysis and cation exchange, can be carried out using the apparatus shown in Fig. 1.^{1,2} The water produced is saturated with hydrogen at 1.6 ppm with oxidation-reduction potential at -600 mV or less.

Here we report on the antioxidative activity observed when rats were given this EHS water for drinking use.

Over a preliminary one-week period, 30 six-week-old male rats (SD strain), each weighing about 200–250 g, were fed on the usual CE-2 diet (CLEA Japan, Tokyo) and given distilled water *ad libitum*. The rats were then trichotomized and fed a similar diet for another week. During feeding, they took the diet and drinking water *ad libitum*, where each water-bottle was devised to isolate the content from atmospheric oxygen. The water was renewed twice a day. Feeding was carried out in an air-conditioned room kept at a temperature of 24 ± 1 °C and a relative humidity of 55 ± 5%, with a 12-h cycle of light (08:00–20:00) and dark (20:00–08:00). The rats were maintained according to the Guidelines for the Care and Use of Experimental Animals of the Japanese Association for Laboratory Animal Science. On day 7, the free radical initiator 2,2-azobis-amidinopropane dihydrochloride (AAPH),³ dissolved in physiological saline at a concentration of 2 mM was administered by intraperitoneal injection. The dose of AAPH injected was set at 50 mg/kg body weight. Then the rats were subjected to fasting for 12 h, during which their urine samples were collected and stored at -80 °C until it was used to measure the concentration of the oxidative stress marker 8-hydroxydeoxyguanosine (8-OHdG).⁴ Also, at 12 h from AAPH injection into each rat, the livers were excised and systemic blood samples were taken from the

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Abbreviations: AAPH, 2,2-azobis-amidinopropane dihydrochloride; ALT, alanine aminotransferase; AST, aspartate aminotransferase; EHS, electrolyzed hydrogen-saturated; TBARS, thiobarbituric acid reactive substance; 8-OHdG, 8-hydroxydeoxyguanosine

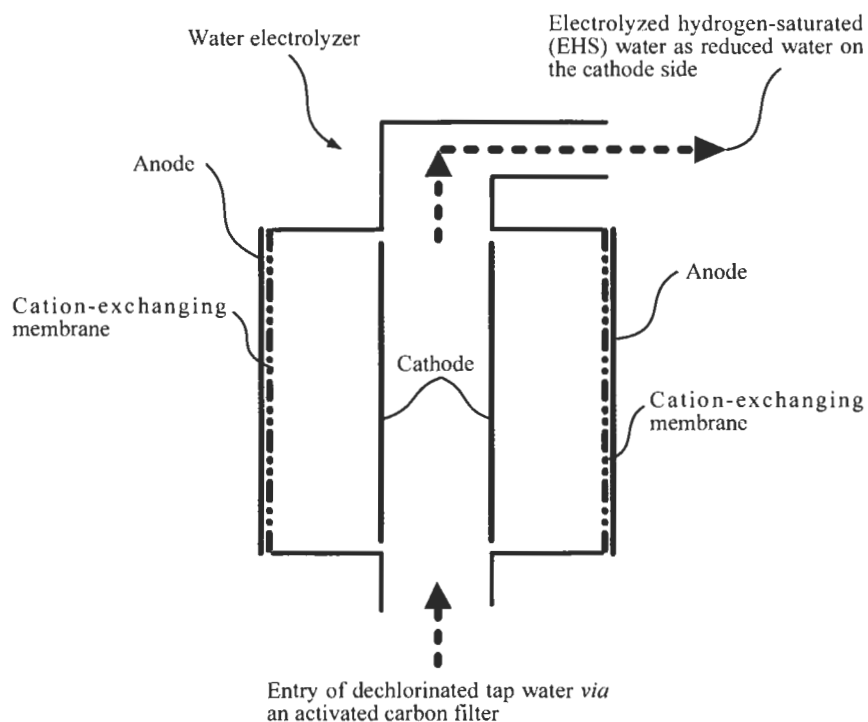


Fig. 1. Device for Use in Producing Electrolyzed Hydrogen-Saturated Water.

Table 1. Data from Feeding Tests

Item	Rat Group		
	Group 1 (Negative control)	Group 2 (Positive control)	Group 3 (Test)
Urinary 8-OHdG (ng/ml)	13.59 ± 0.51	19.84 ± 0.59	18.08 ± 0.40
Hepatic TBARS (nmol MDA/mg protein)	0.148 ± 0.009	0.719 ± 0.025	0.519 ± 0.027
Serum AST (U/L)	66.90 ± 1.44	92.00 ± 7.16	91.70 ± 8.29
Serum ALT (U/L)	29.70 ± 1.65	33.50 ± 2.11	34.50 ± 2.20

Each value is the mean ± SEM.

* $p < 0.05$, ** $p < 0.01$

For symbols and abbreviations, see the text.

abdominal artery, without affliction under anesthesia with 30 mg/kg of pentobarbital. Each liver was stored at -80°C until it was used to analyze the value of thiobarbituric acid reactive substances (TBARS) as an index of lipid peroxidation in the liver.⁵⁾ Each blood sample was cooled to 4°C and centrifuged at 3,000 rpm for 20 min to take serum. With this blood sample, we measured the serum contents of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as indices of hepatic injury.⁶⁾ All the data were statistically analyzed to find the significance for their standard errors of means (SEM) at $p < 0.01$ or $p < 0.05$.

The trichotomized rats belong to group 1 (negative control), given dechlorinated tap water without AAPH injection; group 2 (positive control), given the same

water with AAPH injection; and group 3 (test), given EHS water with AAPH injection.

When feeding data on water intake, diet intake, and body weight gain over the 7-d test period were analyzed, there were no significant between-group differences (Fig. 2). All the rats in each group grew normally, with no appreciable abnormality observed as to appearance or behavior (data not shown).

A significant difference was found when the groups were compared as to urinary 8-OHdG concentration. While the mean value for group 1 differed greatly ($p < 0.01$) from those for group 2 and group 3, a significant difference ($p < 0.05$) was observed between group 2 and group 3 (Table 1). This result indicates that the use of EHS water lessened the DNA damage

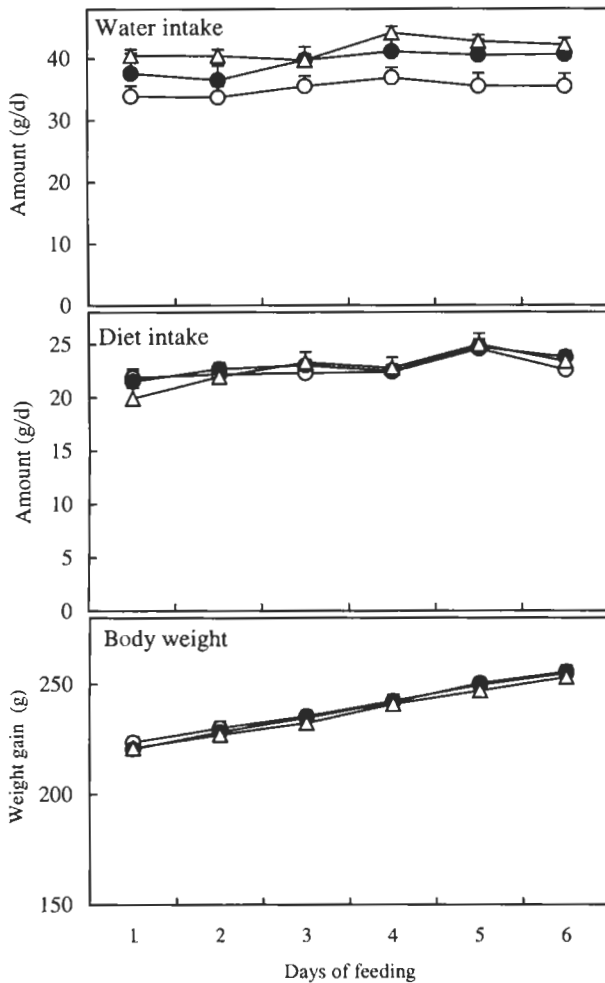


Fig. 2. Feeding Data on Water Intake, Diet Intake, and Body Weight Gain of Rats before They Were Administrated Free Radical Initiator 2,2-Azobis-amidinopropane Dihydrochloride (AAPH) by Intraperitoneal Injection.

○, group 1 (negative control); ●, group 2 (positive Control); △, group 3 (Test). For details, see the text.

inflicted by administration of AAPH. A similar conclusion was drawn from the result that a very distinct difference in the hepatic TBARS value occurred between group 2 and group 3 (Table 1), suggesting an inhibitory effect of EHS water on lipid peroxidation in the liver. But no significant effect was observed between group 2 and group 3 when serum AST and ALT levels were compared between them.

As far as we can determine from our observations in the present study, it is possible that the use of EHS water reduces oxidative stress in the body. Details await further experimentation, which is now under way.

Acknowledgment

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