INFLUENCE OF ALKALINE IONIZED WATER ON RAT ERYTHROCYTE HEXOKINASE ACTIVITY AND MYOCARDIUM

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ABSTRACT — Alkaline ionized water (AKW) produced by the electrolysis of tap water (TPW) was given to pregnant rats throughout gestation. AKW was subsequently given to infants as a test group until 15 weeks old to determine changes in body and organ weights, erythrocyte hexokinase (HK) activity and histological preparations of myocardiac muscle. The results were compared with those for rats given TPW.

Body weight of male and female rats given AKW at 3 to 11 weeks of age after birth significantly increased beyond control group values. Organ weights of offspring at 15 weeks-old showed no statistical difference for either group. HK activity, the rate-determining enzyme in erythrocyte glycolysis, significantly increased in males given AKW at 15 weeks-old. This suggests that AKW intake causes elevation of metabolic activity. Hyperkalemia was observed in males and females given AKW at 15 weeks-old. Especially in males, pathological changes of necrosis in myocardiac muscle were observed.

KEY WORDS : Alkaline ionized water, HK activity, Myocardiac degeneration

INTRODUCTION

Alkaline ionized water (AKW) is commercially available to supplement electrolytes lost due to perspiration. Apparatus for producing AKW is readily available for health purposes.

Watanabe and Shirai (1990) reported that the body weight of littermates 3 weeks after birthing by dam rats given AKW throughout their gestation and lactation periods increased significantly compared with that of controls. Watanabe (1995) reported AKW exerting substantial biological effects on postnatal growth, judging from the increase in intake of food and water in dams given AKW and the body weight of the offspring given AKW from the 14th day of lactation in males and from the 21st day of weaning in males and females, with postnatal morphological development accelerated. Kuchida *et al.* (1993) gave AKW to cattle and noted a much brighter meat color.

The day sperm appeared on the smear was the day designated as day zero of gestation. AKW was subsequently given to gestational rats. Until weaning and 15 weeks after birth, starting from day zero, animals in the test group were given AKW only, while those in the control group were given TPW *ad libitum*. In a preliminary experiment before this study, we investigated changes in weights and histological

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preparations of brain, pituitary, thyroid, thymus, heart, lungs, liver, spleen, kidneys, adrenals, testes and ovaries in male and female rats given AKW and tap water (TPW), respectively, until 15 weeks-old. The effect of AKW on the body weight of mother rats during gestation and after delivery, duration of gestation, litter size, sex ratio of the young and growth of the young was tested. In consequence, no significant differences were observed between the AKW and TPW groups in measurement of weights of various organs. On the other hand, histological preparations indicated that pathological changes were observed in only myocardiac muscle, especially in rats given AKW at 15 weeks-old. Therefore, in this study, as far as organs are concerned, we focused on the heart.

Little is understood about the influence of AKW intake on blood components and body and organ weights in experimental animals. In this study, rats were placed on a diet until 15 weeks-old by the method of Watanabe and Shirai (1990) and Watanabe (1995) to investigate changes in body weight, heart weight, erythrocyte hexokinase (HK) activity, various blood elements and histological preparations of myocardiac muscle. The non-equilibrium reactions of glycolysis are catalysed by HK and some other kinases. HK I in erythrocytes and HK IV(glucokinase) in the liver have different Km values for glucose. Erythrocyte HK is a marker metabolic activity and rate-determining enzyme. Erythrocyte HK activities were thus measured in rats given AKW. The relationship between hyperkalemia and myocardiac degeneration is discussed.

MATERIALS AND METHODS

Animals and feeding

Female rats of Sprague-Dawley strain, weighing 182-197 g (Jcl. SD, Clea Japan Inc., Tokyo, Japan), were purchased at 8 weeks of age and maintained at 23±1℃, humidity of 40-60%, 14 hour illumination, housing 5 rats to a plastic cage with wood chips as bedding and food (CE-2, Clea Japan Inc.) and tap water (TPW) intake *ad libitum*. After 3 weeks of acclimation, animals without abnormal findings were used. Copulation was induced by placing an experienced male rat of the same strain in one cage made of aluminum with 10 female rats more than 12 weeks-old with a regular estrous cycle confirmed by prior vaginal smears. The same male rat was used in all experiments. Smears were studied daily by microscope to confirm copulation. On the day sperm appeared on the smear, females were separated from the male, and this day was designated as day zero of gestation. All pregnant rats were individually housed in polycarbonated cages for delivery. AKW was subsequently given to gestational rats (test group, n = 10). In the controls, the day that sperm appeared on the smear was defined as day zero of gestation, but TPW was given as before (control group, n = 10). Copulated females were divided daily into test and control groups of essentially the same number. The female rats removed were replaced by new female rats so there would always be 10 per 1 male rat. From this day to lactation, AKW was given to mother rats of the test group and TPW to those of the control group. After weaning 21 days after delivery, the dams were autopsied under anesthesia with ether, and various organs in the thoracic and abdominal regions were observed in gross, and after extraction of the uterus, the number of implantation sites was counted.

At 3 weeks after delivery, the mother animal was weighed, and weaning was performed on the same day. Sex ratio (Male/Female) of weanlings and body weight of the offspring were estimated 3 weeks after birth, after dividing a litter born from 10 mother animals in the test and control sections into males and females. The reason why the body weight of the offspring was not weighed until 3 weeks of age is that the mother rat occasionally eats the offspring if we touch the offspring by hand immediately after delivery and during lactation. Consequently, the body weight of the offspring was not weighed by the time of weaning (during 3 weeks after delivery). Male and female F_1 rats were divided at random into groups of 15 animals each. Offspring were housed as 5 rats in each plastic cage. For postnatal rats, AKW and TPW were given to the test and control groups from 3 weeks after birth, respectively. Feeding was continued to 15 weeks after birth. Body

weight of the offspring in the litter born from 10 mother animals given AKW and TPW was measured at 3, 5, 7, 9, 11, 13 and 15 weeks. Consumption of food and water was measured on weeks 3 to15 after birth.

Alkaline ionized water

AKW was obtained by using an apparatus for producing ionized water (Minekaru TBC-R 6103, Tokyo Seiden Co., Ltd., Tokyo, Japan). The principle was that of electrolysis of an electrolyte solution. Ions transferred varied with amounts of reacting substances, hydrogen ion concentration, and flow speed. pH of AKW was 9.0, as measured by pH meter (M-7EII, Hitachi-Horiba Co., Tokyo, Japan) and maximum flow-speed was 140 l/h. AKW was done at noon every day during the experimental period. Immediately, rats in the test group were given AKW only *ad libitum*. Acidic water produced by the flow of anions to the anode was discarded. For purification of AKW, TPW was electrolyzed without drugs.

Water quality test

The Japan Food Hygiene Association, a food-testing organization recommended by the Minister of Health and Welfare according to the Japan Food Hygiene Act and the Japan Drug Act, measured pH, degree of alkalinity and electrolyte concentration of AKW. The methods and items of the tests are summarized in Table 1.

Hematological measurement and erythrocyte HK activity

Whole blood was collected from the jugular vein of rats anesthetized with ethyl ether. They were examined to determine the number of erythrocytes and leukocytes, hematocrit, hemoglobin and glucose. Numbers of erythrocytes and leukocytes and values of hematocrit were measured by the electronic counting method (Sysmex microcell counter CC-110, Toa Medical Electronics Inc., Kobe, Japan), by the capillary method (Toa Medical Electronics Inc.) and by the cyanmethemoglobin method (Cannan, 1965). Blood glucose was measured by the method of o-toluidine (Hyvärinen and Nikkila, 1962). Serum was obtained by centrifugation at 1,500 rpm for 5 min with blood coagulated at 4℃ for 3 hr. Sodium and potassium concentrations in the serum were determined by a radiometer (KNA 1 sodium-potassium analyzer, Radiometer A/S Copenhagen, Copenhagen, Denmark) and chloride by a chloride meter (CL-7 Chloride Counter, Hiranuma Sangyo Co., Ltd., Mito, Japan) according to the instrument manual instructions. Calcium, magnesium and inorganic phosphate concentrations in the serum were determined by the methods of *o*-cresolphthalein complexone (Connerty and Briggs, 1966), xylidyl blue (Mann and Yoe, 1956) and molybdenum blue (Taussky and Shorr, 1953), respectively. Serum protein concentration was determined by a serum protein refractometer. Serum proteins were separated by cellulose acetate membrane electrophoresis (Separax-SP, Jookoo Co., Ltd., Tokyo, Japan) and the electrophoretograms were examined with a densitometer (Model PAN, Jookoo Co., Ltd., Tokyo, Japan). For separation of erythrocytes, a 1 ml aliquot of whole blood containing EDTA and heparin was centrifuged at 1,500 rpm for 5 min, and the buffy coat and top layers were removed. The packed cells were mixed with 2 ml 5% glucose and centrifuged. This washing was repeated twice and the packed cells were used to determine HK activity. HK activity was determined by the method of Bergmeyer *et al.* (1974) with a stroma-free hemolysate obtained by sonicating, freezing and thawing the packed cells. Protein was routinely determined by the method of Gornall *et al.* (1949) with bovine serum albumin as the standard. Hemoglobin was determined by the method of cyanmethemoglobin (Cannan, 1965) with whole blood, and mean corpuscular hemoglobin concentration was calculated based on packed cell volume.

Body and organ weight

After whole blood was collected from the jugular vein of 15 weeks-old rats anesthetized with ethyl ether, body weight of the offspring was measured by using an automatic balance for rats (Shin-Maiko, Yamato Co.,Tokyo, Japan), and the organ was removed to measure its weight with an electronic reading balance (Libror ED-200, Shimadzu Co., Tokyo, Japan).

Histological examination

The total number of rats examined was 60. The test and control groups contained 30 animals (15 male and female rats each). The rats were sacrificed by exsanguination and the brain, pituitary, thyroid, thymus, heart, lungs, liver, spleen, kidneys, adrenals, testes and ovaries were removed. Tissue samples were fixed in 10% formalin, embedded in paraffin and sectioned for histological preparation. They were then stained with hematoxylin and eosin (HE).

Statistical analysis

All quantitative data were statistically analyzed using the Student's *t (Welch)*-test.

RESULTS

Ion concentration of AKW and TPW

Alkalinity and ion concentrations were compared for AKW and TPW (Table 1). The pH of AKW adjusted to 9.0 at the time of electrolysis of TPW was measured as 8.7 by the Japan Food Hygiene Association. This discrepancy may be explained by the time elapsed before measurement. In any case, the difference would not be significant. Alkalinity and calcium, sodium, potassium and magnesium in AKW exceeded those for TPW. Chloride was less than that of TPW.

Table 1. Results of testing qualities of alkaline ionized water (AKW) and tap water(TPW).

	(TPW)	(AKW)
pH [a, e]	7.3	8.7
Alkalinity(mg/l) [b]	38	50
Calcium(mg/l) [c]	17.5	20.1
Sodium(mg/l) [c]	7.8	8.6
Potassium(mg/l) [c]	1.7	2.1
Magnesium(mg/l) [c]	4.1	4.4
Zinc(mg/l) [c, e]	0.03	0.04
Iron(mg/l) [c, e]	0.05(less than)	0.05(less than)
Chloridion(mg/l) [d, e]	9.9	7.8

Note) Water quality was assessed by the Japan Food Hygiene Association, as recommended by the Minister of Health and Welfare based on the Japan Food Hygiene Act and the Japan Drug Act.
[a] : pH meter method.
[b] : Sulfuric acid neutralization titrimetry method.
[c] : Atomic absorption spectrophotometry method.
[d] : Silver nitrate titrimetry method.
[e] : Indicates use of the method for standardization of water quality based on the Japan Waterworks Act.

Body weight

Body weight of the offspring born from 10 mother animals given AKW and those given TPW during gestation and lactation was deter-

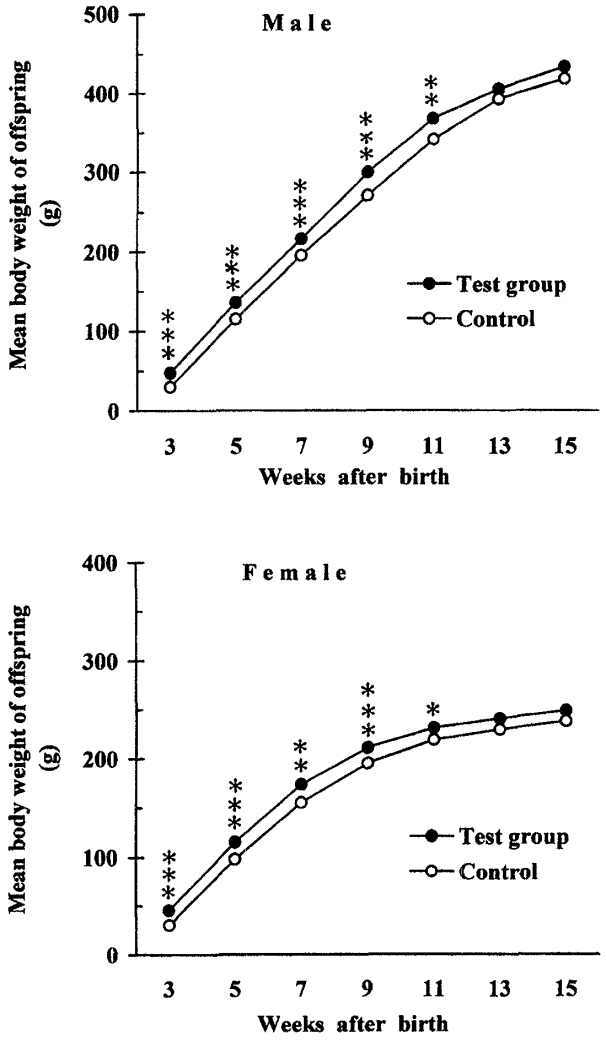
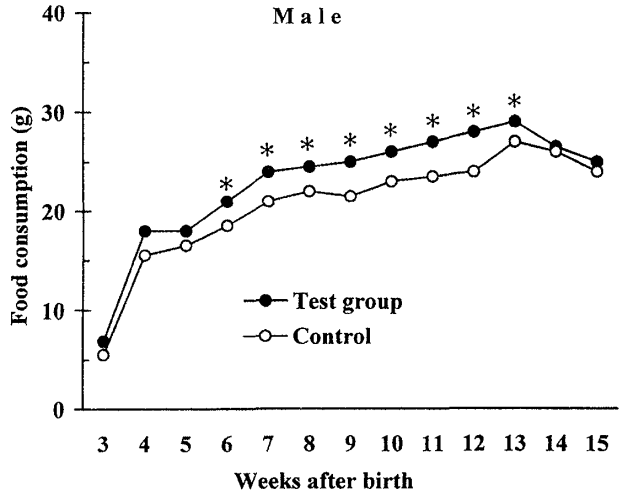


Fig. 1. Mean body weight of male and female F^1 rats given alkaline ionized water (AKW) and tap water (TPW) during 3 to 15 weeks of age after birth. Significant difference from control determined by Student's *t (Welch)*-test was observed for male rats given AKW and TPW from 3 to 9 weeks with $p<0.001$:*** and 11 weeks with $p<0.01$:**, respectively. Significant difference was observed for female rats given AKW and TPW from 3 to 5 and 9 weeks with $p<0.001$:***, 7 weeks with $p<0.01$:** and 11weeks with $p<0.05$:*, respectively.

mined three weeks after birth. Male offspring weighed 45.5±4.4 g (n=61) and female offspring 40.8±3.7 g (n=62) in the test section compared to 38.2±3.8 g (n=62) and 35.8±3.1 g (n=63), respectively, in the control section. In the test sections supplied with AKW, body weight of the male and female offspring was significantly higher than that in the control section supplied with TPW, according to paired *t (Welch)*-test. Body weight of postnatal rats rapidly increased up to 11 weeks after birth, followed by gradual increase to the maximum level. Body weight of male and female rats given AKW at 15 weeks of age exceeded that of the control group. Differences in this parameter for the two groups decreased gradually after 11 weeks (Fig. 1).

After weaning for 21 days, the dams were dissected, and various organs were observed in gross. Further, implantation rate : number of implantation sites / number of corpora × 100(%), sex ratio at weanling : male/female and duration of pregnancy in days were 89.8±4.2 %, 0.983 and 21.4±0.82 in the test group and 90.6±4.5 %, 0.984 and 21.3±0.67 in the control group, respectively.



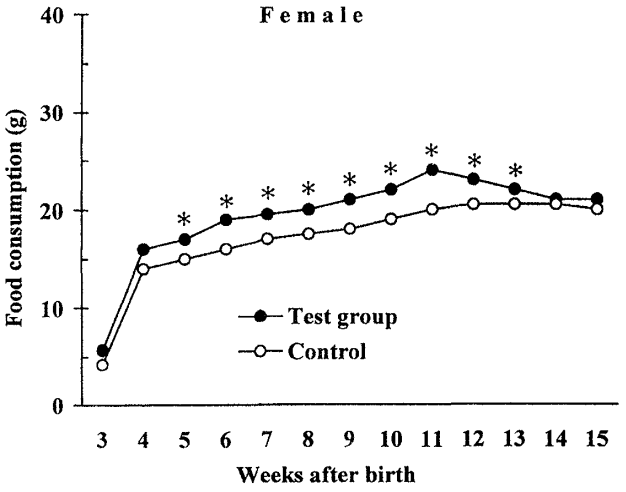
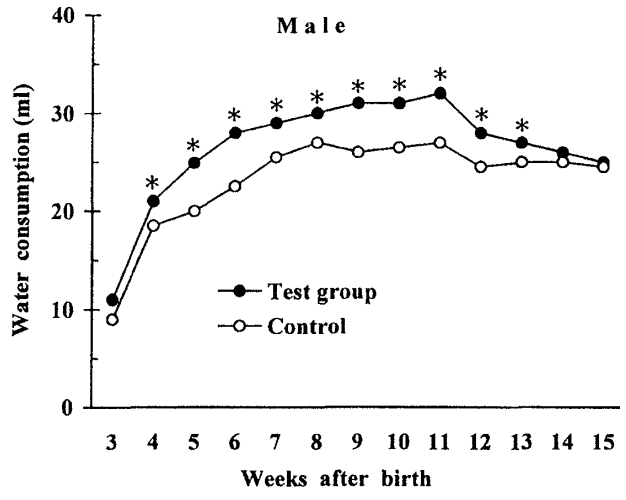


Fig. 2. Mean food consumption of male and female F^1 rats given alkaline ionized water (AKW) and tap water (TPW) during 3 to 15 weeks of age after birth. Significant difference from control determined by Student's *t (Welch)*-test was observed for male rats given AKW and TPW from 6 to 13 weeks and for female rats from 5 to 13 weeks with $p<0.05$:*, respectively.



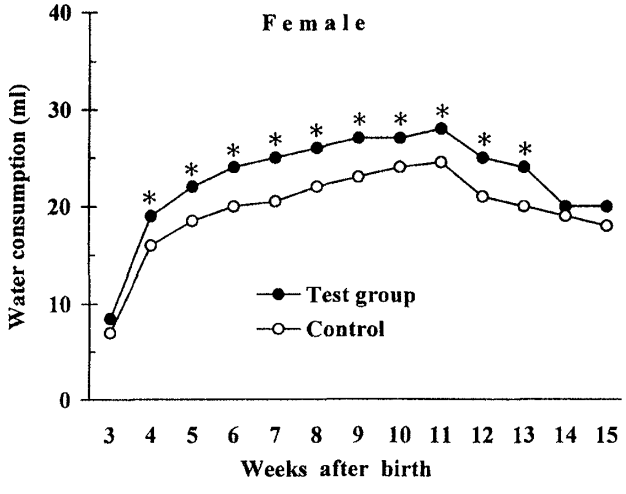


Fig. 3. Mean water consumption of male and female F^1 rats given alkaline ionized water (AKW) and tap water (TPW) during 3 to 15 weeks of age after birth. Significant difference from control determined by Student's *t (Welch)*-test was observed for male and female rats given AKW and TPW from 4 to 13 weeks with $p<0.05$:*.

Food and water consumption

Fig. 2 and 3 showed the mean intake of food and water of rats during 3 to 15 weeks after birth. Consumption of food and water increased significantly from the 5th week after birth to the 13th week in the test group.

Organ weight

Table 2. Absolute and relative organ weights of rats (F_1) at 15 weeks-old given alkaline ionized water (AKW) and tap water (TPW).

		Males		Females	
		AKW	TPW	AKW	TPW
No. of animals		15	15	15	15
Body weight	(g)	438.6±23.3	418.1±18.5	254.6±14.1	239.4±14.6
Brain	(g)	1.806±0.089	1.874±0.102	1.675±0.113	1.695±0.115
	(g%)	0.411±0.025	0.438±0.028	0.657±0.037	0.708±0.044
Pituitary	(mg)	11.8±2.5	10.2±2.4	13.5±4.1	14.4±5.2
	(mg%)	2.64±0.66	2.39±0.56	5.28±1.84	5.91±2.07
Thyroid	(mg)	21.1±4.0	19.3±4.5	16.2±5.7	15.6±5.9
	(mg%)	4.78±1.02	4.58±1.14	6.39±2.47	6.25±2.33
Thymus	(g)	0.460±0.090	0.455±0.063	0.382±0.041	0.373±0.054
	(g%)	0.104±0.021	0.108±0.015	0.150±0.015	0.156±0.028
Heart	(g)	1.231±0.260	1.103±0.250	0.785±0.078	0.834±0.098
	(g%)	0.280±0.059	0.263±0.062	0.308±0.028	0.318±0.034
Lung	(g)	1.535±0.142	1.438±0.180	1.196±0.121	1.129±0.148
	(g%)	0.350±0.031	0.342±0.035	0.470±0.053	0.471±0.055
Live	R(g)	11.103±1.512	10.735±1.109	7.648±0.661	7.332±0.738
	(g%)	2.523±0.238	2.569±0.221	3.007±0.242	3.054±0.225
Spleen	(g)	0.614±0.169	0.592±0.072	0.481±0.061	0.471±0.055
	(g%)	0.140±0.034	0.147±0.019	0.189±0.025	0.196±0.022
Kidneys	R(g)	1.108±0.087	1.059±0.115	0.739±0.067	0.726±0.079
	(g%)	0.252±0.011	0.253±0.027	0.290±0.023	0.302±0.025
	L(g)	1.089±0.074	1.046±0.101	0.728±0.067	0.700±0.072
	(g%)	0.247±0.012	0.250±0.026	0.283±0.026	0.292±0.025
Adrenals	R(mg)	21.7±9.1	23.7±4.9	27.8±6.7	28.4±8.0
	(mg%)	4.94±2.15	5.63±1.29	10.88±2.65	11.86±3.53
	L(mg)	23.3±7.5	24.3±5.5	28.2±8.3	26.6±3.7
	(mg%)	5.29±1.72	5.78±1.36	11.12±3.72	11.08±1.64
Testes	R(g)	1.711±0.418	1.526±0.448		
	(g%)	0.389±0.092	0.367±0.114		
	L(g)	1.699±0.437	1.407±0.419		
	(g%)	0.379±0.092	0.339±0.110		
Ovaries	R (mg)			49.3±10.0	54.4±14.0
	(mg%)			19.35±4.15	22.67±5.90
	L(mg)			47.4±16.9	52.0±13.8
	(mg%)			18.52±6.51	21.58±5.46

Note) Mean ± S.D.

Absolute and relative organ weight of offspring given AKW and TPW at 15 weeks-old is shown in Table 2. No significant difference was noted in the weight of brain, pituitary, thyroid, thymus, heart, lungs, liver, spleen, kidneys, adrenals, testes and ovaries between the test and control sections as determined by Student's *t (Welch)*-test ($P > 0.05$).

Blood biochemical finding

Measurements were made of these ion concentrations in serum of rats (n=12, respectively) at 15 weeks of age. The concentrations of potassium were as follows: male AKW, 6.1±0.6; male TPW, 5.1±0.7; female AKW, 6.4±0.7; and female TPW, 5.2±0.6 mEq/l. Calcium in serum expressed in mg/dl was: male AKW, 11.3±0.4; male TPW, 10.9±0.6; female AKW, 9.6±0.3; female TPW, 9.1±0.7. Chloride in serum expressed in mEq/l was: male AKW, 102.8±3.2; male TPW, 105.2±1.5; female AKW, 103.2±2.9; female TPW, 106.0±1.6. Serum potassium and calcium of the AKW group were significantly higher than for TPW ($p<0.05$), and chloride of

Table 3. Hematological and biochemical finding of rats (F_1) at 15 weeks-old given alkaline ionized water (AKW) and tap water (TPW).

		Males		Females	
		AKW	TPW	AKW	TPW
No. of animals		12	13	12	14
Erythrocytes	($\times 10^4$/mm^3)	868.3±92.0	879.5±77.4	802.0±112.4	798.3±75.7
Leukocytes	($\times 10^3$/mm^3)	16.0±1.5	16.1±1.3	14.7±1.36	14.9±1.3
Hematocrit	(%)	43.4±1.5	42.6±1.7	40.8±3.0	40.1±2.4
Hemoglobin	(g/dl)	9.73±2.32	9.82±1.80	8.11±2.25	7.53±2.26
Glucose	(mg/dl)	86.2±14.4	84.6±18.6	80.2±16.7	78.8±13.4
Na	(mEq/l)	140.3±2.0	141.7±2.8	140.8±2.9	142.1±1.8
K	(mEq/l)	6.1±0.6*	5.1±0.7	6.4±0.7*	5.2±0.6
Cl	(mEq/l)	102.8±3.2*	105.2±1.5	103.2±2.9*	106.0±1.6
Ca	(mg/dl)	11.3±0.4*	10.9±0.6	9.6±0.3*	9.1±0.7
Mg	(mg/dl)	2.9±0.1	3.0±0.3	2.7±0.2	2.7±0.2
Pi	(mg/dl)	8.3±0.8	7.4±1.2	8.7±1.4	8.2±1.2
TP	(%)	7.4±0.3	7.5±0.4	7.4±0.4	7.3±0.5
Albumin	(%)	46.57±2.75	46.49±1.40	50.56±2.35	50.90±2.07
	(g/dl)	3.47±0.24	3.49±0.22	3.77±1.20	3.71±0.25
Globulin α_1	(%)	24.36±1.84	23.07±1.71	22.11±1.30	20.69±1.54
	(g/dl)	1.81±0.17	1.73±0.18	1.65±0.16	1.56±0.13
α_2	(%)	7.65±1.32	7.27±1.14	6.81±1.04	6.81±0.98
	(g/dl)	0.56±0.10	0.54±0.09	0.50±0.07	0.49±0.07
α_3	(%)	3.78±1.19	4.22±1.18	3.25±0.64	3.70±0.65
	(g/dl)	0.27±0.08	0.31±0.09	0.23±0.05	0.26±0.04
β	(%)	14.62±1.31	15.13±1.59	13.55±1.41	13.65±1.01
	(g/dl)	1.08±0.11	1.13±0.10	1.01±0.12	0.99±0.09
γ	(%)	2.96±1.12	3.26±1.13	3.68±1.06	4.19±1.44
	(g/dl)	0.21±0.08	0.24±0.08	0.27±0.07	0.30±0.10
A/G		0.875±0.092	0.869±0.094	1.026±0.098	1.040±0.091

Note) Mean ± S.D.

* Indicates a significant difference between the test and control groups determined by Student's *t (Welch)*-test, with $p<0.05$ in males and females.

the AKW group was significantly less than that of the TPW group ($p<0.05$). Measurements of sodium, magnesium and inorganic phosphate, protein fractions in sera and erythrocytes, leukocytes, hematocrit, hemoglobin and glucose in whole blood showed essentially the same values for the two groups (Table 3).

HK activity

The activity of erythrocyte HK, a rate-determining enzyme in erythrocyte glycolysis, was compared for the test and control groups. The activity of males at 15 weeks was 260.0±53.2 U/100 g hemoglobin for the AKW group (n=12) and 206.3±56.1 U/100 g hemoglobin for the TPW group (n=12), significantly differing. For females at 15 weeks old and given AKW (n=12) and TPW (n=12), the values were 237.1±80.0 U/100 g hemoglobin and 194.3±38.5 U/100 g hemoglobin, respectively, the values not being significantly different (Fig. 4). Some specimen was lost because the hemolysate was excluded from both AKW and TPW groups.

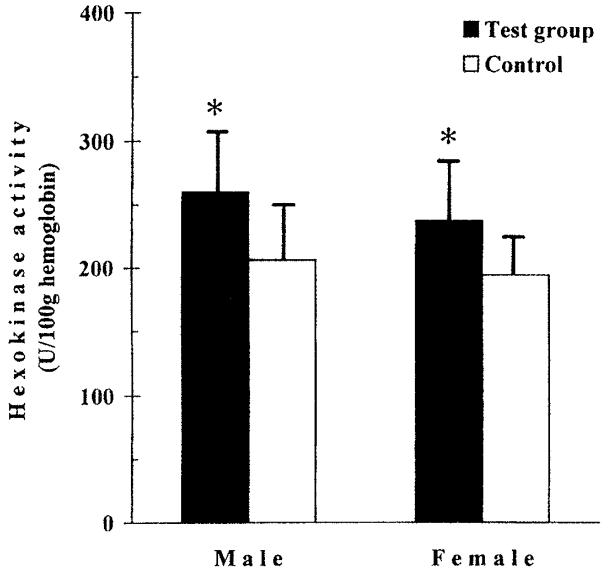


Fig. 4. Mean hexokinase activity in erythrocytes of 15 weeks-old rats given alkaline ionized water (AKW) and tap water (TPW) . Bars indicate mean±standard deviation. Significant difference from control determined by Student, s t (Welch)-test was observed for male rats given AKW (n=12) and TPW (n=12) with $p<0.05$:*. No significant difference observed for female rats given AKW (n=12) and TPW (n=12).

Histological findings

Light microscopic examinations of the histological preparations of brain, pituitary, thyroid, thymus, heart, lung, liver, spleen, kidneys, adrenals, testes and ovaries were carried out. Particularly, as a result, myocardiac lesions were observed in the AKW group. The data on the incidence and severity of myocardiac lesions, such as cell infiltration, focal necrosis, vacuolation of myocardium and vessel wall, fibrosis, homogenizing cell change and edematous dissociation in each group are summarized in Table 4. In male rats 15 weeks of age, given AKW, cellular infiltration of leukocytes in the myocardium was clearly evident, as was fibrosis of myocardiac muscle in the papillary muscle of the left ventricle (Photo. 1 A). In male rats 15 weeks-old, given TPW, the same injuries were observed, but the extent was less in the AKW group (Photo. 1 B). These findings were observed not only in males but also in females, but particularly so in the former (Table 4).

DISCUSSION

Body weight of male and female rats given AKW in postnatal growth significantly increased beyond control group values (Fig. 1). Erythrocyte HK activity of experimental groups increased more than in the control group, especially in males (Fig. 4). HK is a marker enzyme of metabolic activity and rate-determining enzyme in erythrocyte glycolysis (Rapoport, 1968). Its activity thus expresses the utilization rate of glucose (Laris, 1958). Increase in HK activity thus suggests elevation of metabolic activity in the AKW group. No significant difference was observed in heart weight for the two groups, and there were no significant differences between the two groups in weights of other organs. Increase in body weight and metabolic activity in rats given AKW would thus appear to augment cardiac workload.

Serum potassium and calcium concentrations of male and female rats given AKW at 15 weeks of age were significantly higher than in the TPW group, whereas chloride in the AKW group was less than in the TPW group. Serum potassium in the AKW group was particularly

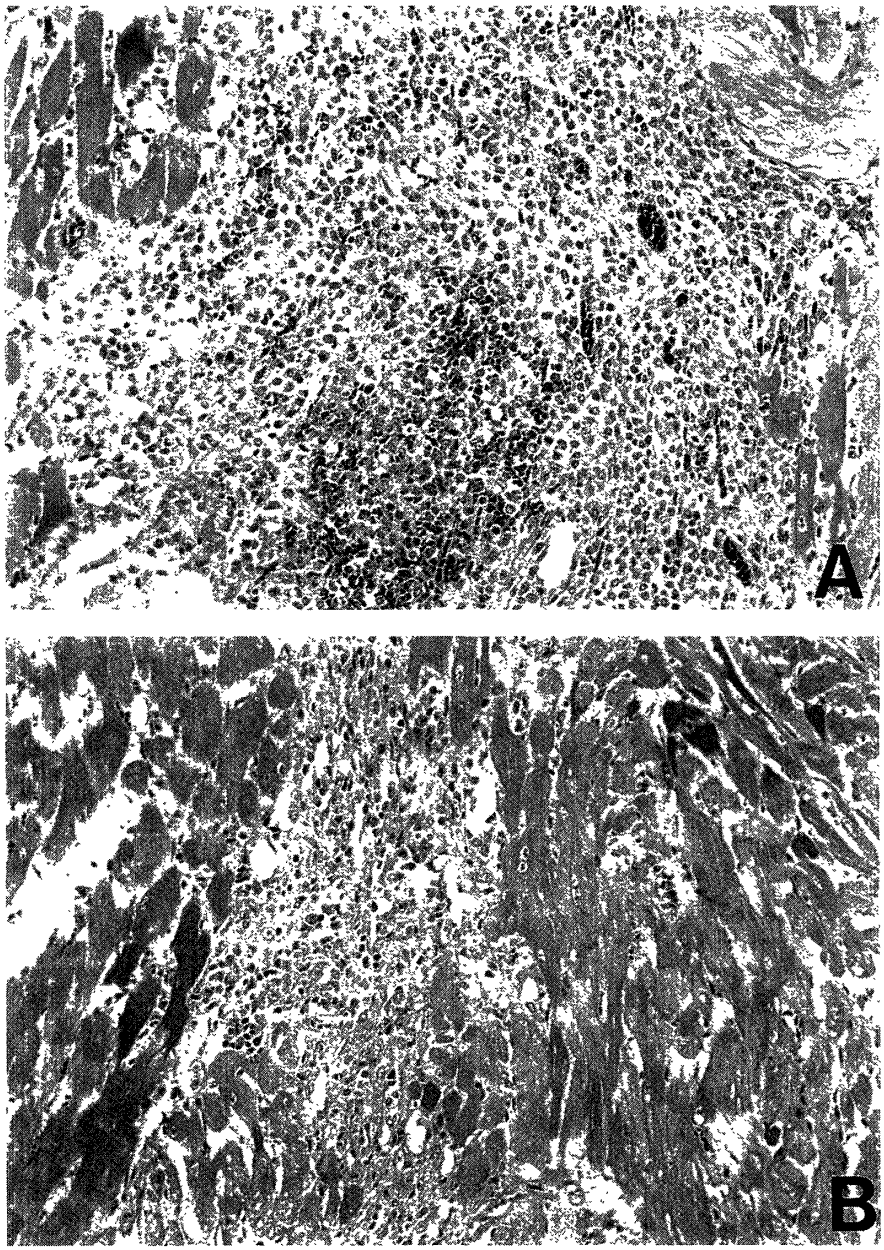


Photo 1. Myocardiac muscle of rats at 15 weeks of age, given alkaline ionized water. (A) Experimental male rat. Cellular infiltration of leukocytes and fibrosis are apparent in papillary muscle, left ventricular myocardium. Hematoxylin and eosin stain. × 50. (B) Control male rat. Comparatively mild infiltration is seen in papillary muscle, left ventricle. Hematoxylin eosin stain. × 50.

indicative of hyperkalemia. In hyperkalemia, the resting potential increases and polarization decreases owing to an increase in intracellular potassium (Lyon, 1983; Weizenberg *et al.*, 1985; Surawicz, 1987). Hyperkalemia in particular causes atrioventricular blockage and ventricular fibrillation to have an adverse effect on myocardiac muscle. This critical condition requires

Table 4. Histopathological finding in the myocardium of rats (F_1) at 15 weeks-old given alkaline ionized water (AKW) and tap water (TPW).

	Males		Females	
	AKW	TPW	AKW	TPW
No. of animals	15	15	15	15
Cell infiltration	− 3 (20.0%)	10 (66.7%)	4 (26.7%)	10 (66.7%)
	± 6 (40.0%)	5 (33.3%)	10 (66.7%)	5 (33.3%)
	+ 5 (33.3%)	0 (0%)	1 (6.7%)	0 (0%)
	++ 1 (6.7%)	0 (0%)	0 (0%)	0 (0%)
Necrosis	− 0 (0%)	8 (53.3%)	0 (0%)	10 (66.7%)
	± 1 (6.7%)	7 (46.7%)	2 (13.3%)	5 (33.3%)
	+ 6 (40.0%)	0 (0%)	6 (40.0%)	0 (0%)
	++ 8 (53.3%)	0 (0%)	7 (46.7%)	0 (0%)
Vacuolation of myocardiac muscle	− 5 (33.3%)	13 (86.7%)	5 (33.3%)	10 (66.7%)
	± 4 (26.7%)	2 (13.3%)	7 (46.7%)	5 (33.3%)
	+ 4 (26.7%)	0 (0%)	1 (6.7%)	0 (0%)
	++ 2 (13.3%)	0 (0%)	2 (13.3%)	0 (0%)
Fibrosis	− 0 (0%)	3 (20.0%)	0 (0%)	5 (33.3%)
	± 5 (33.3%)	11 (73.3%)	6 (40.0%)	10 (66.7%)
	+ 7 (46.7%)	1 (6.7%)	7 (46.7%)	0 (0%)
	++ 3 (20.0%)	0 (0%)	2 (13.3%)	0 (0%)
Homogenizing cell change	− 0 (0%)	7 (46.7%)	0 (0%)	13 (86.7%)
	± 1 (6.7%)	6 (40.0%)	3 (20.0%)	2 (13.3%)
	+ 5 (33.3%)	2 (13.3%)	10 (66.7%)	0 (0%)
	++ 9 (60.0%)	0 (0%)	2 (13.3%)	0 (0%)
Edematous dissociation	−13 (86.7%)	13 (86.7%)	10 (66.7%)	13 (86.7%)
	± 1 (6.7%)	2 (13.3%)	5 (33.3%)	2 (13.3%)
	+ 1 (6.7%)	0 (0%)	0 (0%)	0 (0%)
	++ 0 (0%)	0 (0%)	0 (0%)	0 (0%)
Vacuolation of vessel wall	− 13 (86.7%)	15 (100%)	14 (93.3%)	15 (100%)
	± 2 (13.3%)	0 (0%)	1 (6.7%)	0 (0%)
	+ 0 (0%)	0 (0%)	0 (0%)	0 (0%)
	++ 0 (0%)	0 (0%)	0 (0%)	0 (0%)
	++ 0 (0%)	0 (0%)	0 (0%)	0 (0%)

Note) − : No remarkable changes ± : Slight + : Mild ++ : Moderate

emergency treatment (Lyon, 1983; Weizenberg *et al.*, 1985; Surawicz, 1987). Considering these effects and overload of cardiac workload by increase in body weight and metabolic activity in spite of no increase in heart weight, the protracted intake of AKW in postnatal growth of rats may harmfully affect myocardiac muscle directly and/or indirectly.

Histological preparations of myocardium in male rats given AKW showed cellular infiltration of leukocytes and fibrosis (Photo. 1 A). In other organs, no histological changes were observed between the AKW and TPW groups. The cellular infiltration is due to necrosis, and fibrosis subsequently occurs (Sanditter and Thomas, 1986). Necrosis and vacuolar degeneration of myocardium occur due to abnormal electrolyte metabolism (Matsubara and Hayashi, 1980). In this study, hyperkalemia by AKW intake was thought to contribute to these changes in myocardiac muscle, and furthermore, increase in cardiac workload to accelerate myocardiac change. Myocardiac degeneration may cause leakage of potassium from among cells into the blood. Its concentration in the blood increases, with consequent chronic hyperkalemia. Infiltration and fibrosis were observed in the control group, but to a much lesser extent than in the test group (Photo. 1 B). In the myocardium of rats and rabbits, degeneration, necrosis and fibrosis occur spontaneously, especially in males (Matsubara and Hayashi, 1980). This is in agreement with the present findings for the control group. These changes gradually progress with age, but with no clear effect (Matsubara and Hayashi, 1980).

As mentioned above, increase in body weight and elevation of metabolic activity by protracted intake of AKW in postnatal growth of rats were accompanied by chronic hyperkalemia, and myocardiac degenerations were induced. At this time, it is not clear whether myocardiac degeneration caused by this protracted intake is harmful. Further studies including the determination of myocardiac contractile protein should be conducted to clarify the causes of myocardiac lesions. The body weight increase of the offspring, however, may occur either by the placental transfer to the fetus of water-hydrated cation produced by electrolysis, or nutritional supplementation through the mother's milk up to 3 weeks after birth. It is unclear at present whether the increase in body weight of the offspring was caused by increased production and secretion of milk by the mammary gland and improvement of the quality of milk in response to the supply of AKW with high cation concentration, or a pathological increase in body weight induced by the supply of AKW to the mothers during gestation and lactation.

In addition to the cause of the body weight increase in offspring, the cause of myocardiac necrosis, either by AKW or by some unknown substance dissolved in AKW from the electrode of the electrolytic water ionizer used , remains for further studies.

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