APPROACH TO QUALITY OF LIFE

# Function Water Symposium

Held in Kyoto in 1995

**Vision for the Future of Acid and Alkaline Electrolytic Water**

## DRAFT

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## 1. Lecture by International Guest:
## Magnesium & Fluoride Intake, Through Drinking Water, & The Health

by Heikki Luoma, Dept. of Preventive Dentistry and Cariology, University of Kuopio, Finland

Our two field studies conducted on Finnish men revealed a negative association between cardiovascular (CV) diseases and fluoride concentration as well as magnesium concentration in drinking water. Furthermore, increased risk of myocardial infarction was found to be associated with low level of fluoride and magnesium concentration as compared to those who took higher amounts of these two elements in drinking water.

In an attempt to find any cause-effect relations between intake of the said elements and CV diseases through animal experiments, our early studies by rats showed high calcium accumulation in rats' aortae and kidneys kept on low magnesium and high phosphorous diet. These disturbances were reduced by small addition of fluoride with or without small dose of magnesium to their diet.

Our recent findings in genetically hypercholesterolemic rats, receiving low Mg-high-sugar control diet with some cholesterol, showed that a low addition of fluoride to the diet could reduce some minor serum lipid constituents, generally associated with serum lipid profile of CV diseases.

## 2. Current Situation & Future Direction of Handy Tap Water System for Residential Use

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### A. Preface

Drinking water in mid-rise and high-rise buildings is generally supplied via faucets on every floor after pumped up from the water tank below to the rooftop tank. It is not supplied directly from the water main. Water supply equipment such as the water tanks need to be maintained voluntarily for hygienic reasons by the management. Without proper maintenance, the tap water, which is supposed to be safe and clean, will eventually be contaminated by toxic materials and polluted water.

Or this reason, the Clean Water Act was amended in 1977 to regulate "handy tap water system" that has water tanks larger than 20 cubic meters. The new regulation mandates those who installed the tanks to maintain them properly and have them inspected periodically. In addition, Executive Order of the Clean Water Act was amended in 1985 to extend the regulation to include "Handy tap water system" with water tanks larger than 10 cubic meters. Later in January of 1987, the National Government stipulated "Guidelines for Drinking Well Water". According to these guidelines, water systems that are not regulated either by "Clean Water Act" or "Hygienic Building Maintenance Act" (under Building Management Law) have also been regulated to go through proper maintenance and inspection as "Small-scale Tank Water".

### B. Number of Handy Tap Water Systems ('94 figures)

The installer of the handy tap water system is not required by law to register it. Although the exact figure is not clear, there are a number of small-scale tank water systems and handy tap water systems in the city for multi-family residential, retail, office, hotel and school buildings. Small-scale tank water systems no more than 10 cubic meters are counted at 6,007, whereas handy tap water systems that exceed 10 cubic meters are 2,687.

### C. Maintenance Including Regular Cleaning & Inspection

The installer of the handy tap water system is required to maintain it routinely as well as to take legal inspection at least once a year for more than 60 items. In Kyoto, this periodical inspection is conducted by a designated inspection agency. So far, 1,895 facilities have taken this inspection resulting in the rate of 70.5% against all the facilities. If the legal inspection finds any problems with the facility and the agency gives advice, it has to make improvements immediately under the supervision of the Health Department. Last year, 21 facilities were given instructions to make improvements, all of which have already done so.

### D. Conclusions

The law imposes each installer of the handy tap water system various burdens of maintenance. However, new installations as well as modification of existing system are not required any registration. Therefore, it is necessary for the City to establish some means to identify the current situation of installation to be able to give directions to the installers. From this point of view, City of Kyoto is not enforcing a regulation to require registration for new systems and record keeping of the related documents according to the "Handy Tap Water System Administration Handbook". The City is also educating the public to promote adequate management of the system by identify unknown facilities with the help of "Investigation Guidelines" as well as promoting legal inspections during the intensive supervision period as part of the annual project plan.

All of us in the Bureau of Public Health are fully aware that the education and guidance for the water system installers should be intensified further, and existing situations be thoroughly identified, to maintain the high standard of water hygiene for residents, and protect them from any damages by inadequate drinking water.

## 3. Quality of Source Water for Municipal Supply & Health

by Katsuhiko Yokoi, Mieko Kimura & Yoshinori Itokawa, Kyoto University Graduate School of Medicine
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Solute quality of source water for municipal supply is one of the possible factors affecting human health and disease. Relations between standardized mortality rates of malignant neoplasm, and parameters of source water quality for municipal supply and environment in Japan were investigated.

Data by prefecture in 1985 were taken from the reports by the Ministry of Health & Welfare and the Prime Minister's Office of Japan. Among source water parameters, potassium permanganate consumption ($r = 0.481$), iron ($r = 0.505$) and manganese ($r = 0.334$) were significantly ($p< 0.05$) correlated with the mortality rates for male malignant neoplasm. Potassium permanganate consumption ($r = 0.312$), iron ($r = 0.405$) and zinc ($r = 0.390$) in source water significantly related to the mortality rates for female malignant neoplasm. These results suggest that source water quality for municipal supply water and related environmental factors affect development and evolution of neoplasm.

### A. Purpose of Study

Drinking water has historically been the route of infection for water-related diseases, but the problem has almost been solved by chlorination of tap water. However, there is a potential correlationship between the components of drinking water and the occurrence or prevention of chronic diseases. For example, in the regions where residents take mineral water, mortality rates of anemic heart disease are low. On the other hand, the relation between components of water and malignant neoplasm, which is the primary cause of death in the region, is not clear. We selected all range of environmental factors including quality of source water from various statistics by prefecture, and tried to explain mortality rates of neoplasm in each prefecture.

### B. Subject and Method of Study

As to mortality rates of various neoplasm, we referred to the Mortality Rate Table for Malignant Neoplasm (by sex) in 1985 published in Mortality Rates by Prefecture Adjusted by age issued by the Ministry of Health & Welfare in 1990, Bureau of Statistics and Information. The seven items of water quality, chloric ion, potassium permanganate consumption, iron, manganese, z inc, hardness, and pH, have been calculated by weighted average sing the Amount of Water Supply by Reservoir (sorted by water quality in different Prefectures) published in Water Statistics of 1985 by the Ministry of Health & Welfare, Department of Hygiene and Tap Water Institute. The 16 parameters associated with social and economic factors were referred to "Prefectures in Statistics" by the Prime Minister's Office of Statistics and "Science Almanac" edited by Tokyo Astronomical Observatory.

All the statistics were processed by Macintosh SYSTAT version 5.2 (SYSTAT Inc., Illinois, USA). First of all, we calculated the correlation between mortality rates and various parameters. Next, we extracted the major components from each of social and economic parameters and came up with coefficients in the correlation between the obtained components and mortality rates for malignant neoplasm.

### C. Study Results and Analysis

Parameters that were significantly correlated ($p< 0.05$) with mortality rates of malignant neoplasm for either men or women were as follows: among source water parameters, the items that were significantly correlated with mortality rates of neoplasm for men were potassium permanganate consumption ($r = 0.481$), iron ($r = 0.506$), manganese concentration ($r = 0.334$). The items that were correlated significantly with mortality rates of neoplasm for women were potassium permanganate consumption ($r = 0.481$), iron ($r = 0.405$), zinc concentration ($r = 0.390$). Among social and economic factors, the parameters that were significantly correlated with mortality rates of men were rates of general assistance ($r = 0.349$), rates of workmen's deaths and injury 9r = 0.379), car ownership rate per household ($r = -0.506$), whereas parameters significantly correlated to mortality rates of women were per capita income in the Prefecture ($r = 0.377$), rate of agricultural population ($r = -0.442$), the latitude of the area ($r = 0.406$), and temperature ($r = -0.313$).

There were many common correlation patterns among these parameters. We applied analyses of major components for each of water quality parameters and socio-economic parameters, and processed by Vari Max rotation to come up with the simple structure. In the first group of socio-economic components (S1), per capita income in the Prefecture, number of ambulance dispatch, road area ratio, and gross product shipping per manufacturing employee contributed positively to the correlation, whereas rate of agricultural population per capita acreage of livable area, contributed negatively. These were inferred as urban factors. In the second group of components (S2), the longitude and latitude of the area contributed positively as opposed to the temperature that contributed negatively. These were inferred as geographical factors. In the third group (S3), population rate of general assistance and rate of labor injuries and deaths worked negatively, whereas car ownership rate per household worked positively. These were inferred as everyday-life factors. In the fourth group (S4), rate of forest and farmland area had positive and negative correlation respectively. The fifth group (S5) showed positive contribution by the number of auto accidents per capita contributing positively to the correlation which was inferred as auto accident factor. As to source water quality parameters, the first component (W1) such as potassium permanganate

consumption, iron and manganese concentration positively contributed to the correlation and was inferred as water quality deterioration factor. Regarding the second component (W2), hardness and pH of water positively contributed and was categorized a hardness-related factor. The third component (W3) showed concentration of zinc and chlorine iron positively contributed and was categorized as zinc-related factor.

S3 (r = 0.528) and W1 (r = 0.554) significantly correlated to mortality rates of men, and S2 (r = 0.407), W1 (r = 0.416) and W3 (r = 0.416) to those of women. Especially the deteriorated water quality factor (W1) contributed to the increase of deaths by malignant neoplasm for both men and women. According to the above results, correlation between death rate by malignant neoplasm and source water quality factor as well as socio-economic factor was suggested.

## 4. Effects of Calcium Alkaline Ionized Water on Quality of Bone Mass in Rats

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Male Wistar rats, 3 weeks old, were divided into 12 groups. Four different synthetic diets (i.e. calcium concentration of each diet was 0, 30, 60 and 100% of adequate amount). Three kinds of water, tap water, water containing calcium lactate, and calcium alkaline ionized water, was assorted and given ad libitum to the 12 groups.

No significant differences were observed in the gain of weight and consumption of food and water among the groups. Comparing the groups that consumed the diet with the same calcium concentration, the weight and calcium concentration in the femur and libia were increased more significantly in rats that were given calcium alkaline ionized water than those given tap water and calcium lactate water.

### A. Purpose of Study

Osteoporosis is defined as “the state of decreased bone mass and fragility of bones due to the deterioration of bone tissues that can easily cause bone fractures”. Osteoporosis is caused by various situations. One of the causes is known to be the abnormal level of metabolism related to calcium (Ca). In other words, lack of Ca intake, reduced absorption of Ca from the intestines, increased level of urinal discharges of Ca cause the problem. Moreover, the reduced level of Ca absorption can bring a lower level of Ca in the blood resulting in the secondary hyperthyroidism to trigger osteoporosis. The risk factors of osteoporosis are considered to be two kinds: one that prevents the bone mass from growing, the other that promotes reduction of the bone mass.

Our experiments this time were conducted as described below. Alkaline ionized calcium water (ionized water) was given to the rats, and the function of calcium in the ionized water was examined.

### B. Study Method

Sixty male Wistar rats, all of them 3 weeks old, were divided into 12 groups. Different diets were prepared at 4 levels of calcium concentration ranging from 100% of normal calcium concentration own to 60%, 30% and 0%. The rats were given the diets voluntarily. Three kinds of water, tap water (with approximately 6 ppm of Ca), water containing calcium lactate (4 ppm of Ca), and alkaline ionized water (40 ppm of Ca, pH=9) generated by a device certified by Alkaline Ionized Water Association Unified Standard were given to them on a voluntary basis.

Rats’ weight, amount of food and water taken, and calcium concentration in the drinking water were measured every day. During the experiment period, metabolism experiments were conducted by a Ballman gauge during the entire period. After four weeks, the blood was taken from each rat under Nembutal anesthesia, then tibiae and libia and other tissues were removed to measure the bone mass and calcium concentration in the bones and other tissues by an atomic absorption spectrophotometry.

### C. Study Results

As a result of comparing the groups of rats that were given three kinds of water among those fed with the same percentage of calcium in the diet, no significant difference was recognized for their weight increase, amount of food and water taken. As to the bone mass of their tibiae and libia, and calcium concentration in the bones, those given alkaline ionized water have seen significant increase as compared with those given tap water or calcium lactate water. No significant difference was observed for the amount of calcium in their tissues.

Nothing is known about the system of increase in bone mass for those given alkaline ionized water. However, we are planning to examine it in relation to the cause of osteoporosis crisis.

## 5. Effects of Alkaline Ionized Water on Intestinal Microcirculation

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Using intravital microscopic system, effects of alkaline ionized water on intestinal microcirculation were examined. Wistar rats were used for the experiment. Their 12 cm long ileal segments, each at 20 cm from the cecal valve were placed on a plastic plate and observed.

Submucosal microvessels of the rat ileum were observed by an intravital fluorescence microscope equipped with a high-speed video camera system after intra-arterial infusion of endotoxin (2mg/kg/hr). Intestinal mucosal injury was produced 60 minutes after the endotoxin infusion.

Preceding these mucosal damages, however, velocity of red blood cells (REC) has significantly decreased after 30 minutes in both arterioles and venules, and reached their minimal values after 60 minutes.

Intraluminal infusion of alkaline ionized water (pH 9.0) into the small intestine did not produce any significant changes in intestinal microcirculation by itself. The changes in intestinal microcirculation after LPS administration was examined in rats that were chronically treated by alkaline ionized water as compared with those with tap water. The micro circulatory damage by LPS was slightly attenuated by the treatment with chronic alkaline ionized water, although there was no significant difference between two groups.

Using intravital microscopic system, effects of alkaline ionized water on intestinal microcirculation were tested experimentally. Wistar male rats were used for the experiment. They underwent a laparotomy under pento-barbital anesthesia and were taken out their ileum loops. These loops were laced on a plastic plate equipped with a controlled temperature tank. Submucosal microcirculation in the rats' ileum was observed by an intravital fluorescence microscope equipped with a high-speed video camera, and then recorded by the video to undergo an image analysis.

As a lesion model, endotoxin (E.coli LPS) was infused constantly at 2 mg/kh/hr from the carotid, and the lesion of microcirculation was observed. To examine the effects of alkaline ionized water on the improvements of microcirculation, rats were raised for four weeks by alkaline ionized water (pH 9.0), and were compared with the control group raised by tap water. As parameters of intestinal microcirculation, we examined diameters of blood vessels, velocity of red blood cells, and adhesion of leukocytes to the vessels.

Infusion of alkaline ionized water (pH 9.0) by itself did not bring any significant changes to the intestinal microcirculation. Approximately sixty minutes after infusion of endotoxin, ulcerous hemorrhage was observed at the mucosa of the small intestine. However, earlier at about 30 minutes after the infusion, velocity of red blood cells has decreased in both arterioles and venules, and an increase in the number of leukocytes adhered to the venules was observed.

Effects of alkaline ionized water on the recovery from endotoxin lesion was examined by rats that were raised by the ionized water. The result was that the ionized water did not cause reduction of velocity of red blood cells within intestinal microcirculation, bit it tended to somewhat repress leukocyte adhesion, although no significant difference was observed.

## 6. Physiological Effects of Alkaline Ionized Water: Evaluation of Animal Experiment by Rats

by T**akashi Hay**kawa, Satuski Segawa, Misako Mori, Seiji Hyashi and Haruhito Tsuge
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Alkaline ionized water (AIW) is an effective type of functional water. It has been approved by the Ministry of Public Health and Welfare in Japan. However, there has not been enough evidence to satisfy recently increasing interests and demands for AIW.

This experiment was performed to evaluate physiological effects of AIW by animal experiment using rats with special emphasis on functions of gastrointestinal tracts. Five-week old male Wistar rats were given free access to MF diet (produced by Oriental Yeast Co., and AIW or tap water for 8 weeks.

As a result, gain of body weight, intakes of food and water, weights of the liver and kidneys did not differ significantly although the weights of small intestine, colon plus rectum, and cecal content of short-chain fatty acids have decreased in the AIW ingested groups.

Since there is no significant difference in the intestinal microflora, the effect of AIW would not be through a decrease in intestinal microorganism but through other conditions that are partially responsible for alkalinity and presence of calcium ion in AIW. These results support one effect of AIW. That is, abnormal fermentation is suppressed in gastrointestinal tracts by drinking AIW.

The production system of alkaline ionized water is medical equipment approved by the Ministry of Public Health and Welfare. The alkaline ionized water produced by this system has effects on abnormal fermentation in gastrointestinal tracts, chronic diarrhea, indigestion, acid control, and suppression of gastric hyperacidity. In recent years, there are increasing interests in and demands for the alkaline ionized water, whereas the public knowledge about its efficacy is still poor. Therefore, we tried to evaluate the physiological effects of AIW by animal experiments using rats especially focusing on the function of digestive organs.

**A. Effects of Long-term Intake of AIW**

Five-week old male Wistar rats were raised for 8 weeks with free access to MF diet (produced by Oriental Yeast Co.), as well as AIW (pH 9, 10 and 11) and tap water. On the last day of experiment, their blood samples were taken, and internal organs removed. During the period of experiment, there is no difference among the rats in growth, intake of food, and general appearance. The intake of water was less in the group of pH 11, although there was no difference among other groups. The weights of the liver and kidneys did not show any difference among them, although the weights of small intestine, colon plus rectum, and cecal contents showed some decrease in the group given alkaline ionized water (Fig.1). Especially the amount of short-chain fatty acids (acetic acid, propionate acid, butyric acid) in the cecal contents measured as a parameter for intestinal fermentation has decreased, or showed some tendency to decrease, as compared to the control group (Fig.2).

**B. Effects of Alkaline Ionized Water on Microflora of Enteric Bacteria**

To examine the impact of AIW on the intestinal fermentation of rats raised by the MF diet whether it is by the changes in the microflora of enteric bacteria or not, we tested the microflora of cecal contents for rats that were given AIW for a short period (1 week) as well as a long period (8 weeks) as compared to those given tap water following Mitsuoka method. As a result, we found 5 kinds of aerobic bacteria such as enterobacteriaceae, streptococcus, staphylococcus, bacillus, and lactobacillus in the short-term test. Especially lactobacillus was predominant. AIW did not bring any significant difference in terms of the type and amount of bacteria. As to anaerobic bacteria, 3 kinds were found which were bifidobacterium, bacteroidaceae, and B.fragilis group. Some of other anaerobic bacteria were detected more often in the rats given AIW, although there was no significant difference in the type and amount of bacteria as in the case of aerobic bacteria. Intake of AIW did not result in significant difference in the long term test either.

**C. Effects of pH and Calcium for Long-term Intake of AIW**

The AIW used for this experiment has different pH and calcium concentration (50 ppm) from those of tap water. In order to analyze the mechanism of AIW's repression against intestinal fermentation, we examined the effects of its pH and calcium concentration.

Five-week old Wistar rats were given tap water, water with the same calcium concentration as AIW (+Ca), alkali water at pH 10, AIW at pH 10 with the same calcium concentration (pH 10+Ca), and alkaline ionized water at pH 10 (AIW), and were raised for 8 weeks by MF diet. As a result, the weight of cecal contents was significantly less than the tap water group (Fig. 3). The amount of short-chain fatty acids in the cecal contents were significantly less than the tap water group for the groups of AIW and pH 10+Ca and pH 10 seemed to work synergistically.

These facts seem to support the repressive effects of AIW against abnormal gastrointestinal fermentation without changing the microflora of enteric bacteria as expected.

## 7. Effects of Alkaline Ionized Water on Acute Gastric Mucosal Injury in Rats

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**A. Summary**

Effects of alkaline ionized water (AIW) on acute gastric mucosal injury induced by aspirin HCl was investigated in rats. Acute gastric mucosal injury was inflicted by intragastric administration of aspirin and HCl.

To investigate the effects of AIW, 5 subject groups were treated for 2 weeks with 1) AIW (pH 9.4, Ca 50 ppm), 2) tap water, 3) alkaline water (NaOH, pH 9.4), 4) lactate calcium solution (Ca 50 ppm), and 5) alkaline plus lactate calcium solution (pH 9.4, Ca 50 ppm).

Pretreatment with AIW for 3 weeks significantly reduced the les, lipid peroxide in the mucous membrane increased significantly ion area induced by aspirin-HCl as compared to the group treated with tap water. Neither the increase in lipid peroxides nor the accumulation of neutrophils was inhibited by AIW. In addition, there were no significant differences at erosive areas between the AIW group and the group 3), 4) or 5).

These results indicate that AIW treatment is effective in preventing gastric mucosal injury induced by aspirin-HCl. However, further examination is necessary to clarify the exact mechanism of AIW.

**B. Description**

We have made a presentation at the Symposium '94 on the effects of AIW on experimental gastric mucosal injury in rats. Among others, we pointed out the fact that AIW reduces the gastric mucosal injury caused by aspirin which is one of the non-steroid anti-infection drugs (NSAIDs). NSAIDs are often used for the routine treatments of lumbago, arthritis and rheumatism and others, but their side effects such as gastric mucosal injury (hemorrhage, sore and ulcer) are problematic. Much is not known about mechanism of the gastric mucosal injury caused by aspirin, and many studies are still conducted.

Recent studies indicate that activated neutrophils at the time of aspirin dosage as well as various free radicals produced by the neutrophils are somehow involved in the formation of the lesion. For this symposium today, we examined the effects of AIW on the gastric mucosal injury by comparing with other groups and from the viewpoint of repression of lipid peroxide reactions.

Gastric mucosal injury in the experiment was created by giving male Sprague-Dawley rats aspirin and chloric acid after a 24 hour fast. For the comparison sake the following 5 groups were prepared: 1) AIW group (pH 9.4, Ca 50 ppm), 2) tap water group (pH 7.1, Ca 3 ppm), 3) alkali water group (NaOH was added to tap water to bring the pH to 9.4), 4) calcium group (lactate calcium was added to tap water to bring the calcium concentration to 50 ppm), and 5) alkali + calcium group (pH is adjusted at 9.4, Ca concentration at 50 ppm).

In each group, the rats were given free access to the water, and used for the experiment after 2 weeks. During the 2 weeks, there were no abnormal findings in the blood test nor an increase of body weight. pH of their blood or gastric mucus did not show any significant differences among the groups. At the same time as the gastric mucosal injury developed, lipid peroxide in the mucous membrane increased significantly as well as neutrophil accumulation level. The mucosal injury was repressed significantly enough by AIW, although it did not have significant effects of repression against the increase of lipid peroxide and neutrophil accumulation. Also, there were no significant differences between AIW group and group 3), 4), or 5).

Since not much is known about the mechanism of AIW's protective effects on gastric mucous membrane, we could not provide a good explanation. However, we know that AIW does not have any effects of protecting the mucous membrane when applied just once and the function water does not eliminate free radicals by itself. Therefore, we suspected a possibility that AIW induced some kind of internal mechanism of protecting the gastric mucous membrane. We also examined the intrinsic antioxidant function of the mucous membrane, but there were no significant differences among the groups. More studies are needed on these matters.

## 8. Effects of Alkaline Ionized Water on Gastrointestinal Complaints

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Abdominal complaints are considered as one of the most common physical problems among seemingly healthy population. In fact, when certain community populations were analyzed based on the questionnaire about bowel diseases created by Mayo Clinic, unexpectedly high incidents of GI complaints of dyspepsia or irritable bowel syndrome was demonstrated. AIW Electrolyzers were first approved for their effectiveness on certain abdominal complaints such as gastric hyperacidity, indigestion and abnormal intestinal fermentation in 1966.

The Research Committee of AIW, chaired by Yoshinori Itokawa, Dept. of Hygiene, Kyoto University, has been conducting studies to re-evaluate their clinical effectiveness. We hereby report the results obtained from a pilot clinical study programmed by the Committee.

Seventeen volunteers suffering from any combination of the common gastrointestinal complaints such as nausea, heartburn, abdominal pain, epigastrial heaviness, abdominal fullness, bloating, appetite loss, and abnormal habits of passages, were given 1 liter of AIW for 2 weeks. Although further double-blinded study is needed under well-controlled conditions to confirm the efficacy of AIW, 47% of the cases were evaluated as effective as determined by overall improvements of each GI complaint as well as the bowel movement habits.

Heartburn, nausea, epigastrial heaviness, abdominal fullness, abdominal pain, diarrhea or constipation (abnormal bowel movement) are the most common gastrointestinal complaints experienced routinely. Since they are not organic diseases and also self-controllable, most people let them go without seeing doctors.

In our study focused on certain population (faculty and students of a private university) using May Clinic Bowel Disease Questionnaire (808 men, 131 women at ages of 32.5 +/- 12.1 totaling 939), we found a high rate of complaints. 34.5% had epigastrial complaints represented by dyspepsia, and 17.8% had complaints at the lower digestive tracts represented by irritable bowel syndrome. As to the abdominal pain, 61% of those who turned in the questionnaire experienced it at least once in the past year, of which 53.2% answered that they experienced 6 times or more in the past year. Regarding the abdominal pain, 87.3% picked up either "does not bother" or "does not affect the everyday life", and they seem to let it go without seeing the doctor.

As to the bowel moment on the other hand, 13.3% had constipation, 14.5% diarrhea, 9.5T alternate abnormal bowel movement, totaling 37.3% complaining about some kind of abnormal bowel movement. 56.1% of those who have chronic constipation and 40.8% of those who have chronic diarrhea (judging from their specific bowel movements) suffer also from abdominal pain. For other complaints, 39.9% had abdominal fullness, 13.7% nausea, 7.6% heartburn experienced in the past year.

The AIW Electrolyzer was given a manufacturing license in 1966 according to the Pharmaceutical Affairs Law based on its function to control gastric acid and effects on indigestion, hyperacidity, abnormal gastrointestinal fermentation, and chronic diarrhea. Therefore, this device is expected to have some effects on the above-mentioned disorders. The Research Committee of AIW, chaired by Yoshinori Itokawa of Dept. of Hygiene at Kyoto University, has been conducting studies to re-evaluate their clinical effectiveness. This time, we are going to report the results from the preliminary clinical testing.

The subjects of the test, 17 volunteers who suffer from gastrointestinal complaints (2 men and 15 women) were given 1 liter of AIW at pH 10 every day for 2 weeks. The AIW was confirmed to be safe by the previous basic clinical testing. The subjects' impression about their physical conditions after the 2 weeks ranged from "improved" (6%), "somewhat

improved" (36%), "no changes" (29%) to "no comments (29%). Their doctors' diagnosis ranged from "much improved" (18%, "improved" (29%), "somewhat improved" (41%), to "no changes (12%).

Looking at the improvements of conditions by symptoms, epigastrial heaviness has reduced from 10 persons to 4, abdominal fullness from 14 to 10, incomplete evacuation 11 to 9, abdominal pain 5 to 4, appetite loss 5 to 2, heartburn 4 to 3, belching 4 to 3, and also mentally speaking, irritation 6 to 1, anxiety 2 to 0, hypertension 3 to 1, depression 4 to 2, insomnia 15 to 10, all of which have decreased in number. On the other hand, the number of people who suffered from symptoms increased in case of sick feeling from 2 to 3, intestinal growling 7 to 9. As to the bowel movement, four people had once a day, 3 had more than 5 times a day, 2 had 4 times a day, 5 had 2 times a week, and 3 had once a week. However, the condition of constipation or diarrhea has been improved after 2 weeks of drinking AIW where 10 people had the bowel movement once a day, one person had 4 times a day, 5 had 2 times a day, and one person had 2 times a week. No critical side effects, self-conscious or not, nor abnormal values of clinical testing were identified.

Because of the possibility of the placebo effect caused by drinking one liter of water every day, the above results are not immediately considered as effects of AIW. The final evaluation is yet to be made by the ongoing double-blinded clinical tests. However, medications for gastrointestinal complaints not attributable to organic diseases have some problems in terms of application, side effects, and cost-benefit balance. From this point of view, AIW is expected to prove effective.

## 9. Basic Clinical Evaluation of Alkaline Ionized Water

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The goal of this study was to evaluate the effect of alkaline ionized water (AIW) on gastric acidity and biochemical profile. Mean intragastric pH went up whereas the pH 3 holding time during 24 hours was reduced for the volunteers who were given 1.0 liter of AIW per day at pH 9 compared with those given tap water. There were no differences, however, in this effect between AIW at pH 10 and pH 11, and tap water.

Serum potassium concentration has increased for the volunteers who were given 1.0 liter AIW at pH 10 and 11 for 7 days, but not at pH 9. Additional study on AIW at pH 9.0 to 9.5 showed no abnormal findings, even with serum potassium concentration in 2, 3, 5 and 7 days for all the volunteers. These results suggest that AIW at pH 9.0 to 9.5 may be effective and safe for ameliorating the symptoms.

### A. Preface

Effects of AIW on gastrointestinal symptoms are widely known. However, there have been no clinical studies on its mechanism and effects on our entire body system. To be able to make better use of AIW, the above-described analyses are necessary. We conducted these analyses by examining changes of intragastric pH as well as stool, urine and blood tests.

### B. Basic Clinical Test 1

**Purpose:** To examine AIW's effects on intragastric pH and its safety (in regards to side effects).

**Method:** Each of 6 volunteers were given 1.0 liter of AIW at pH 9 10, or 11 per day (400 ml in the morning, and the rest it taken at any time) for 7 days. On the seventh day of the experiment, a pH-sensor (made by Eurotech) was placed in each volunteer's stomach for 24 hours. The volunteers are expected to spend a routine day, and changes in their gastric pH for the entire day are recorded and analyzed. Also, blood, urine and stool tests as listed in the Table 1 were conducted before and after drinking AIW.

Table 1: Test Items

| | | |
|---|---|---|
| 1) | Blood Tests | |
| | Peripheral Blood: | leukocyte counts, erythrocyte counts, hemoglobin, hematocrit, platelet counts |
| | Biochemistry: | total protein albumin, ZTT, TTT, total bilirubin, total cholesterol, CPK, GOT, GPT, LDH, ALP, *y*-GTP, ChE neutral fat, nitrogen in urea, creatinine, uric acid |
| | Serum: | CRP |
| 2) | Urine Tests: | pH, density, protein, sugar, urobilinogen, bilirubin, ketone body, occult blood |
| 3) | Stool Tests: | human-hemoglobin method |

**Results:** The intragastric pH increased sharply just once right after AIW was taken (Fig.1). Especially with AIW at pH 9, 24-hour mean intragastric pH went up in all cases compared to those before taking AIW, whereas the pH3-holding time (ratio of time during 24 hours when the intragastric pH is 3 or less) dropped down in all cases (Fig.3). This indicates AIW had acid control effects. However, with pH 10 and 11, neither 24-hour mean intragastric pH nor pH-3 holding time showed as much difference as in the case of pH 9. No one had subjective symptoms by taking AIW. At pH 9, no abnormal changes were identified by blood, urine and stool tests. At pH 10, 5 of 6 cases had an increase of serum potassium concentration, 2 of which showed high blood potassium symptom. Since serum potassium concentration increased further at pH 11, AIW was not given any more for 3 cases (Fig.2). There were no abnormal changes in other testing results.

**D. Basic Clinical Test 2**

**Purpose:** To confirm safety of the AIW Electrolyzer in the market at pH 9.0 9.5 because serum potassium concentration went up by taking AIW at pH '10 and 11 in the previous Basic Clinical Test 1.

**Method:** 8 volunteers were given 1. liter of AIW per day (400 ml in the morning and the rest is at any time) at pH 9.0 to 9.5 for 7 days. All the tests listed in the Table 1 were conducted before taking AIW as well as on the second, third, fifth and seventh day of the experiment.

**Results:** There appeared no side effects recognized by the volunteers in all cases. There were no abnormal changes in blood, urine and stool tests nor in the serum potassium concentration which showed abnormal level in the previous Test 1.

**Conclusions:** AIW at pH 9 had an acid control effect depending on the changes of gastric pH level. AIW at pH 10 and 11 did not show as much effects as in the case of pH 9. When AIW at pH 10 or 11 was taken, serum potassium concentration increased. However, AIW at pH 9.0 to 9.5 produced by the AIW Electrolyzer in the market did not result in any abnormal changes in the blood test, assuring short-term safety.

Judging from all these results, AIW's efficacy against gastric symptoms are found to be optimum at pH 9.0 to 9.5. However, not much is known about the overall effect of AIW on the human body, and more detailed studies are needed.

* Refer to the original document for the following figures and tables:

Fig.1: Daily changes of gastric pH (measured)
Fig.2: Changes of serum potassium concentration
Table 2: Average gastric pH
Table 3: pH 3 holding time (%)

## 10. Physical Chemistry of Electrolyzed Acid Aqueous Solution

by Hiroyasu Nomura, Shinobu Koda, Yuko Nishimoto, Haruhisa Miyake, Shigeaki Yonemore
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Physio-chemical properties of electrolyzed acid aqueous solutions were reviewed. By measurement of Raman spectra of water, the structure of electrolyzed acid aqueous solution (EAAS) was found to be almost consistent with that of regular water at the corresponding pH. Oxidation-reduction potential of electrolyzed acid aqueous solution at pH 2.7 was calculated by Nernst equation. Concentrations of HCl0 and $Cl_2$ were roughly estimated by the mass balance of chlorine in the solution. The calculated oxidation-reduction potential ca.1.4 volt was slightly higher than that obtained by the experiment. The results of ESR measurement of EAAS indicated that a signal of 5.5'-dimethyl-1-pyroline-oxide spin adduct was observed, and changed with the time.

EAAS is water with oxidation-reduction potential of about 1300 mV and pH 2.7 produced at the anode in the electrolysis of salt water. In recent years, this solution has been drawing public attention for its bactericidal and medical effects as one type of function water. However, the intrinsic difference between regular water and EAAS is not quite understood. We hereby examine the structure and properties of EAAS from physio-chemical point of view.

**A. Structure of Water**

Raman spectra of water at the same pH level as EAAS show similar pattern within the wide range of frequency band between 100 to 4000 $cm^{-1}$. It became clear that the structure of EAAS is almost identical as that of regular water.

**B. pH and oxidation-reduction potential**

When solution of only NaCl is electrolyzed, the following reactions take place at the cathode:

$$H_2O \leftrightarrow \frac{1}{2} O_2 + 2H^+ + 2e^-$$

$$2\,Cl^- \rightarrow Cl_2 + 2e^-$$

$$Cl_2\,(aq) + 2e^- \rightarrow 2Cl^-$$

$$Cl_2\,(aq) + H_2O \leftrightarrow HClO + H^+ + Cl^-$$

$$Cl_2\,(aq) + H_2O \rightarrow ClO^- + Cl^- + 2H^+$$

Therefore, EAAS produced at the cathode includes $Na^+$, $Cl_2$, and HClO. The oxidation-reduction potential and pH are determined by the equilibrium of the above reactions. Electro-chemically, oxidation-reduction potential of the solution is verified by Nernst equation. It is dependent on the concentration of $Cl_2$ and HClO as well as pH, and is given by the following equation:

$$E = 1.63 - 0.06\ pH + 0.03 \log \frac{(HClO)^2}{Cl_2}$$

Inferred from the effective chlorine concentration of EAAS at pH 2.7, the concentrations of HClO and $Cl_2$ are $1 \times 10^{-3}$ and $8 \times 10^{-5}$ mol/l respectively. The standard oxidation-reduction potential is found at about E = 1.41 V. Although there are some problems with the way the concentrations are calculated, Nernst equation explains the high level of oxidation-reduction potential of EAAS.

**C. Solubility of OH Radicals**

5,5-dimethyl-1-pyrroline-oxide (DMPO) is well known as spin-trap agent for OH radicals. ESR spectra of EAAS were measured using DMPO. As a spin adduct of DMPO, DMPO-OH was detected. It was found that the signal intensity of DMPO-OH changes with time, thus production of DMPO-OH depends on passage of time. These results indicate the existence of a precursor to product OH radicals, and the DMPOOH was produced from DMPO reacting with some kind of chemical compounds.

Considering these points, EAAS produced by electrolysis of salt water is a kind of solution that contains chlorine, hypochlorous acid, chlorine ion, and its bactericidal effect comes mainly from the hypochlorous acid.

## 11. Analysis of Function Water

by Yuko Nishimoto, Dept. of Chemistry, Faculty of Science, Kanagawa University

Electrolyzed strong acid aqueous solution (ESAAS) is called function water because it has a strong bactericidal function and effect on viruses, immune systems and atopic dermatitis. This function water has unique characteristics such as high positive oxidation-reduction potential, strong acidity, and high concentration of dissolved chlorine and oxygen. However, any analytical studies have not been conducted in detail.

In our current investigation, we examined analytical methods and conditions of ESAAS. Measurements of pH, ORP, DO and O-NMR were carried out. Concentrations of $Na^+$, $Cl^-$, and residual chlorine were measured. We also examined effects of electrolytic current on source water.

In recent years, various kinds of water with different functions are available and actually utilized. However, the relation between its function and properties is not clear in many cases. We hereby report the results of our study on the effects and their mechanism regarding ESAAS produced by electrolyzing water, as well as its relation with the physical characteristics of source water.

For this experiment, we altered the existing device to be able to adjust electrolytic current. First of all, we examined ESAAS produced under standard conditions with the help of sodium chloride as promoting reagent. After studying the analytical methods and conditions, pH, exudation-reduction potential, amount of residual oxygen, concentrations of chlorine ions and their effective concentrations, amount of effective chlorine, sodium and potassium were measured both in case of tap water and pure water. We also examined stability of the solution when it is preserved. As to the changes of characteristic values, we examined electrolytic current, types of promoting reagents for electrolysis, and effects of concentration level of the solution. We also conducted a state analysis of water by measuring components in the solution by 17P-NMR. Judging from these results, we have finally come to infer the mechanism of function water, in addition to studying how we can obtain the same characteristic values of water without electrolytic process.

According to the study, we found that there are no significant changes in the characteristic values of water caused by differences in electrolytic current or concentration level of the reagent. Therefore, we can assume the quality of function water is quite stable. Also, we found that the function water can be stored for about 5 days in a brown bottle in a dark and cold place. We also confirmed that the function water can be mitigated by strong basic solution when it has to be drained.

The characteristics of ESAAS are considered to be low pH, high oxidation-reduction potential, and high level of dissolved oxygen. The oxygen level changes according to the water temperature. However, since there is no report so far that the medical and bactericidal effects of function water differ by season, the real cause of the function seems to lie somewhere else other than dissolved oxygen. We did not observe any abnormality in the function water either.

As to pH, oxidation-reduction potential and effective chlorine concentration, we found that the same level of composition can be achieved by mixing components rather than by electrolysis. The problem yet to be solved would be how to confirm the medical and bactericidal effects of function water. The advantage of function water produced by electrolysis seems to be that stable quality can be obtained from just tap water and sodium chloride without using any chemicals that require careful handling. This study also implies a possibility of making function water by mixture in case there is no place to install the electrolysis equipment.

# 12. Properties of Water in Polymer Membranes

by Ryutaro Kiyono, Masayasu Tasaka,
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Solvent transport was measured in water. This was conducted between two different temperatures (thermoosmosis) across hydrophobic poly-tetrafluoroethylene (PTFE) membrane as well as modified PTFE membranes prepared by the introduction of hydrophilic groups. The direction of thermoosmosis across the modified PTFE membrane decreased as the amount of hydrophilic groups in the membrane increased. This is because the introduced hydrophilic groups enhance the affinity of the membrane matrix for water molecules, and thus the transported entropy of water in the membrane approaches the partial molar entropy of water in the external solution.

## A. Preface

When two solutions separated by a membrane have different temperatures, a flow of solvent (thermoosmosis) driven by the temperature difference is observed. The magnitude and direction of thermoosmosis depend much on the molecular structure of water in the membrane as well as the external solution. Therefore, the molecular activities in the membrane can be studied by measuring thermoosmosis.

In our study, we measured thermoosmosis across hydrophobic polymer membranes as well as membranes with higher affinity by introducing hydroxyl groups, and examined how water in the membrane changes when the property of membrane shifts from hydrophobic to hydrophilic.

## B. Experiment

We used three kinds of membrane for the experiment. First, hydrophobic Gore-Tex membrane, manufactured by Japan Gore-Tex, made of poly-tetrafluoroethylene (PTFE) was used. Secondly, we used GT-TE membrane. This is made of the Gore-Tex membrane soaked in a chemical agent called Tetra-Etch II (manufactured by Junkou Co., Ltd.,) to gradually introduce affinity by hydroxyl groups. The last membrane is WPW-045 (manufactured by Sumitomo Denko) made of PTFE with many added hydroxyl groups.

GT-TE membranes, with different affinity, were made by soaking Gore-Tex membranes in the special solution for a minute each. Three kinds of solution were prepared at different volume ratio of Tetra-Etch to the diluent ethanol at 0.02, 0.5, and 1. One of the membranes was placed between two acrylic half cells (upper 160, lower 50cm$^3$), and thermoosmosis was measured by creating temperature difference across the membrane. Effective area of the device is 28cm$^2$. Pure water was used for the external solution.

## C. Study Results and Analysis

When non-equilibrium thermodynamics are applied to the volume flow of liquid through an uncharged membrane under the condition of temperature gradient, the volume flow (with water as external solution) is given by the following equation:

$$-J_v = \underline{c}\,0\ 1_v\,0\ (S0 - s0)\ (1 / \delta)\ \Delta T = D\ \Delta T$$

$\Delta T$ : Temperature difference across the membrane
$\underline{c}0$ : Concentration of solution in the membrane
$1_v0$ : Velocity of solution in the membrane
$S0$ : Mol entropy of water in the external solution
$s0$ : Average transport entropy of solution in the membrane
$\delta$ : Thickness of membrane
D : Thermoosmosis coefficient

Since $\underline{c}0$, $1_v0$, and $\delta$ are all positive, direction of thermoosmosis depends on the difference of entropy between inside and outside of the membrane. If the average transport entropy of solution in the membrane is larger than the mol entropy of water in the external solution, the direction of thermoosmosis is from high to low temperature side, and vice versa.

In case of the hydrophobic Gore-Tex membrane, thermoosmosis flow was observed from high to low temperature side. This indicates $S0 - s0 > 0$, which means the average transport entropy of solution in the membrane is higher than the mol entropy of water. Therefore, it is implied that water molecules are in an unstable condition not to be able to react with the hydrophobic membrane. Regarding GT-TE membranes with various level of affinity, thermoosmosis was observed directing from high to low temperature side. However, the volume of flow decreased as the affinity increased.

By soaking porous PTFE membranes such as Gore-Tex in the agent Tetra-Etch II, high-affinity groups such as hydroxyl can be introduced through radical polymerization in and around the membrane. Since the introduced groups will enhance the affinity between the membrane and water molecules in it, the molecular state of water in the membrane gets closer to that of external water as compared to the case of using hydrophobic membrane. This seems to be why the flow of thermoosmosis dropped down as the affinity increased.

## 13. Analysis of Free Radicals in Electrolyzed Acid Aqueous Solution by Electron Spin Resonance (ESR) Spectroscopy

by Shigeaki Yonemori, Haruhisa Miyake
Research Center, Asahi Glass Co., Ltd.

Active oxygen radicals in an electrolyzed acid aqueous solution (EAAS) have been investigated by electron spin resonance spectroscopy (ESR). A hydroxyl radical ( ・OH ) is detected as DMPO (5,5-dimethyl-1-pyrroline-N-oxide) spin adduct by spin trapping. The DMPO-OH spin adduct is gradually increased after adding DMPO to the solution. The impact of the electrolyte and the current density was examined. For the spin trapping experiment, sodium sulfate was used instead of sodium chloride as a promoting reagent for the electrolysis. The DMPO-OH spin adduct has increased proportionally to the current density level.

### A. Preface

Studies on medical effects of EAAS such as bactericidal and granulation effects are conducted in various fields. However, there are only a few reports on its properties. It is reported that, when sodium chloride is used as promoting reagent for electrolysis, free radicals come out in addition to chloric products such as hypochlorous acid directly produced by electrolysis. Among these free radicals, we focused on hydroxyl radicals ( ・OH ), and analyzed them by electron spin resonance spectroscopy (ESR). To measure the hydroxyl radicals, we applied spin trap method which is used to detect active oxygen radicals under the water in the ecosystem.

### B. Experiment

To produce electrolytic water, ion-exchange type equipment (Flosel type manufactured by Asahi Glass Engineering Co. called “Oasis-bio” and Batch-cell type made by ourselves) was used. It is a membrane separation type, and minimized the mixture of solution between anode and cathode sides. Nippon Denshi’s JES-TE300 was used for ESR equipment. Radical trap agent was refined from Labotech’s 5.5-dimethyl-1-pyrroline-N-oxide (DMPO).

### C. Study Results and Analysis

Using hydroxyl radicals (DMPO) contained in EAAS as spin trap agent, we measured ESR spectrum. It showed characteristic four peaks (aN = 1.49, aH = 1.50, g = 2.005) indicating the formation of OH radicals ( ・OH ). Fig.1 shows the peak intensity of ESR for this DMPO-OH spin adduct. We found that this peak intensity increases with time after mixing EAAS and DMPO. The chemical reaction of DMPO with OH radicals is so quick that we believe hydroxyl radicals do not exist in EAAS from the beginning, but are produced after DMPO is added to the solution.

Usually, sodium chloride solution is used for promoting reagent of electrolysis. However, if spin trap method is applied to this system, the spin trap agent will be dissolved due to the production of chloric acids, adding to the complication of ESR spectrum. Then we considered using only sodium sulfate usually contained in tap water at about 10 ppm as promoting reagent to be added to pure water. We compared the solution of sedum chloride plus sodium sulfate and the solution of sodium sulfate only using super-pure water, almost the same spectra were obtained.

Also, we examined the correlation between the number of days EAAS was stored in the dark and cold place, and the peak intensity of ESR for DMPO-OH to find out that there are no changes even after 10 days of storage. Furthermore, we examined the relation with the current density, which is one of the electrolytic conditions. It was found that both ESR peak intensity of DMPO-OH and the number of hydroxyl radicals have increased as the current density is raised.

Thus, we analyzed hydroxyl radicals of EAAS and ESR, and confirmed their existence. It became clear that hydroxyl radicals are stable for more than 10 days when stored in the dark and cold place, and they increase in numbers when the spin trap agent is added to the solution. In addition, the relation between hydroxyl radicals and electrolytic conditions were presented. Under constant electrolytic conditions, we believe that electrolyzed water with stable amount of hydroxyl radicals can be produced.

## 14. Study on Propriety Instrumentation of Function Water

by Michio Aoyama, Nippon Rensui Co., Standard Equipment Division

### A. Summary

Following the basic study of function water, its peculiar thermal characteristics were clarified. A further investigation was undertaken to determine “temperature conversion factors for function water instrumentation” by reviewing the data on thermal characteristics of function water.

### B. Preface

The most convenient and general way to identify and control the feature of electrolysis equipment and quality of electrolyzed strong acid aqueous solution (ESAAS) might be the measurement of pH (concentration of hydrogen ion) and ORP (oxidation-reduction potential). ESAAS can be used for many purposes, and could change its quality according to the production process and environmental factors.

One of the themes of this study is the necessity of a sensor especially designed for ESAAS rather than a general sensor used in the past. We focused on the thermal characteristics of the sensor, and its response time as well as the special shape and stability level the sensor is supposed to have.

**C. Study Process and Results**

(1) We manufactured a prototype of sensor that is considered to be the best for measuring ESAAS.
Shape: Designed to fit the maintenance requirement
(Extended the refill cycle of the liquid in the sensor)
Mechanism: Designed to fit the characteristics of ESAAS
(Understanding fluidity and thermal characteristics of ESAAS)
Stability: Designed to withstand a long-term measurement
(Selection of materials, etc.)
Characteristic: Convenience of being able to measure pH and ORP at the same time
(Two pair of electrodes for pH and ORP are placed in a sensor)

(2) Using the prototype sensor, we underwent various experiments on thermal characteristics, response time and long-term stability of ESAAS, and analyzed all the date. (For details, see "Reports of Funded Studies" 1994 published by Function Water Research Foundation).

(3) Results
We could test the sensor to our satisfaction due to our studies this time and our accumulated knowledge. As to the overall capability of the sensor, we could obtain the expected results for response time and maintenance requirement. We conducted the experiments under the same condition this time, but it should be noted that the resulting values change depending on the conditions.

**D. Conclusion**

ESAAS is expected to be used by many people in the future. Therefore, it is important to understand the water quality to be able to use it safely and effectively. Also, it is critical to know the characteristics of ESAAS and maintain it properly to be able to repeatedly produce the constant quality. It is our best hope that this study will help on these matters.

## 15. Principal & Applications of Devices Releasing Activated Oxygen

by Makoto Hosoya, Office Hosoya Co., Ltd. & Shoji Kubota, The Society of Water Design

Hydrogen peroxide is decomposed into water and oxygen with the help of a catalyst as shown in the equation:

$$2\ H_2O_2 \xrightarrow{CAT} 2\ H_2O + O_2$$

By the device that produces activated oxygen called Oxidator, the above reaction occurs in the following two stages:

(1) The catalyst decomposes hydrogen peroxide in a Plexiglas container producing oxygen in gas. An air cushion at the bottom of the container acts as a valve while preventing water from penetrating the unit.

(2) The ceramics of the Oxidator releases pure oxygen ($O_2$), and activated oxygen (hydroxyl radicals) have caused various interesting phenomena. The results are as follows:
1. The amount of dissolved oxygen is always maintained in the water.
2. ・OH reacts with algae and organic matters that pollute water. Therefore, the water is kept clean, free from bad smell.
3. ・OH is effective in killing bacteria.
4. ・OH reacts with dissolved ionized organic matters in the water and deposits them.
5. Plants grow up rapidly.

Hydrogen peroxide is decomposed into water and oxygen with the help of a catalyst as shown below:

$$2\ H_2O_2 \xrightarrow{CAT} 2\ H_2O + O_2$$

The Oxidator that produces activated oxygen makes the above reaction in two stages as follows:

(1) By a solid catalyst, hydrogen peroxide in the container is decomposed to emit oxygen gas necessary for pushing it out.

(2) Due to the high pressure of oxygen created in the air-tight container, the hydrogen peroxide is pushed out through the air cushion at the bottom of the container and reach a special ceramic container outside to produce pure oxygen ($O_2$) and activated oxygen (hydroxyl radicals ・OH).

・OH is one of the forms of activated oxygen, and categorized as free radical. Oxygen can be dissolved only to the extent of saturation point, whereas the amount of dissolved oxygen produced by catalytic decomposition of $H_2O_2$ is increased. $O_2$ is stable and inactive, but ・OH is especially active among activated oxygen and is quick to react.

According to the measurement by O-NMR, there is a clear difference by time between source water and produced water. A strong impact of ・OH on the water is implied. Also, by a surface tension test, the tension of produced water is reduced

significantly from that of source water.

On the other hand, there was not much difference in the results of both O-NMR and surface tension tests between source water and hydrogen peroxide water. These facts leave us to infer that there is a chemical composition other than $H_2O_2$ in produced water. Measurement of free radicals using ESR method made it clear that ・OH is actually produced.

In the process of producing ・OH at the same time as oxygen by decomposing $H_2O_2$ , many interesting phenomena and effects occur as follows:

1. The maximum dissolution of oxygen gas in water is much higher than in the case of regular oxygen.
2. By oxidation, ・OH decomposes algae and deposited organic matter that pollute water in the tank or pond. Therefore, it can keep water clear for a long time. It also prevents bad smell caused by increased nutrients in water.
3. By continuously increasing the amount of ・OH dissolved in water, bactericidal effects can be enhanced.
4. ・OH reacts with iron in the so-called “red water” and precipitates it by agglutination. This way, it can revive the red water that is otherwise unusable.
5. Growth of aquatic plants and other plants is speeded.

## 16. Inactivation Effects of Acid Aqueous Solution on Feline Virus in Blood, Feces, Urine & Sputum

by Hiroyuki Koyama, Infectious Disease Division, Dept. of Veterinary Medicine, Kitasato University

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This time, we injected feline herpes virus (FHV) or feline calisi virus (FCV) into cat’s blood. Then we judged the inactivation effect by measuring the titer of virus right after adding EAAS to the solution. We also examined what causes the inactivation effect of EAAS.

Blood, urine, saliva and stool were obtained from mature SPF cats that show no reaction to virus antibody. The titer of undiluted virus solution are $10^{7.5}$ and $10^{6.7}$ $TCD_{50}$ / 25 μ 1. EAAS was produced by Oasis O2 manufactured by Asahi Glass Engineering Co., Ltd. The pH was between 2.54 to 2.56, oxidation-reduction potential (ORP) from 1156 to 1160 mV, residual chlorine (Cl) 32 to 35 ppm. Immediately after the EAAS was added to FCV or FHV solution to dilute to 10 times in volume, both viruses were inactivated. The results are summarized as follows:

(1) Blood containing EDTA and undiluted solution of FCV or FHV were combined by the same volume. Then EAAS was added twice as much all the way to 1000 times as much. By measuring the titer of virus immediately after adding EAAS, we found that the infectious titer was at 200 times dilution or more for FCV and 100 times or more for FHV.
(2) In the same way, EAAS was added to the mixture of urine and virus. In this case, the infectious titer was at 50 times dilution or more and 20 times or more for FHV.
(3) In the same way as 1., EAAS was added to the mixture of saliva and virus, the result was the same as the case of urine.
(4) 48g of normal stool was combined with phosphate buffer solution (PBS) to make an emulsion. This was further diluted to 25% emulsion by adding more PBS, and the same amount of virus solution was added. When EAAS was combined to this solution, the infectious titer proved to be at 200 times dilution for both FCV and FHV.
(5) By stirring for a long time, EAAS was modified to have pH of 2.55 to 2.67, ORP at 800 to 886 mV, Cl at less than 0.05 ppm. Even with 200 times as much EAAS as undiluted virus solution, both viruses were not inactivated. This seems to be caused by the changes of ORP and Cl.
(6) Contrary to the above experiment 1. through 4., 500 times as much EAAS was added to blood. Immediately after EAAS has changed to have pH 2.52 to 2.56, ORP of 634 to 660, Cl at 0.88 to 1.0. Even adding this solution to undiluted virus solution at 200 times volume, neither virus was inactivated. However, when EAAS was combined with blood at 2000 times volume, the pH changed immediately to 2.48 to 2.59, ORP to 1.111 to 1.124, Cl to 20. When this solution was added to undiluted virus solution at 50 times more volume, both viruses were inactivated. In this experiment, both ORP and Cl seem to be involved in affecting inactivation of virus.

## 17. A Field Trial for Estimating Possibility of Controlling Powdery Mildew of Cucumber by Function Water

by Yoshikazu Yamaki, Annette Schörner
University Orchard, Faculty of Agriculture, University of Tokyo

Function water was sprayed onto cucumber plants in order to evaluate its effects on powdery mildew. Acid water only, or alternately with alkaline water was sprayed at the intervals of 2 to 4 days. Two kinds of control methods were used, one is no-fungicide spray, the other conventional fungicide spray. Function water, although not as effective as fungicide, apparently reduced powdery mildew symptoms of cucumber leaves from the 18th day after planting the plants for about 2 weeks.

Repressive effects of function water against powdery mildew of cucumber were tested in the farm. Cucumber plants were cultivated in Ninomiya Orchard in the University Farm of Faculty of Agriculture, University of Tokyo located in Ninomiya-cho, Naka-gun, Kanagawa Prefecture. Four experimental areas were set as follows:

(1) Conventional fungicide spray area
(2) Strong acid water spray area
(3) Strong acid water and strong alkaline water alternate spray area
(4) No-spray area

The plants were replanted on May 25th. pH of strong acid water is about 2.4, ORP is about 1,120. As to strong alkaline water pH is about 11.5, ORP about 850.

Schedule of function water spray: May 27, 31, June 3, 6, 10, 12, 14, 16, 19, 21, 24, 27, 29, July 2, 7, 10, 12, 14, 18, 21, 24
Schedule of Fungicide spray: May 27, June 2, 7, 12, 16, 21, 24, 30, July 12, 18, 24
Schedule of Insecticide spray: June 2, 5, 9

The symptoms of powdery mildew were recorded only for fully developed leaves and those that have no less than 50% of the surface area. In the early stage until the fourth measurement (12 days after replanting), no effects of spray were observed. On the 16th to 31st days of replanting, the symptoms spread rapidly in the no-spray area, whereas in the fungicide spray area the symptoms were apparently repressed. In the function water spray area, the symptoms were somewhat controlled.

As the sticky-leaf disease became prominent, fungicide was sprayed on the 34th day of replanting which further aggravated the powdery mildew. 37 days after replanting, however, the symptoms were most controlled in the fungicide spray area. There were no significant differences were observed In three other areas. On the 45th day, the symptoms worsened so much in the fungicide spray area that there were no significant difference any more from the alternate spray area. One of the reasons for no significant difference between function water spray area and no-spray area on the 37th day as well between fungicide spray area and alternate spray area on the 45th day seems to be that the fungicide against sticky leaf disease helped powdery mildew to propagate.

This study has been made possible by the grant from Function Water Research Foundation and equipment (to produce oxidation potential function water) by Remodeling 21 Co., Ltd. For the nursery and cultivation of cucumber, we received advice of Mr. Hideo Inao of Tama Farm of Faculty of Agriculture, University of Tokyo. We are grateful for their help.

## 18. Application of Acid Electrolyzed Water in Dentistry

by Akihiko Shiba, The Third Dept. of Prosthodontics, Faculty of Dentistry, Showa University

Acid electrolyzed water (AEW) is produced by electrolysis of tap water. We verified that AEW has a strong and rapid bactericidal effect with wide range of anti-bacterial spectrum, and furthermore, with biological safety. We investigated application of this AEW to the dental field.

The result of this study indicated that AEW is effective against endodontic treatment, irrigation of periodontal pocket, washing and sterilization of the denture, and hand-sterilization and sterilization of treatment apparatus.

AEW is produced by electrolyzing tap water. This AEW is drawing more attention these days in the dental field as a replacement for conventional drugs. The reason for it is its effect is strong and rapid with wide range of anti-bacterial spectrum. In addition, it is the least harmful to the organism as compared to the conventional drugs.

I have been studying bactericidal and hygienic effects of AEW in the medical field for about 7 years. Here are some of the various findings about application of AEW to the dental field.

### A. Sterilization Time and Effects, Anti-bacterial Spectrum

AEW's sterilization effect takes place in 5 to 10 seconds. This effect is believed to come mainly from residual chlorine gas. The chlorine gas attacks amino acids exposed on the surface of microorganism and bacteria, and kills them instantly.

The major player is this chlorine gas, although pH and ORP take some roles. This is confirmed by the reaction specificity test of AEW. According to the test, AEW reacts with glycine that has amino group, but not with acetic acid that does not have it.

The antibacterial spectrum of AEW is very wide-range. AEW is effective against bacteria, anti-acid bacteria, eumycetes, and virus, especially against MRSA, HBV (B-type hepatitis), HIV (AIDS virus) that are infectious in the hospital. Also against oral bacteria, it is found effective against aerobic bacteria, yeast bacteria and anaerobic bacteria by our experiments.

**B. Safety of AEW**

Safety for organism is an important issue. Twelve items of safety test have been conducted including his, cytotoxic, and mutability tests. Concluding from the results, AEW has a little more risk against organism than physiological saline, but the degree is negligible.

**C. Medical Application**

Among applications of AEW to medical fields, treatment of atopic dermatitis by AEW is outstanding as often televised about the effect. In addition, AEW is used for treatments of cuts, burns, confinement sore, nostril catarrh and atopic rhinititis. Above all, AEW is applied for cleaning and sterilization before, during and after operations as well as prevention of infections in the hospital.

**D. Dental Application**

Application of AEW to dental field is also diverse. It is already widely used for dental and periodontal treatments, cleansing after tooth extraction, cleaning periodontal damages, inflammation, tumor, as well as washing hands and sterilization of equipment and dental impression or mold, and for prevention of infections in the hospital. We are not the exception to use AEW clinically.

## 19. Effects of Acid Electrolyzed Water in Chronic Periodontitis Treatment – Preliminary Report

By Ken-ichi Saito, Toshiyuki Nemoto
Dept. of Oral Surgery, Kanto Teishin Hospital

This study investigated effectiveness of acid electrolytic water (AEW) used for treatments of chronic periodontitis. Seven patients were treated by AEW. They rinsed their affected part with approximately 20 ml of AEW inserted through a thin needle into the pocket or fistula of their gingival once a week.

In about a month, some difference was observed in the lesion healing from the pre-treatment conditions. Five of seven patients demonstrated progressive healing with evidence of reduced infectious condition at the lesion.

Clinical application of AEW is more popular these days since it has wide range of antibacterial effects against pathogenic bacteria. We have applied AEW for treatments of chronic periodontitis (so called pyorrhea, etc.) that many of the middle aged and the elderly in Japan suffer from. We have not found any effective treatments against this disease. Following is a summary from our report.

**A. Study Subject**

The patients' group consisted of 4 men and 3 women age 38 to 56. Chronic periodontitis is suppurative inflammation that appears around fistulae or the root of the teeth. It includes those with limited lesion around periphery (peripheral type) and those with extended lesion all the way down to the tip of the root (root-tip type). Four of the patients had peripheral type, three root-tip type. The latter three had fistulae in the gum.

**B. Study Method**

About 20 ml of AEW was inserted through a fine dental syringe needle into the pocket or fistula of the patient's gingiva, and the lesion was cleansed by water pressure. In all cases, the gingiva pocket was curretted first before the cleaning. These procedures were followed once a week, and AEW's effectiveness was evaluated by comparing the lesion's conditions before and one month after the treatment on items such as reddishness, swelling, pain, amount of pus, and disappearance of fistulae. No anti-bacterial agents nor chewing pills were given to the patients before or after the treatment.

**C. Study Results**

For the peripheral type, three out of four cases saw reduction of reddishness and swelling, and the amount of pus decreased significantly. The other one case did not have much improvement. For the root-tip type, two out of three cases saw disappearance of fistulae. These fistulae have never been cured by anti-bacterial agent or root canal treatment, but have disappeared by the cleaning for once or twice. As to the other one case, fistula still remained but reduction of reddishness and swelling was recognized.

**D. Conclusion**

In treating chronic periodontitis by AEW for 7 patients, 5 out of 7 cases saw apparent improvement of the inflammation at the lesion. This can be recognized as AEW's effects on the disease. We are planning to test more cases in the future, and also conduct bacteriological research and control studies at the same time.

## 20. Analysis of Virucidal & Bactericidal Action by Function (Electrolyzed Oxidizing ) Water

by Yoshinobu Shimizu, Dept. of Oral Bacteriology, Tohoku University School of Dentistry

### A. Abstract

Electrolyzed oxidizing (EO) water obtained at the anode side by electrolysis of water containing 0.05% of sodium chloride using a diaphragm between the cathode and anode has shown virucidal and microbicidal activities. Comparing virucidal and bactericidal effects of EO water with those of hypochlorous acid using herpes simplex virus type 1, polio and streptococcus faecalis, the minimum concentration of chlorine contained in EO water was less than that of hypochlorous acid. This result indicates that virucidal and microbicidal activities of EO water are different from hypochlorous acid, and substances such as HClO, Cl, $Cl_2$, $H_2O_2$, $OH^+$ (hydroxyl radical) and so forth, seem to synergistically exert virucidal and microbicidal effects by balancing under competitive acidic conditions.

### B. Preface

EO water is obtained at the anode side by electrolyzing salt water using a diaphragm. It shows nonspecific virucidal and bactericidal effects. On the anode side, OH is consumed by a reaction described as $4OH \rightarrow 2\ H_2O + O_2$, and $H^+$ is accumulated to bring pH to acid side. In addition, hydroxyl radicals ($OH^+$) and $H_2O_2$ are produced as well as $Cl_2$, HClO, $ClO_2$ and other oxidants since NaCl is added to the water. HClO has a strong sterilization effect, and sodium hypochlorite (NaClO) is a stable compound as one of sodium salts. EO water (035, 025, 002 type) produced from 0.05% salt water has been found to contain $ClO^-$ at about 8 ppm. There is a good possibility that $ClO^-$ is showing the virucidal and bactericidal effects. After producing HClO by combining HClO and NaClO ($NaClO + HCl \rightarrow NaCl + HClO$), we compared various types of EO water for their pH, oxidation-reduction potential (ORP), change of mV, amount of Cl, and virucidal and bactericidal effects.

### C. Study Results

As more HCl (0.6N) is added to each concentration sample of NaClO, pH dropped down and mV went up. 0.9 ml of NaClO or NaClO + HCl solution (pH 3.0, 1,000 mV) and 0.1 ml of herpes simplex virus type 1 (HSV-1) and polio virus or streptococcus (S.faecalis) have been put together to react in the room temperature for a minute.

Then, the minimum concentration of chlorine (mg/l) to show virucidal and bactericidal effects was examined. This value for NaClO solution was 2.25 against HSV-1. As to NaClO + HCl, the solution of pure water and HCl with equal volume (pH 2.8) also showed virucidal effects against HSV-1). Against olio virus, the minimum concentration value was 8.8 for both NaClO and NaClO + HCl. Against S.faecalis, NaClO showed 9.00 and NaClO + HCl 4.35.

Judging from these results, virucidal and bactericidal effects of both NaClO and NaClO + HCl are almost the same, and HSV-1 ceases to be infectious even in chloric acid solution of pH 2.7. Then we examined the amount of chlorine included in the solution at minimum concentration as to have virucidal and bactericidal effects for each of 5 kinds of EO water with different salt concentration, electric current and voltage applied. All of them have pH of 3.00 or less and voltage of 1,000 mV or more. As to the concentration of chlorine (mg/l), sample A was 43.80, B 4.19, C 8.15, D 4.20 and E 3.44. Measuring the minimum concentration of chlorine (mg/l) as to have virucidal and bactericidal effects, A was 2.74, B 2.10, C 1.02, D 1.05, and E 0.86 against HSV-1. On the other hand, A was 10.95, B 4.19, C 4.08, D 2.10, E 1.72 against polio virus, although only A and D were different from these at 5.48 and 1.05 respectively against S.faecalis.

Lastly, some EO water at concentrations not explicable by the density of $ClO^-$ showed virucidal and bactericidal effects. This indicates that those effects are not caused only by $ClO^-$ but also by synergy among $OH^+$, $H_2O_2$, $ClO_2$ and other substances. EO water is always produced by the same principle but have all kinds of characteristics depending on the type of machine with different diaphragms, electrodes, voltage and electric current. Its metal-corrosive nature and safety against organism are some of the problems to be solved, but they also seem to be dependent on the type of machine.

## 21. Efficacy of Electrolyzed Acidulous Oxidizing Water

by Takashi Okubo, Dept. of Surgery, NTT Tokai General Hospital
Kazuhisa Inuzuka & Hiroki Kawai, The Central Laboratory for Clinical Investigation, Anjyo-Kousei Hospital, Aichi, Japan

In contrast to electrolyzed highly acidic oxidizing water that has been used as disinfectant, acidulous oxidizing water (AOW) has hydrogen ion concentration (pH) of 5.5, redox potential of 800 to 1,000 mV and dissolved chlorine concentration of 50 ppm and up.

In the room temperature, the amount of residual chlorine remained high even during the activation test, and the sterilization effect lasted for a long time in a stable condition. In particular, chlorine concentration of the retained gas in the upper part of the preservation reservoir was one-hundredth of that of conventional highly acidic water. This proved that the residual chlorine of AOW is hardly volatile.

In addition, when organic substances are mixed in deactivation tests, AOW proved to be 100 times stronger than highly acidic water. This means AOW is barely inactivated by organic substances. When combined with other general disinfectants, AOW with no excessive acidity is found more effective and less corrosive than acidic water.

From these points of view, we believe that AOW can make up for drawbacks of electrolyzed highly acidic oxidizing water. This new function water is something worth more attention.

**A. Preface**

Electrolyzed highly acidic oxidizing water that has been used as disinfectant has pH ranging from 2.3 to 3.2, residual chlorine concentration from 7 to 50 ppm, ORP from 1,000 to 1,200 mV, whereas AOW has pH of 5.5, residual chlorine concentration of 50 ppm or more, and ORP of 800 to 1,000 mV. AOW is designed to take advantage of residual chlorine by creating the environment near pH 5, which represents the highest possible density of hypochlorous acid. It is expected to have the same level of disinfective effect as hypochlorous acid solution at the density of 200 ppm (Fig.1). This is a report on the effects of this new type of function water.

**B. Study Method**

Effects of AOW was examined by comparison tests with highly acidic oxidizing water conventionally used on the checklist including disinfective effects, inactivation by organic matter, disinfective effects in the hospital environment, metal corrosion, concentration of produced chlorine.

**C. Study Results**

In inactivation tests under the room temperature, AOW had high residual chlorine concentration, and showed stability with a long lasting sterilization effect. Especially the concentration of chlorine gas trapped at the top of the container was about 100 ppm in case of highly acidic oxidizing water as opposed to around 1 ppm in case of AOW. Therefore, it was found that the residual chlorine barely has volatility in the latter case (Table 1).

In inactivation test by mixing organic matter, the highly acidic water lost its disinfective effect at the density of 0.01% or more, whereas AOW did not lose the effect even at 1% (100 times). This indicates the fact that AOW is less subject to inactivation by organic matters (Fig.2&3).

Combining with other general disinfectants, it is understandable that AOW with moderate acidity is more advantageous. As to the corrosive function against metal, the corrosion potential was somewhere between 470 and 800 mV, which is lower than that of sodium hyperchloride. That is, AOW has low metal-corrosion function.

**D. Analysis**

From all these results, AOW is considered to be a new type of function water that can make up for the drawbacks of conventional strong acidic water in terms of inactivation by organic matter as well as metal corrosion function. In addition, AOW's concentration of residual chlorine is so high that it is effective for preventing infections in the hospital.

* Refer to the original document for the following figures:

Fig.1: Equipment to produce AOW
Fig.2: Disinfective effect of AOW
Fig.3: Disinfective effect of highly acidic oxidizing water

Table 1: Concentration of chlorine gas by environmental conditions

| Type of acidic water<br><br>Place of measurement | Highly acidic oxidizing water<br>pH = 2.39, ORP = 1,122 mV<br>residual chlorine 20 ppm, 25°C | Acidulous oxidizing water (AOW)<br>pH = 5.55, ORP = 890 mV<br>residual chlorine 80 ppm, 25°C |
|---|---|---|
| Upper opening of 100 liter tank | 100 ppm | 1 ppm |
| Opening of small portable tank | 30 | 1 ppm or less |
| Near faucet, small portable tank | 3 | ditto |
| Bottom of hand washing sink | 3 | ditto |
| Near faucet, hand washing sink | 7 | ditto |
| Empty container of acidic water | 9 | 1 ppm |
| Mop washing water in bucket | 1 - 3 | 1 ppm or less |

## 22. Infection Control by Electrolyzed Strong Acid Solution in Intensive Care Unit

by Keiji Kumon, Naoki Yahagi, Yasuhiko Watanabe, Masaki Haruna, Jyunki Matsui, Hideaki Hyashi, Yukoko Kishizoe, Masakakazu Sakamoto, Miyuki Watanabe, Surgical Intensive Care Unit, National Cardiovascular Center

We studied prophylactic effects of oral cavity lavage by electrolyzed strong acid solution (ESAS) for patients of silent aspiration pneumonia intubated after cardiovascular surgery as compared to the effects of conventional method applying iodine solution for infection control in the surgical intensive care unit. One time, lavage by ESAS resulted in a slight decrease in colony counts in the oral cavity to the same extent as in the case of conventional method. After the lavages for several times, however, the colony counts decreased significantly. The effects of lavage by ESAS exceeded those by iodine solution. We concluded that the oral cavity lavage by ESAS is recommended as an alternative for preventing silent aspiration pneumonia for intubated patients in the intensive care unit.

### A. Preface

Serious patients in the intensive care unit have lower immunity against infections. They are also connected with various artificial devices such as intravenous catheter, tracheal intubation, bladder balloon catheter, and other drains necessary for evaluating quick changes of patients' conditions and treatment. There are all kinds of possibilities for pathogens to enter the human body. Therefore, the patients are susceptible to the infections, and once infected, they easily get in critical conditions that are difficult to treat. This is why very tight infection control is expected in the intensive care unit.

We issued a report last year about the effects of cleaning hospital environment and hand sterilization by ESAS and also the effects of ESAS on the treatment of silent aspiration pneumonia which is a serious infectious disease. This time, we report the results of our study on the effects of oral cavity lavage by ESAS to prevent silent aspiration pneumonia which is the major complications of serious patients who need artificial respiration as compared with that by conventional method by iodine solution.

### B. Study Subject and Method

57 adult patients who had gone through cardiovascular operation were examined. 1 of them were given oral cavity lavage by 20 ml of ESAS, 21 of them were given 40 ml, 14 of them were given 20 ml of iodine gargle diluted to 30 times, 11 of them 40 ml of the same gargle. The bacterial culture samples were taken starting from the first oral cavity lavage in the ICU, and continued to be taken several times around the times of the lavages.

Carrymate was used for the method of sampling. Secretion of the oral cavity was taken by a swab inserted through the corner of the mouth or a bite block. The virus were cultivated in media of chocolate agar, Sabouraud agar, blood agar, and MacConkey agar. Only aerobic virus were cultivated by seed swab method. The lavage of oral cavity was done by injecting cleansing liquid through a syringe into the corner of the mouth and sucked at the same time.

For both one-time and several-time lavages, changes of colony counts of gram-positive virus and negative virus were compared between the conditions before the first and after the last (second to sixth) lavages. The amount of virus colony was divided into 5 stages. $10^4$ or more was categorized as stage 5, $10^3$ or more 4, $10^2$ or more 3, 10 or more 2, less than 10 1, and negative 0. The results are shown as average ± standard deviation. Statistical studies were conducted by paired tests with less than 0.5 as significant correlation.

### C. Study Results

The effects of several-time lavage were recognized for both groups of ionized gargle diluted to 30 times and ESAS. However, the degree of decrease in bacteria colony counts was more distinct for the groups that used ESAS, especially against gram-negative virus (Table 2).

Table 1: Effect of one-time lavage of oral cavity

| | | n | Before lavage | After lavage | P |
|---|---|---|---|---|---|
| Diluted iodine solution | | | | | |
| 20 ml | G (+) | 44 | 4.18 ± 1.08 | 3.93 ± 0.93 | 0.027 |
| | G (-) | 44 | 4.41 ± 1.13 | 4.23 ± 0.94 | NS |
| 40 ml | G (+) | 30 | 3.07 ± 1.89 | 3.00 ± 1.76 | NS |
| | G (-) | 30 | 4.80 ± 0.48 | 4.67 ± 0.55 | NS |
| ESAS | | | | | |
| 20 ml | G (+) | 34 | 4.06 ± 0.81 | 3.91 ± 1.14 | NS |
| | G (-) | 34 | 3.97 ± 0.94 | 3.68 ± 1.20 | 0.034 |
| 40 ml | G (+) | 68 | 3.34 ± 1.41 | 3.24 ± 1.39 | NS |
| | G (-) | 68 | 3.78 ± 1.24 | 3.71 ± 1.29 | NS |

G (+): gram-positive virus G (-): gram-negative virus NS: Not Significant mean ± SD

Table 2: Effect of several-time lavages of oral cavity

| | n | Before lavage | After lavage | P |
|---|---|---|---|---|
| Diluted iodine solution | | | | |
| 20 ml G (+) | 11 | 4.55 ± 0.69 | 3.55 ± 1.04 | 0.004 |
| G (-) | 11 | 4.91 ± 0.30 | 4.27 ± 0.79 | 0.013 |
| 40 ml G (+) | 8 | 3.25 ± 1.75 | 2.88 ± 1.89 | NS |
| G (-) | 8 | 4.88 ± 0.35 | 4.63 ± 0.52 | NS |
| ESAS | | | | |
| 20 ml G (+) | 11 | 4.37 ± 0.67 | 3.27 ± 1.56 | 0.019 |
| G (-) | 11 | 4.27 ± 0.65 | 3.00 ± 1.55 | 0.015 |
| 40 ml G (+) | 19 | 4.05 ± 0.85 | 2.79 ± 1.58 | 0.002 |
| G (-) | 19 | 3.84 ± 0.83 | 3.37 ± 1.54 | NS |

G (+): gram-positive virus G (-): gram-negative virus NS: Not Significant mean ± SD

**D. Analysis**

Judging from the results of our study, the disinfectant effect of oral cavity lavage by ESAS is comparable to that by conventional iodine solution diluted to 30 times. In case of lavages for several times, ESAS showed more effective reduction of bacteria colony counts than iodine solution after the lavages. It seems that ESAS is good for oral cavity lavage.

However, in case of one-time lavage, the colony counts did not drop down as much as expected by both 30 time dilution of iodine solution and ESAS. Some of the reasons for this seem to be deteriorating self-cleansing function in the oral cavity after the operation, its long-lasting dryness, difficulty of care during the operation. Furthermore, it seems to be the result of increased colony counts of bacteria because of bleeding in the oral cavity due to the damage by intubation, in addition to the fact that both ESAS and 30 time dilution of iodine solution were inactivated immediately after contacting organic matter in the oral cavity.

On the other hand, the amount of solution used for one-time lavage was increased from 20 ml to 40 ml, and it did not result in much improvement in the data. However, after the lavages repeated 2 to 6 times, significant reduction of colony counts was recognized for both gram-positive and gram-negative virus in each group with a few exceptions. It became clear that the oral cavity lavage is effective for preventing aspiration pneumonia caused by silent aspiration, which is virus found in the oral cavity. Nevertheless, patients who just had an operation have such poor conditions in the oral cavity that the number of lavages should be increased as necessary, as we believe, according to the degree of contamination in there.

**E. Conclusion**

It was confirmed that the lavage of oral cavity by ESAS to prevent aspiration pneumonia for serious patients who need artificial respiration is no less effective in reducing the colony counts of bacteria than that by conventional iodine dilution.

## 23. Effects of Function Water on Skin Ulcers

by Toshiaki Sato, Hisashi Aoyama, Haruki Asanuma
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There have been great difficulties in managing infections of burn patients. Effects of antibiotic agents are uncertain. Recently, function water has been used as disinfectant in many hospitals, and its effects are getting recognized. In this study, we tried to identify changes of bacterial flora on burned skins by applying function water, and found out it affects bacterial flora distinctively. (No bacteria were identified during the first 3 hours after disinfection.) To the best of our knowledge, function water is useful in managing disinfection of burn patients.

**A. Preface**

In treating burns, prevention of infections is still an important issue. Increased resistance of bacteria against multiple antibiotics combined with uncertain effects of drugs applied to the burn lesion due to its specificity make the treatment very difficult. The antibiotics injected in the body would not get to the lesion surrounded by thick layers of dead tissues where the blood does not circulate well, and they hardly reach the effective concentration for treatment. Antibiotics for external application also have hard time getting into the depth of dead tissues.

**B. Purpose of Study**

Function water has been used these days for cleaning medical equipment, interior surfaces, hands of medical staff, etc., and its effects are getting recognized. To examine the effects of function water on infected lesion of burn patients, we applied it to the lesion of third degree burn, and measured its changes of bacteria counts by time.

**C. Study Method**

Once the scab of third degree burn after 4 weeks has been cut off, function water and iodine solution were applied to each ulcerated skin. Using food stamps sold in the market, bacteria colony counts in the lesions were measured before and every one hour after the application up to 8 hours later. In addition to these two tests, a control sample was prepared without applying any solution for measurement. The food stamp was pressed hard on the skin, then the temperature was kept at 37°C for 48 hours to cultivate the bacteria. The number of colonies was counted by naked eyes. Aside from these food stamp tests, regular bacteria cultivation tests were conducted for each part.

**D. Study Results**

In the lesions where function water or iodine solution was applied, no bacteria were found for three hours after the application. The number of bacteria colonies increased gradually 4 hours after the application, and tended to return to almost the original number in 8 hours. For the control sample, there was no tendency for the colony counts to drop down.

**E. Conclusion**

According to our measurement, function water had almost the same effect of reducing bacteria colony counts as the iodine solution, and was considered to be clinically effective. This time, only function water was applied to preempt the effect of physically removing bacteria by cleansing. If we come up with better way of applying the solution, it can become an effective means to fight infections in the burn lesion.

* See the graph in the original text: Changes of colony counts by time on the ulcerated skin.

## 24. Treatment of Infectious Ulcer with Function Water

by Kiichi Inagawa, Takahiko Moriguchi
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Chronic ulcer caused by infections by MSRA (Methicillin Resistant Staphylococcus Aureus) and others is hard to heal. We often see chronic ulcer resistant to surgical treatments or various conservative treatments. Therefore, we tried conservative treatments using function water to our satisfaction.

The lesion was washed by function water once a day. 12 cases of infectious ulcer are reported here. Out of these, 5 cases were healed, 3 cases well granulated and 4 cases had the infection disappear.

The definition of function water is not clarified yet. So far, the best definition seems to be “solution that is produced by combination of water and electrolyte that is given physiochemical treatment and a certain level of activation energy, and can be applied to life science.”

The function water we used this time is produced in the electric field for physiochemical treatment. More specifically, acidic solution with low pH was produced at the anode by electrolysis of electrolytes such as sodium chloride and potassium chloride using irreversible electrodes separated by a membrane. Because chlorides are used as electrolyte, chloric acid, active chlorine ion group, and active oxygen ion group are produced. This is similar to the process that occurs in organism as a result of redox reactions.

For example, it is already confirmed at the molecular level that the bactericidal effect of neutrophil is caused by active oxygen ion group. The active oxygen ion group in acidic solution shows the similar function. Therefore, this acidic solution is considered to be useful as disinfectant and getting more popular in the medical field. Above all, there are a number of reports about its effects on infections in the hospital as well as the promotion of granulation on the skin.

This time, we treated 12 cases of chronic ulcer resistant against various conservative treatments by acidic solution (hereinafter called function water. They consist of 2 cases of confinement sore (1 at sacrum, the other at ischium), 3 cases of burn (1 at sinciput, 1 at knee, the other at knee), 3 cases of external skin injury (2 at leg, the other at foot), 3 cases of skin fistula (1 at sinciput, 1 at knee, the other at leg), and 1 case of infection by inserting a tissue expander (at cheek).

Pus came out of each lesion. In 6 cases, MRSA was detected in the pus. As a treatment, the lesion was washed by function water once a day. As a result, 5 cases saw the lesions covered by epithelium, 3 cases did not go as far but had granulation, and the rest of 4 cases saw remission of pus and foul odor. During the treatment, no abnormality was identified seemingly caused by side effects of function water whether is self-conscious or not. It is reported that lavage of the lesion by function water demonstrates an excellent débridement effect as compared to that by only salt water iodine solution. In our tests, the débridement effect was recognized since, according to our experience, it was easier to remove the dead tissues in the lesion than otherwise, and also remission of pus and foul odor was identified.

Furthermore, the speed of this function deserves special mention. In all case, remission of pus and foul odor was identified with 3 days of function water treatment. However, if infection reached deep into the tissues, pus could not be stopped completely. It seems possible that this problem is solved in the application method is somehow improved. Finally, function water can be applied on burn lesions without damaging newly formed granulation. Rather, epithelium was formed quickly by the granulation effect of function water.

## 25. Experiences of Treating Infectious Wound & Chronic Ulcer with Function Water

by Katsumi Tanaka, Tohru Fujii,
Dept. of Plastic & Reconstructive Surgery, Nagasaki University School of Medicine

In cases of infectious wounds and chronic ulcers such as diabetic and ischemic ulcers, it is necessary to treat them by washing and cleansing before application of ointment therapy or performing an operation. Bathing, washing and wet dressing (wet to dry dressing) with function water was applied to 37 clinical cases. Then we observed conditions of the wounds and bacterial changes.

In this study, all cases saw improvement in regards to bactericidal effects and granulation. Among these, the wounds were conservatively healed in 7 cases, whereas the rest of 30 cases had the wounds gradually cleansed and reduced. In most cases, the bacterial counts have dropped down to aseptic condition. It is applied that function water has bactericidal effects in clinical cases and effectively promoted wound healing.

### A. Preface

Cleaning the lesion is needed first before the operation or ointment therapy to treat infectious wounds and chronic ulcers. We tried to treat them by bathing, cleansing or wet dressing with function water, and observed remission of infections and formation of granulation to evaluate effectiveness of this method. Our study reports on how to apply function water, list up the clinical cases, and drew a conclusion on the effectiveness of function water.

### B. Study Subject and Method

Function water was applied to 37 patients who were treated at the Department of Plastic and Reconstructive Surgery in Nagasaki University, School of Medicine during 12 months period from August 1994 to July 1995. Among them, 28 were men, 9 women, age 3 to 94 averaging at 46.

17 cases had infectious wounds due to external damages or operation, 7 had chronic ulcer caused by blood vessel malfunction, 3 had gangrene or chronic ulcer accompanies by diabetes, 8 fresh burns, and 2 confinement sore.

The methods of applying function water differ slightly by clinical case. In general, bathing or cleaning by function water and gauze exchange by wet to dry dressing were applied to the patients of infectious wounds and chronic ulcers. In case of fresh burns, function water was used to clean and remove secretion and pus on the lesion at the time of bandage exchange. The method and frequency of applying function water were decided on a case-by-case basis and were modified whenever the symptoms changed.

### C. Evaluation

Criteria were set for each symptom to conduct economic observation. Among these criteria, lesion's characteristics were categorized into location, size, condition of granuloma (color, bleeding), amount and character of secretion, degree of pain, foul odor, extent of epithelium formation. As to bacterial infections, bacteria were cultured on a regular basis. They were identified and the patient's sensitivity was checked. At the same time, types of disinfectants and side medicine, dressing materials, and operation procedures were described.

Regarding the side effects, we made notes about stimuli against skin and membrane, sharp pain, and allergy.

### D. Study Results

The period of treatment by function water was 1 week minimum to maximum 4 months. In all the 37 cases, each lesion was cleaned and reduced. None of them got worse. The bacteria turned negative or got reduced in 19 cases. In the worst 3 cases where infections spread to the entire body, bacteria colony counts did not decrease dramatically, but the lesions have been reduced and cleaned. 7 out of 37 cases have healed conservatively whereas the other 30 have gone through operations.

As to the side effects, 1 case had reddish skin immediately after the application of function water, and another had irritation at the lesion. Since the symptoms were so light that the application has been continued and eventually they healed by themselves without deterioration. No abnormality was found for other treatment cases.

There are more to be clarified about bactericidal effects and efficacy of function water. However, we believe that cleaning the lesion promotes its healing when function water is used for the treatment of infectious wounds and chronic ulcers.

## 26. Treatment of Skin Infection Ulcer with Function Water

by Hiroyuki Kanazawa, Yoshiyuki Minamimoto, Kouichi Honda, Hiroharu Igawa, Dept. of Plastic & Reconstructive Surgery,
Hokkaido University School of Medicine & Tsuneki Sugihara, Keiseigeka Memorial Hospital

Function water has unique characteristics including high positive oxidation-reduction potential, strong acidity, high concentration of dissolved chloride and oxygen. Skin ulcer with infection by MRSA (Methicillin Resistant Staphylococcus Aureus) is hard to heal. We, plastic and reconstructive surgeons, treat skin ulcers resistant to surgical procedures of various conservative therapies. We treated the ulcers by function water and have obtained satisfactory results. We hereby report 13 cases of ineffective ulcer.

This clinically applied therapy proved to be so effective against the symptoms that it can be conveniently applied to many more.

**A. Preface**

In the past several years, Methicillin Resistant Staphylococcus Aereus (hereinafter called MRSA) is becoming the main cause of infections in the hospital. Since our ward combines plastic and reconstructive surgery with dermatology, patients of chronic diseases and skin ulcer due to confinement sore as well as those who need high-tech and long-term treatments are concentrated.

Under theses circumstances, it is known that strong acidic electrolyzed solution (hereinafter called function water) has a powerful bactericidal effect, can be easily produced, and is very cost-effective. The bactericidal effect of function water comes from physical function of oxidation-reduction potential, which is proved to have very low toxicity compared to chemical agents.

Therefore, in our department with many skin ulcer patients, it is expected to be widely used if clinical effects and safety of function water are established. This time, we would like to present some cases of conservative treatment against infectious skin ulcer by function water together with our analyses.

**B. Study Subject and Method**

Function water was applied to 13 patients of MRSA-infected skin ulcer from December 1994 to September 1995. 9 of them were men, 4 women, age 16 to 76 averaging at 44. 4 cases were ulcer from confinement sore, 2 burn ulcer, 6 infectious ulcer due to injury or operation, the other was ulcer caused by blood vessel malfunction.

Function water was produced by the membrane-type electrolyzer installed in the hospital. It was stored in shaded air-tight plastic bottles at room temperature. The entire volume was renewed every week.

**C. Study Results**

The period of application of function water ranged from 1 week to 1 month. 12 out of 13 cases saw reduction of secretion, cleaning of the lesion, benign granulation, etc. In addition, the bacteria turned negative with reduced colony counts. In one case, almost no changes were observed during the treatment by function water. No particular side effects were recognized in all cases.

**D. Analysis**

Because of its high oxidation-reduction potential, function water kills bacteria by taking away their electrons and oxidizing them several seconds after the first contact. Function water itself gains electrons and gets neutralized to turn to low concentration salt water. Unlike regular bactericidal chemical agents (disinfectants), function water does not have problems of osmosis or residual of chemical substances.

Among others, bactericidal effects of function water are reported to be effective against not only general germs such as yellow staphylococcus, coliform (Escherichia coli) and bacillus pyocyaneus (Pseudomonas aeruginosa), but also against drug resistant bacteria such as MRSA. Function water is also said to have even virucidal and eubactericidal effects.

We have applied function water to treat 10 patients of infectious skin ulcer by washing the lesion. In comparison with the effects by salt water, we had the following impression about function water:

(1) The lesion was cleaned and benign granulation occurred more rapidly.
(2) Epithelium formation from surrounding tissues was made more quickly.
(3) The total period of treatment was shorter.

As to MRSA, lavage by function water made it turn negative quicker than that by conventional method. However, we also had a hunch that it is not easy to completely kill bacteria in deeply infected lesions.

## 27. Electrolysis Conditions of Tap Water & Composition of Alkaline Water

by Zempachi Ogumi, Yoshiro Tomida, Div. of Energy & Hydrocarbon Chemistry, Graduate School of Engineering, Kyoto University, & Kenji Kikuchi, Dept. of Materials Science, University of Shiga Prefecture

Impacts of electrolytic conditions on the composition of electrolyzed water were examined. The structure of the experimental cells was similar to that of commercially available apparatus. However, the quantity of electricity passed per unit water volume was ten times larger than that designed for commercial use aiming at testing under accelerated conditions.

Metal dissolution of electrodes into electrolyzed water was examined. Platinum-coated titanium, ferrite and stainless steel were tested as electrode materials. As to ferrite electrodes, the content of Ni (II) went up immediately after the electrode polarity was reversed while ion concentration decreased with electrolysis time. Using nickel-free ferrite electrodes,

no nickel was detected. Stainless steel anode resulted in high concentration of Cr (VI) and Ni (II) above regulated maximum in electrolyzed water. Concentrations of formaldehyde and acetaldehyde as well as trihalomethane in electrolyzed alkaline water has increased during electrolysis. This increase was dependent on the properties of the separator.

Chlorine came out at the anode by permeating through the separator causing a haloform reaction with organic matter to produce trihalomethane. Electroosmosis seemed to play an important role in this transport.

**A. Preface**

We manufactured a prototype apparatus to produce alkali ionized water with similar structure to those sold in the market. Electrodes and membrane sold at stores were used. By this apparatus, we studied electrolytic conditions and the composition of electrolyzed water.

**B. Experiment**

Many alkaline-ion electrolyzers use tap water supplied to the cells after going through active carbon. In our study, we followed this process to supply source water to our apparatus with or without calcium lactate. The electrolysis was conducted by constant electric current at 0.3A. Since the flow of source water was slow at 2 liter/hour, the quantity of electricity passed per unit volume of water was 10 times larger than that of a regular electrolyzer in the market.

**C. Study Results and Analysis**

Source water was electrolyzed for an hour using a separator (septum) made of polyester with high affinity treated by sucrose acid ester. Metal ions were analyzed in the produced solution. During this process, the electrolyte moves from the anode cell to the cathode side by electroosmosis.

In the electrolyzed water produced with electrodes made of platinum and iridium-coated titanium, no oxidation dissolution of electrode materials was detected. $Fe^{2+}$, $Fe^{3+}$ and $Ni^{2+}$ were found in both anodic and cathodic solutions. When stainless steel was used for electrodes, ions such as $Fe^{2+}$, $Fe^{3+}$, $Ni^{2+}$ and $CrO_4^{2-}$ were detected in anodic solution, whereas no $CrO_4^{2-}$ was found in cathodic side. This means $Fe^{2+}$, $Fe^{3+}$ and $Ni^{2+}$ that were produced by oxidation dissolution of electrode materials in the anode cell have shifted to cathode side, but $CrO_4^{2-}$ remains in the anode cell. Using ferrite electrodes that do not include nickel, or negative ion exchange membrane for the separator, no $Ni^{2+}$ was detected in the cathode cell.

In the next step, we examined changes of metal ions in the electrolyte when the electrode polarity was reversed. In case of electrodes made of titanium coated with platinum and iridium, no oxidation dissolution of the electrode materials was detected. When ferrite electrode was used on the other side of platinum-coated electrode, high concentrations of $Fe^{2+}$, $Fe^{3+}$ and $Ni^{2+}$ were detected in cathodic solution (alkaline) immediately after the polarity of ferrite electrode was switched from negative to positive. In the same token, $Fe^{2+}$, $Fe^{3+}$, $Ni^{2+}$ and $CrO_4^{2-}$ were detected in case of stainless steel electrodes. In both cases, ion concentration in the negative cell decreased rapidly (Fig.1).

We also examined changes of organic matter in the source water. First of all, we studied the effects of calcium lactate to be added to tap water treated by active carbon. Even without adding calcium lactate, formaldehyde and trihalomethane not included in tap water were found in the electrolyte. When acid lactate was added to the source water, concentration of chloroform in the cathodic solution went up from 0.003 ppm to 0.059 ppm, acetaldehyde concentration in the anodic solution increased from 0.02 ppm to 0.81 ppm, whereas residual chlorine dropped down from 1.0 ppm to 0.02 ppm. At the anode, chloride ions are electrolyzed and produce chlorine, which is transported to cathode side by electroosmosis.

In the alkaline solution contained in the cathode cell, the chlorine produces trihalomethane by haloform reaction. Taking advantage of these facts, the speed and effects of electroosmosis by different membranes were studied. The types of membrane were polyester sheet (SP) treated by sucrose ester to give hydrophilicity, vinylidene-fluoride coated polyester sheet (FP), mixed fabric sheet of polytetra-fluoroethylene and polyethylene-terephtharate (TE), and polyethylene sheet (PS). The results are shown in the Table 1 below. Wiedeman constants, which indicates the speed of electroosmosis in our measurement for SP, FP and TE were 1.38, -0.15, 0.21 $cm^3\ S^{-1}A^{-1}$ respectively. If the constant is very small, trihalomethane concentration got smaller when FP and TE were used for the membrane. In other words, it was found that the speed of electroosmosis was an important factor to determine the haloform reaction.

Furthermore, to confirm the effect of electroosmosis, the source water was supplied to the pressurized cathode cell so that the flow of electrolyte through the membrane was from cathode to anode side against the direction of electroosmosis. As a result, no formaldehyde, acetaldehyde nor trihalomethane was found in the cathodic solution.

**D. Conclusion**

Using a ferrite plate as anode, high concentration of $Ni^{2+}$, and using stainless steel, high concentration of $Ni^{2+}$ and $CrO_4^{2-}$ appeared at the anode. Especially after the anode turned to cathode by reverse electric current, high concentration of $Ni^{2+}$ and $CrO_4^{2-}$ remained in the alkaline water for a while. However, as time goes by, the concentration gets lower rapidly. By this electrolysis, concentrations of formaldehyde, acetaldehyde and trihalomethane increase. Since the rate of increase depends on electroosmosis, it became clear that the speed of chlorine molecules produced at the anode by oxidizing chloride ions to move into the cathode cell is an important factor.

## 28. Safety Evaluation of Function Water

### I. One month & Six-month Repeated Oral Dose Tests for Electrolyzed Basic Aqueous Solution in Rats

by Atsunobu Matsuo, Hitoshi Yamamoto, Noguaki Ni-I, Takeshi Hisa, Motohiro Nishikawa, Noriko Ktsutou, Yohkazu Matsushima, Shigeru Morita, Yukio Yanagimoto, Life Science Laboratory

In order to evaluate effects of function water on human health, electrolyzed basic aqueous solution (EBAS) with various pH (11.0, 10.0, and 9.0) was given orally to male and female rats of Sprague-Dawley strain at 20 ml/kg/day in 1-month and 6-month toxicity tests where the control group was given deionized water during the period. In addition, a recovery group was tested for 2 weeks.

Body weights, food and water intakes were measured once a week for the first 3 months and thereafter once a month. Biochemical examinations such as urinary, hematological and serum exams were also performed at the end of both tests. By autopsy, major organs and tissues were removed and weighed. They were fixed in 10% neutral formalin for a pathological exam. No apparent adverse effects of EBAS were observed in both of the toxicity tests.

Water is drawing public attention in the health-oriented trends these days. Among others, alkaline ionized water is used by many to promote health in everyday life. It is reported that alkaline ionized water has some effects such as repressing abnormal intestinal fermentation as well as controlling gastric acid. Therefore, we performed repeated oral dose tests of EBAS for 1 month and 6 months using rats. Here is our report on the tests.

#### A. Test Materials and Method

(1) Lab animals and their management

All the rats used in the experiment were male and female SD rats (SPF: bought from Japan Charles River Co., Ltd.). They were kept in the lab for 7 days before the test. 4 groups of 15 male and 15 female rats were prepared for each of 1month and 6-month tests (one each was a control group). In each group, 5 rats were designated for recovery tests. They were all raised in a barrier-system breeding room with the temperature at 22 ± 3°C, humidity 55 ± 10%, ventilation ratio 12 times/hour or more (all fresh air), and 12 lighting hours/day. They were given free access to solid food sold as NMF (produced by Oriental Yeast Industry Co., Ltd.) and tap water.

(2) Preparation of test materials and method of dosage

Solution of calcium lactate (Wako Junyaku Industry Co., Ltd.) was added to the electrolyzer certified by Alkaline Ionized Water Association Unified Standard. Alkali-ion solutions with pH 11.0, 10.0 and 9.0 (calcium ion at 30 ± 10 ppm in all cases) were prepared for the test. The rats were forced oral dose of them for 1 month or 6 months. Solutions of pH 11.0, 10.0 and 9.0 were given at the rate of 20 ml/kg/day using a gastric balloon made for rats. Purified water was given at 20 ml/kg/day to the control groups. For each of the test groups, a recovery group was tested for two weeks.

(3) Test items

Tests were conducted on the items ranged from general observation, measurements of body weights, intake of food and water, urinal, hematological and serum exams. Weight of organs, their weight percentage, and pathological tests were also examined.

#### B. Test Results and Analysis

In all of the above-described tests for both 1-month and 6-month groups, alkali ionized water did not result in any toxicity. Among these tests, the serum exam showed some increase in GOT (glutamate-oxaloacetate transaminase) and changes in the quality of some electrolyte, although these changes were within the normal range.

## 29. Safety Evaluation of Function Water

### II. Four-week Repeated Oral Dose Tests for Electrolyzed Basic Aqueous Solution in Crab-eating Monkey

by Hitoshi Yamamoto, Atsunobu Matsuo, Hideki Hashimoto, Yasuhiko Goto, Akitsugu Ikeda, Yihkazu Matsushima, Shigeru Morita, Yukio Yanagimoto, Life Science Laboratory

In order to evaluate the effects of function water on our health when ingested by large volume, electrolyzed basic aqueous solutions (EBAS) with various pH (11.0, 10.0, 9.5) were orally given to 9 groups of crab-eating monkeys (one individual per group) for 4 weeks at daily dose level of 20 ml/kg, 50 ml/kg (25 ml/kg twice a day), and 100 ml/kg (50 ml/kg twice a day). Body weights were measured every week. Biochemical tests such as urinary, hematological and serum exams were conducted on the days 0, 1, 2, 3, 7, 14, 21 and 28 during the experiment.

Ingestion of the test solution with pH 11.0 at 20 ml/kg/day caused a transient increase in serum GOT (glutamate-oxaloacetate transaminase) or GPT (glutamate-pyruvate transaminase) activity in the early period of the experiment. Moreover, ingestion of the test solution at 50 ml/kg/day, not 100 ml/kg/day, caused a transient increase in serum GOT activity in the early stage. This indicates there is no dose-response relation.

Following the previous tests of 1-month and 6-month repeated oral dose of EBAS, we conducted 4 week repeated oral dose tests using male crab-eating monkeys as large mammals. The purpose of the tests was to study the effects of EBAS with different pH and amount of dose on organism. Here are the results of the study.

**A. Study materials and Method**

(1) Lab animals and their management

Four male crab-eating monkeys, all quarantined and healthy with the weights around 5 kg were kept in separate cages for a week in preparation for the tests. They were raised in a naturally-lit clean room with the temperature at 20 to 25°C, humidity at 50 to 70%, ventilation ratio 10 times or more/hour (all fresh air) with 8 lighting hours per day. They were given free access to solid food sold as PS (produced by Oriental Yeast Industry Co., Ltd.) and tap water.

(2) Preparation of test materials and method of dosage

Solution of calcium lactate (Wako Junyaku Industry Co., Ltd.) was added to the electrolyzer certified by Alkaline Ionized Water Association Unified Standard. Alkali-ion solutions with pH 11.0, 10.0 and 9.0 (calcium ion at 30 ± 10 ppm in all cases) were prepared for the test. To examine the effect by pH, 1 monkey in each group was forced oral dose of alkaline ionized water for 4 weeks with different pH into the stomach at the rate of 20 ml/kg/day using 6Fr multi-purpose tube (manufactured by Atom Co., Ltd.). After the test, recovery period was set for another 4 weeks. As to the group that was given pH 11.0, another monkey was added for pathological anatomy.

To examine the dose-response relation, the test materials were given to the above animals at 50 ml/kg/day for another 4 weeks, and the effects were studied after the 4-week recovery period. Then, after another 4-week recovery period, the test materials were given at 100 ml/kg/day for 4 weeks, and the effects were studied again.

(3) Test items

Besides daily general observation and body weights measurement once a week, urinal, hematological and serum exams were conducted before the test as well as the day 1, 2, 3, 7, 14, 21 and 28 of the test for each of the materials. Also in the recovery period, additional exams were conducted on the day 14 and 28. As to the group given pH 11.0 solution at 20 ml/kg, pathological anatomy was performed. Together with visual observation, weight of organs, their weight percentage, and pathological tests were examined.

**B. Test Results and Analysis**

When three types of alkaline ionized water (pH 11.0, 10.0, 9.5) were given at the constant rate of 20 ml/kg/day, the group of pH 11.0 showed a temporary increase of GOT and GPT in the serum in the early period of the test. For other test items, no abnormality worth noting was found. Monkeys that were given solution of pH 11.0 for weeks were dissected for pathohistological exams of main organs without special findings on abnormality.

In case of increasing the amount of alkaline ionized water to 50 and 100 ml/kg/day for each pH, the group of 50 ml/kg/day showed a temporary increase of GOT activity in the serum in the early period of the test. In case of 100 ml/kg/day, no increases of GOT activity nor the dose response relation was found. For other test items, there was no abnormality worth mentioning.

Judging from these results, we believe alkaline ionized water has little toxic effects on organism at pH less than 11.0.

## 30. Basic Examination of Bactericidal Properties of Strong-acid Ionic Water

by Toshio Takahashi, Taiichi Kodaka, Joji Iizuka, Eiichi Yamada, Shigeo Kaneko, Sumio Tominaga
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**A. Summary**

Strong-acid ionic water (hereinafter called ionized water), when used alone, was found to have a bactericidal effect only 5 seconds after contacting bacteria in vitro. This effect is drastically reduced, however, in the presence of other substances such as serum, soap or skim milk.

After MRSA or P.aeruginosa-polluted hands are washed with ionized water, it was proved that there is no difference in the amount of bacteria compared to washing hands by tap water if concentration of residual chlorine is 10 mg/liter. When the concentration is increased to 40 mg/liter the bacteria could be partially removed by washing hands with ionized water for more than 40 seconds.

**B. Preface**

Infections in the hospital by MRSA and other bacteria have been a serious problem not only in the hospital but in the society in general. We, as medical professionals, are working hard on the counter measures.

It is said that washing hands of medical staff is most important in preventing infections in the hospital. However, other problems, such as skin chapping, dermatitis, and increased resistance of bacteria against disinfectants are arising.

These days, some reports are found about bactericidal and clinical effects of ionized water as a disinfectant that is mild to our hands and is hard to invite increased resistance of bacteria. In our hospital, ionized water has been used for washing hands, cleaning hospital environment, and treatment of patients since the completion of our new building in May of 1994. Here is a report on our basic study on the effects of ionized water.

**B. Study Method**

(1) Experiment in vitro: Step 1

10 μ liter of bacteria broth at the density of 9 x $10^8$ cfu/ml was added to 1 ml of ionized water (pH 2.5, ORP 1,310 mV, residual chlorine at 10 mg/l) and combined with 1 ml of SCDLP bouillon culture bed (by Eiken Chemicals). After inactivating the ionized water immediately, the bacteria were cultured for 24 hours at 35°C. By visual observation of solutions in vitro, the turbid ones are recognized to have positive growth, whereas clear ones went through subculture and the bacteria were confirmed dead.

To study effects of other substances that coexist in the ionized water, the same tests were repeated after adding 1%, 0.1% and 0.01% of serum, 0.01% and 0.001% of soap, 0.1% and 0.01% of skim milk. The following bacteria were used in the tests:

| | | |
|---|---|---|
| 1. standard bacteria colony | | 4 colonies of 4 strains |
| 2. gram-positive bacteria | (clinically collected) | 27 colonies of 12 strains |
| 3. gram-negative bacteria | (clinically collected) | 40 colonies of 21 strains |
| 4. enteric pathogens | (clinically collected) | 12 colonies of 8 strains |
| 5. yeast-like eumycetes | (clinically collected) | 12 colonies of 6 strains |

(2) Experiment in vitro: Step 2

After the solution of MRSA, or P.aeruginosa, was applied to hands and was washed away by ionized water, bacterial growth was examined. The same exam was conducted for a control group that was given MRSA applied to hands that were sterilized by quick-drying rub-in type disinfectant (Welpas by Maruishi Pharmaceutical).

To study changes of colony counts of bacteria by time, the same solution of MRSA was applied to hands and the growth of bacteria was examined after washing them by ionized water. For a control group, tap water and povidon iodine were used to wash hands after the application of the same MRSA solution.

(3) Sterilization of hospital environment

Ionized water was sprayed at the places listed below in the hospital room of MRSA patients. It was wiped out by paper towel, and MRSA bacteria were examined.

1. on the floor near the bed
2. around the headboard of the bed
3. shelves near the headboard

**C. Study Results**

(1) Experiment in vitro: Step 1

Effects of ionized water on bacteria were confirmed for both standard and clinically collected colonies except Bacillus subtilis after only 5 seconds of contact. As to the effects of coexisting substances, no effects of ionized water were identified under the condition of 0.1% of serum, 0.01% of soap, and 0.1% of skin milk.

(2) Experiment in vitro: Step 2

1. removal of bacteria
2. existence of bacteria after washing hands
3. change of bacteria colony counts by time after washing hands

It all differed by concentration of residual chlorine, amount of bacteria and duration of hand wash. We will report at the Symposium. We will also present the difference in case of using quick-drying rub-in type disinfectant, tap water and povidon iodine.

(3) Sterilization of hospital environment

In both of the hospital rooms we examined, MSRA bacteria deriving from the patients were detected at all the areas wiped out around the time of ionized water application.

**D. Conclusion**

Ionized water showed bactericidal effects in vitro by contacting bacteria just for 5 seconds. However, the effects were dramatically reduced by other substances in the solution.

In washing bacteria-polluted hands by ionized water, the bactericidal effect of ionized water depends on concentration of residual chlorine contained in the solution. If the concentration is at 40 mg/l, the bacteria could be partially removed by washing hands for 40 to 60 seconds, although there was not much difference from washing by tap water in some cases. Quick-drying rub-in type of disinfectant solution had a sure bactericidal effect in a short period of time.

We believe that ionized water is not suitable for sterilizing hospital environment because its bactericidal effect is offset by coexisting substances in the solution.

## 31. Utilization of Electrolyzed Weak Acid Aqueous Solution for Sterilization of Hemodialysis Equipment

by Yoshihei Hirawawa, Yutaka Ikeda, Shigeru Hoshina, Kidney Center, Shinrakeun Hospital

### A. Summary

Electrolyzed weak acid aqueous solution (EWAS – pH 4.5 to 5.5, chlorine concentration 50 ppm) was used to sterilize hemodialysis equipment. We compared its effect of sterilization with that of sodium hypochlorite solution (pH 8.5 to 9.0), chlorine concentration 500 ppm). The bactericidal effects were almost the same for both solutions. Within 5 minutes of starting sterilization, viable bacteria have completely disappeared. From these facts, 20 minutes of sterilization seem quite enough. Since EWAS has low concentration of chlorine, the time required to wash away chlorine was shorter than sterilization by sodium hypochlorite solution. EWAS has such a weak solubility against $CaCo_3$ that washing away deposits of calcium salts may not be necessary.

Even though the delivered dialysate is sterile, proliferation of bacteria occurs in the dialysate contained in the dialyzer due to the incomplete sterilization of the coupler with an O-ring. We created a new type of coupler with a silicon gum packing instead of an O-ring. Since these couplers can be sterilized completely, bacterial proliferation did not occur during dialysis treatments.

### B. Preface

In hemodialysis treatments, control of bacterial proliferation in the dialysate is an important issue. Especially these days, high flux dialyzers have been used more often to prevent complications. Therefore, the needs are growing for asepsis of the dialysate. Not to mention that sterilization of hemodialysis equipment is needed for the safety of patients and staff, it is also required that the equipment is durable, does not give impacts on the environment of sewage treatment, sterilize in a short time, and is conveniently handled.

The counter measures currently taken against pollution of environment by disposed dialysate include water treatment by reverse osmosis (RO) membrane, sterilization and cleaning of the tank of sodium bicarbonate, the raw material of the dialysate, supply of dialysate by ultra filtration (cutoff value is 10,000 or less by molecular weight), daily sterilization of flow area of dialysate including the dialyzer and patient monitor device by sodium hypochlorite solution (residual chlorine concentration at 500 ppm, flow velocity at the monitor 500 ml/min) for 30 to 40 minutes and cleaning by reverse osmosis, and cleaning of the flow area by 0.3% acetic acid solution every other day to dissolve and remove attached calcium salt.

We have been examining the advantage of EWAS as disinfectant over sodium hypochlorite solution, and the repressive effect against proliferation of bacteria in the dialysate achieved by improving couplers in the dialyzer.

### C. Study Method

(1) Effect of sterilizing the dialyzer

After using EWAS at pH 4.5 to 5.5 with concentration of HClO at 50 to 60 ppm produced by non-membrane type electrolyzer (manufactured by Idee Co., Ltd. – Fig.1) for hemodialysis, the dialysate was contained in both single-use and multiple-use equipment for 24 hours to culture bacteria inside. Then the bactericidal effect of EWAS was examined as compared to that of sodium hypochlorite.

(2) Change of residual chlorine concentration and pH in the cleaning process

In the process of sterilizing the dialyzer and cleaning by RO water, residual chlorine concentration and pH in the disposed dialysate were measured by time, and were compared with the case of sterilization by sodium hypochlorite.

(3) Solubility against calcium bicarbonate

0.1 g of $CaCo_3$ was placed in a funnel with paper filter, and 30 ml of EWAS was poured per minute for 30 minutes. Concentration of $Ca^{++}$ in the filtered water was measured to study the solubility of EWAS against $CaCO_3$.

(4) Effect of the improved coupler

The conventional coupler has a built-in O-ring to tightly connect to the dialyzer. However, since the sterilization around the O-rings is incomplete in the process of disinfecting equipment, proliferation of bacteria is unavoidable in the dialyzer solution. Instead of these O-rings, we have used silicon-gum packing and created improved couplers that are easy to sterilize inside. We examined the results.

### D. Study Results

(1) Sterilization effect of EWAS

EWAS was supplied at 500 ml/min for 20 minutes to the dialyzer where the dialysate is kept for 24 hours after hemodialysis so that bacteria have proliferated. Sterilization effect of EWAS was compared with that of sodium hypochlorite solution (also at 500 ml/min for 20 minutes – Fig.2). The detected bacteria were mainly Psudomonas, Enterobacter and Staphylococcus.

For both methods, satisfactory sterilization was achieved. There was no difference between them, although, 2 to 4 hours after the cleaning and sterilization, bacteria grew back.

(2) Concentration of residual chlorine
Changes in concentration of residual chlorine by time in the liquid waste from the patient monitor No.1 and No.20 in the process of disinfecting and cleaning (by RO water) the multi-use dialyzer is shown in Fig.3. Concentration of residual chlorine in case of sterilization by EWAS was one-tenth of that in case of using sodium hypochlorite. The washing time to achieve 0 concentration of residual chlorine was shortened by 15 minutes applying the former method.

(3) Solubility against calcium bicarbonate
Compared with 1% to 0.3% acetic acid solution, EWAS has about half the solubility against $CaCO_3$ (Fig.4). EWAS was expected to dissolve calcium salts attached to the equipment in the sterilization process.

(4) Effect of the improved coupler
The number of bacteria colony and endotoxin concentration in the dialysate using conventional couplers is shown in Fig. 5. The dialysate flowing into the dialyzer is aseptic and contains almost no endotoxin. However, after passing through the dialyzer, it contains high concentration of proliferated bacteria and endotoxin. Since there is not much time between daytime hemodialysis and the nighttime counterpart, no sterilization is conducted. Thus the dialyzer used for nighttime hemodialysis is further polluted by bacteria. Fig.6 shows diagrams of the new-type of couplers using silicon-gum packing (A and B types). Thanks to these new-type couplers, bacterial growth in the solution contained in the dialyzer is almost completely repressed (Fig.7).

**E. Analysis**

It is possible these days to provide dialysate that is aseptic and free of endotoxin. However, the current situation is that bacterial proliferation in the solution of dialyzer in use for hemodialysis is not controlled. The reason for this is the structural defect of conventional couplers, that is the use of an O-ring that is hard to sterilize. By our new-type of coupler with silicon-gum packing instead of an O-ring, and also by improving the connector, we achieved expected sterilization effect. Thanks to these couplers, bacterial proliferation in the solution of dialyzer in use is well controlled.

The second problem of sterilizing the dialyzer is that it takes about 2 hours for its cleaning and sterilization. Therefore, it is hard to schedule the sterilization process between daytime and nighttime hemodialyses. Using EWAS instead of sodium hypochlorite, this time has been shortened rendering sterilization before the nighttime homeostasis quite possible.

The third problem with the current sterilization method is the impact of high concentration of sodium hypochlorite on sewage treatment system. This problem is mostly solved by the use of EWAS. The staff's safety is also enhanced by it.

Period cleaning of the hemodialysis equipment is done by acetic acid to dissolve calcium salts deposited in the pipes. Since EWAS has weak solubility against $CaCO_3$, it is expected to prevent such precipitation.

Although EWAS has many advantage in sterilizing hemodialysis equipment, additives should be adjusted to produce EWAS at required pH since pH of RO water varies by season and region.

**F. Conclusion**

Using EWAS (pH 4.5 to 5.5, chlorine concentration 50 to 60 ppm) for sterilization of hemodialysis equipment, we obtained the following results:

(1) For single-use and multi-use hemodialysis equipment, the bacteria were all perished within 5 minutes. It seemed that the intended purpose is achieved by 20 minutes of sterilization. Bactericidal effect of EWAS was almost the same as that of sodium hypochlorite at 500 to 600 ppm (pH 8.5 to 9.0).

(2) The time spent to reduce the chlorine concentration to 0 was significantly shortened as opposed to using sodium hypochlorite. The chlorine concentration in the waste solution in the process of sterilization is so low that its impact on sewage treatment tanks is negligible.

(3) EWAS's solubility against $CaCO_3$ is about half that of 0.3% acetic acid. Therefore, EWAS makes necessity of cleaning the dialyzer by acetic acid less frequent.

(4) The improved coupler can well be sterilized inside and is useful since it does not promote bacterial proliferation in the solution of the dialyzer in use.

* Refer to the original documents for the following figures:

Fig.1: Flow chart of equipment to produce EWAS
Fig.2: Cleaning and sterilization effects of single-use hemodialysis equipment
Fig.3: Comparison of residual chlorine concentration for multi-use hemodialysis equipment
Fig.4: Comparison of solubility against calcium bicarbonate ($CaCo_3$)
Fig.5: Changes in bacteria colony counts and concentration of endotoxin in the dialysate
Fig.6: Improved couplers for hemodialysis
Fig.7: Bacteria colony counts in dialysate using improved couplers

## 32. Cleaning Endoscopes by Acid Electrolyzed Water

by Jun Okada, Kinuko Sato, Yukihiro Sakurai, Clinical Laboratory & Endoscopic Center, Kanto Teishin Hospital

Infections mediated by endoscopes are reported to be on the rise. We use acid electrolyzed water (AEW produced by Oxylyzer made by Miura Electronic Co. in Tokyo) for our routine treatment in order to investigate its usefulness for disinfection of endoscopes. Endoscopes were cleaned in the following four ways: glutaraldehyde cleaning, routine washing with running water (conventional way), cleaning by AEW applied through the aspiration channel, and the same method with a channel cap soaked in AEW. Then, cultured bacteria were processed by AEW supplied through the aspiration channel after each of the above methods.

No bacteria such as Helicobater pylori have developed after AEW was applied. The result was the same as in the case of cleaning by glutaraldehyde. Other cleaning methods results in bacterial growth of 30 to 88% in the test samples. Meanwhile, the endoscope was examined carefully by analyzing each channel after our routine inspection and disinfection with AEW for 1,000 times. There were no changes in the endoscope itself compared with those in normal use.

In conclusion, washing with AEW is as effective as glutaraldehyde cleaning. It is rapid, convenient and safe for the patients and the medical staff. In addition, AEW had a minimum effect on the endoscope. We believe this cleaning method is effective for disinfection of endoscopes used for many patients.

Inspections of upper and lower digestive tracts by an endoscope are conducted more often these days. Using a single endoscope, many of them have to be done in a limited time. In our hospital, 8,000 inspections are conducted for upper tracts by a gastrocamera, and 2,000 for lower tracts by an intestinal endoscope. Among them, inspection of upper tracts is repeated 40 to 50 times a day, and the average use of a single fiberscope is 8 times or more.

There are guidelines set by Japan Society of Digestive Organ Endoscope in 1985 regarding the method of cleaning and sterilizing endoscopes. Our hospital has been cleaning ours according to these guidelines. However, the risk of infections mediated by endoscopes is more of our concern, and establishment of an effective cleaning and sterilization method has been an issue for a long time.

As active members of the “Aqua-Acid Water Study Group” founded in 1992, we have been studying cleaning and sterilization effect of endoscopes for upper digestive tracts focusing on the effectiveness of aqua-acid water (strong acid water). The results have been presented in various conferences and study sessions (ex. Environmental Infections Society, Endoscope Study Group). We are pleased to report those results this time. The AEW used for our study is produced by the Oxylyzer made by Miura Electronic Co.

### A. Study Method

We have examined the effect of AEW on symptoms except infections in the following methods:

(1) After cleaning and disinfecting the endoscopes by a conventional method (with 0.2% solution of Tego 51 and soap water, etc.), fresh AEW was sucked from the channel entrance in the amount of 10, 30 and 50 ml for the test of bacteria cultivation.

(2) After cleaning the endoscopes, the channel caps were exchanged with those soaked in AEW, and 50 ml was sucked. Then the process (1) was repeated.

### B. Study Results

The possibilities of detecting bacteria or eumycetes in the solution were 88, 30, 58 and 47% by the conventional method and AEW suction method of 10, 30 and 50 ml respectively. The major bacteria found include yeast-like eumycetes, non-fermentation bacteria, staphylococcus, streptococcus and bacillus subtlis. There were no significant correlation among suction amount of AEW, bacteria colony counts and detection possibilities. On the other hand, no bacteria were found including Helicobater pylori by the method (2) described above.

### C. Analysis

Based on the results of our preliminary examination, we conducted further study by applying a method where 50 ml of AEW was sucked after exchanging the channel cap with those soaked in AEW, and then air and water were supplied to the endoscope and its lenses were polished. We have been using this method for our routine cleaning since two years ago. As to the surface corrosion of the interiors of the endoscope, its manufacturer Olympus has conducted durability tests for more than 1,000 times and reported that there is no materials deterioration or damage worth noting as compared to the conventional method.

### D. Conclusion

Excellent results were obtained by combining the conventional method of cleaning with that of using channel caps soaked in AEW and sucking it into the endoscope. However, it was considered important to send air and water into the tube after the cleaning in order to get rid of AEW completely. This is a convenient, effective and safe method of cleaning endoscopes by AEW, and is especially effective in disinfecting them after the use for other patients. In addition, we studied methods of cleaning intestinal endoscopes by AEW. We will report on the results at the same time.

## 33. Investigation on Testing Methods for Bactericidal Effects on Function Water

by Hisaaki Sato, Kaoru Kubozono, Tadamasa Yamada, Mamiko Nakaguchi, Hirakawa Tamami, Notbutoshi Maebara
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We have found phenol coefficient and perishing rate constant of bacteria to evaluate bactericidal effects of function water. Comparing these types of function water, phenol coefficient of type A (pH 2.7, ORP 1,160 mV, Cl 20 ppm), type B (pH 3.2, ORP 1,080 mV, Cl 80 ppm) and type C (pH 2.5, ORP 1,130 mV, Cl 4 ppm) against Salmonella Typhimurium were 0.27, 0.44 and 0.07 respectively.

In the effective dilution of function water, concentration of chlorine was approximately 1.0 ppm. In A and B types of function water with high concentration of chlorine, ORP in the effective dilution was less than 1,000 mV. The bacteria perishing rte constant of function water could not be determined since the bacteria were killed immediately upon exposure to function water.

These results reveal that phenol coefficient is more available method of testing bactericidal effects of function water at above 1,000 mV of ORP and 1 ppm or chlorine contents than others.

0.05% solution of NaCl (as promoting reagent) is electrolyzed with an anode and a cathode with a membrane separator between them. The solution that is produced in the anode side at pH of 2.7 or less and ORP of no less than 1,100 mV is known to have strong bactericidal effects. However, a method of comparative measurement is not established yet. In pursuit for measurement criteria of bactericidal effects of function water, we examined phenol coefficient and perishing rate constant to compare them. In addition, we measured ORP and chlorine concentration of three types of function water to examine the relation between bactericidal effects and those values.

Function water type A, produced by Company A (pH 2.7, ORP 1,160 mV, chlorine concentration 20 ppm), type B produced by Company B (pH 3.2, ORP 1,080 mV, chlorine 80 ppm), and type C produced by Company C (pH 2.5, ORP 1,130 mV with 4 ppm of chlorine) were used to measure phenol coefficient and perishing rate constant against Salmonella Typhimurium (ST), Staphylococcus aureus (SA), Escherichia coli (EC) and Pseudomonas aeruginosa (PA) using a conventional method. The phenol was bought in the market (Kanto Chemistry, finest grade). Dilution of function water was done by pure water (comparative resistance 18.3 M Ω・cm, ORP 467 mV, chlorine 1 ppm or lower) produced by Toraypure LV-08 (Toray). Adjustment of bacteria culture was done by Dalbecco's buffer with phosphoric acid and salt water with divalent positive ions removed, and Heart infusion broth (Difco) was used for bacteria culture.

Function water A, B and C had bactericidal effects when diluted to the maximum of 24 times, 40 times and 6 times respectively. Phenol coefficients calculated by 90 times the effective concentration of phenol were 0.27, 0.44 and 0.07 respectively. Chlorine concentration of 24 time dilution of type A, 40 time dilution of type B, 6 time dilution of type C were 1.0, 1.5 and 0.75 ppm respectively. ORP was 960, 860 and 1,000 ppm in that order. Calculated in the same way, phenol coefficients of function water A, B and C against SA, EC and PA were 0.18, 0.14, 0.23; 0.24, 0.24, 0.40; 0.04, 0.06, 0.06. With regard to measurement of the perishing rate constant, the bacteria that showed $10^7$ cfu/ml at the time of reacting with function water were reduced to less than $10^2$ cfu/ml in 5 minutes after the reaction making it impossible to calculate the constant.

In consequence of these tests, measurement of phenol coefficient is considered to be an effective method in evaluating bactericidal effects of function water. In this measurement process, function water with high value of ORP, given the same chlorine concentration, showed stronger bactericidal effects. So did function water with high chlorine concentration given the same ORP. This implies that both chlorine and ORP are engaged in the bactericidal activities of function water, and those effects come out at ORP 1,000 mV or more and chlorine concentration of no less than 1 ppm.

## 34. Hand Disinfectant Effects of 2 Kinds of Electrolyzed Acidic Aqueous Solutions by Glove Juice Method

by Kazuhiko Otoguro, Fusayo Suzuki, Yoko Akimaru, Kazuo Kodama, Jong-Chol Cyong, Hideo Kobayashi, Kanki Komiyama,
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& Yoichi Yajima, Kaiyu Clinic

Comparative studies on disinfectant activities of electrolyzed acidic aqueous solution (EAAS pH 2.5 to 2.6) and 0.2% solution of benzalkonium chloride (Welpas) were carried out by the glove juice method. After determining baseline level of bacterial flora on the hand skin of 128 volunteers for three times, the skin was washed either by flowing EAAS I or II for 90 seconds or a single use of 3 ml of Welpas. Bacteria on the washed hand were collected by the glove juice method. Then the number of bacteria colonies formed in the agar plate were counted. The activities of EAAS I and II were compared with those of Welpas.

Bacterial reduction rates of EAAS and Welpas were 65.1% and 58.7%, whereas those of EAAS II and Welpas were 70.2% and 57.6%. Logarithmic reduction values of EAAS 1 and Welpas were 0.60 and 0.74. Those of EAAS II and Welpas were 0.57 and 0.52 respectively. There were no significant difference among these 3 types of solution.

This indicates that EAAS I and II may be useful for disinfecting hands.

Effects of disinfecting hands of healthy adults with two kinds of EAAS were compared by using disinfectant called Welpas and by glove juice method.

**A. Study Materials and Method**

(1) Test solution and control solution
The test solutions are EAAS I (pH 2.6 ± 0.5, residual chlorine concentration 20 ± 2 ppm, ORP 1,140 mV) produced by Medi Wostam (Taiyo Engineering Co., Ltd. And Hoshizaki Electric Co., Ltd.), and EAAS II (pH 2.5 ± 0.2, residual chlorine concentration 40 ± 10 ppm, ORP 1,120 to 1,140 mV) produced by Oxylyzer (Miura Electronic Co., Ltd.). As a control sample, 0.2% solution of benzalkonium chloride was used.

(2) Testing agency and subjects
The tests were conducted both in Kitasato Institute Bio-Iotric Center and Shin-Akasaka Clinic using crossover method. The subjects were selected from healthy adult men and women without any skin diseases. 32 of them were assigned to one type of EAAS at either of the locations totaling 128 for the test.

(3) Test method
Measurement was done by modifying Kanki's method which is partial modification of FDA's glove juice method. First, existing number of bacteria colonies (BL value) on the right hands before disinfection were counted three times. Then the hands were disinfected by the test solution in preparation for the next bacteria colony counts.

(4) Method of sterilizing hands by EAAS
1. Rub and wash hands and two-thirds of the arms with flowing EAAS for 15 seconds.
2. After wetting the sterilization brush by EAAS, brush the right hand for 60 seconds (10 seconds for the palm, another 10 for the back of the hand, 20 for finger side and between fingers, 10 for ⅔ of the arm down).
3. Wash ⅔ of the arm with flowing EAAS for 15 seconds.

(5) Method of disinfecting hands by Welpas
Take 3 ml of Welpas on the palm and massage it into the palm, back of the hand, fingers, between fingers, fingertips and ⅔ of the arm until it is dry.

(6) Colony counts of existing bacteria on the hand
Number of bacteria colony was counted in the samples for BL value measurement collected from the subjects as well as samples after sterilization by EAAS that appeared on Brain Heart Infusion Agar plate. Then the number of colonies in 20 ml of the sample solution was calculated.

(7) Evaluation method of disinfectant effects
Bacteria reduction ratio which is (BL value — # of colony counts / BL value x 100% and index reduction value which is ($\log_{10}$ BL value / # of colony counts after disinfection) were calculated to conduct paired t-tests.

(8) Subject and objective symptoms
All the volunteers filled out each report form to note whether there were any subjective symptoms or not. They also saw the doctor just before the test as well as 6 hours after the application of EAAS.

**B. Study Results**

(1) Subjective and objective symptoms
There appeared no subjective nor objective symptoms that area clinically important in the test period.

(2) Disinfectant effects
Average bacteria reduction ratio of EAAS I was 65.1% and that of Welpas was 58.7%. They had almost the same disinfectant effects. Average bacteria reduction ratio of EAAS II and that of Welpas were 70.2% and 57.6% respectively, showing significant difference ($P < 0.01$). Average index reduction ratio for EAAS I was 0.60 and that of Welpas was 0.74in almost the same range. As to EAAS II and Welpas, the ratio were similar at 0.57 and 0.52 respectively.
Based on these results, EAAS is expected to have better bactericidal effects than others in terms of routine disinfection of medical staff's hands.

## 35. Bactericidal Effects of Soft Water (HClO), A Product of Electrolyzed Water on Pathogenic Microorganisms & its Clinical Applications

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Bactericidal activities were compared between soft acid oxidizing water (soft water: pH 5.0 to 5.5, ORP 800 to 1,100 mV, HClO concentration 30 to 80 ppm) and hard acid oxidizing water (hard water: pH < 2.7, ORP > 1,100 mV, HClO concentration 20 to 50 ppm). In vitro examination, the soft and hard water exhibited equally strong bactericidal activities against many species of pathogenic bacteria such as S.auerus, E.coli and S.shigella. However, the soft water showed much higher level of activity than the hard water against Bacillus subtilis and Clostridium botulinum.

The soft water left in the room temperature exhibited intense bactericidal activities and maintained the level for a month, whereas the hard water lost its activities 2 days later.

In addition, the soft water showed distinctive bactericidal effects especially when applied to wash hands of several healthy people as well as to clean the rooms of laboratory animals.

Environmental pollution including hospital infections and food contamination has become a serious issue these days. We are studying water that has a strong bactericidal effect and available conveniently in the great amount from tap water system to tackle this problem.

This time, we examined bactericidal effects of the conventional hard water (pH < 2.7, ORP > 1,100 mV, HClO concentration 20 to 50 ppm) with the newly developed soft water (pH 5.0 to 5.5, ORP 800 to 1,100 mV, HClO concentration 30 to 80 ppm) against various bacteria. Other than the tests in vitro, hand-washing and spray tests were conducted. We herby report some of the results.

**A. Study Method and Materials**

In vitro tests were started by combining bacteria broth and the sample water in the ratio of to 9. After certain period of reaction time, 10-time volume of the liquid medium was added to neutralize the water's effects. Then the number of bacteria colonies was counted.

Preservation tests of oxidizing water were conducted by placing the water in air-tight plastic containers both in the room temperature and in a dark and cold place. After certain period of time, the sample water and bacteria broth were mixed at the ratio of 9 to 1. Then, after a while, 10-time volume the liquid medium was added to it and the number of bacteria colonies was counted.

Hand-washing tests were conducted for 3 groups of hand-washing time for 10, 20 and 30 seconds. Bacteria were collected from palms and fingers by swabs before and after the washing, and the number of colonies was counted.

For spray tests, the multi-purpose compact sprayer SanaJet manufactured by Sanden Co. was used. The tests were conducted in the animal pen to measure the number of airborne bacteria colonies for 30 minutes each before, during, immediately after and 2 hours after the spraying.

**B. Study Results and Analysis**

Soft water showed better bactericidal effects than hard water by in vitro tests. Especially against Bacillus subtilis and Clostridium botulinum that germinate, soft water brought a distinctive result in 2 minutes, whereas hard water did not do anything in the same period.

As to the preservation tests, soft water showed enough effects against MRS and pathogenic E.coli for 2 months in a cold and dark place, and 1 month in air-tight containers in the room temperature. On the other hand, hard water's effects lasted only for a week in a cold and dark place, and 2 to 3 days in air-tight containers in the room temperature.

Hand-washing tests showed intensive effects of soft water especially by washing for more than 20 seconds. Hard water showed unstable effects even when hand-washing lasted more than 30 seconds.

Spray tests had significantly better effects using soft water whereas hard water and tap water did not reduce the number of bacteria and even tended to increase it.

From these tests we have come to the conclusion that soft water based on a new concept demonstrates higher bactericidal effects than conventional hard water. Since hard water is difficult to preserve and also has a problem of producing toxic chlorine gas, the soft water is expected to become the mainstream of bactericidal acid water in the future.

## 36. Studies on Mechanism of Antibacterial Activity of Acidic Electrolyzed Water

by Kunimoto Hotta, Dept. of Bioactive Molecules, National Institute of Health, Tokyo

Acidic electrolyzed NaCl solution (EW) was studied on its physiochemical aspect of antibacterial activity. Although it has been claimed that high ORP and low pH are the two major factors of the activity, neutralization of EW resulted in a significant decrease in ORP without losing much of its antibacterial activity. This activity was demonstrated without direct contact with bacteria, although it has been restricted drastically when $NaSO_4$ was substituted for NaCl. Therefore, it is unlikely that low pH and high ORP play critical roles in the activity. Rather, hypochlorous acid, chlorine gas and hydroxyl radicals generated in EW are said to be critical elements in the activity.

It was also found that DNA, RNA and proteins were almost completely degraded in E.coli cells treated with EW. Damages done to the cell envelope were also indicated. Similar results were obtained with hypochlorous acid that was acidified by HCl.

EW, which is a product of electrolyzing low-density sodium chloride (usually 20 ml, 0.115% or lower) has capability of instantly killing bacteria including MRSA as well as virus under the laboratory conditions. It has been said that this activity is due to low pH at 2.7 or under, and high level of ORP of 1,000 mV or more. To verify this, we neutralized EW to find out that the bactericidal effects are maintained despite decreased ORP. This result tells us that low pH and high ORP are valuable indices for producing EW, but not the major factor of its bactericidal activity. We tried to clarify the cause of antibacterial activity of EW by experiments to come up with the following results.

By electrolyzing sodium sulfate solution instead of salt water, we could also produce acidic water with pH 2.7 or under, although the level of antibacterial activity was low. The smell of chlorine that comes out of EW will eventually dissipate in an open area, and the antibacterial activity will be lost with the same low pH. In addition, the bacterial growth was repressed

without direct contact with EW. This made us judge that chlorine in the solution is an important factor for its antibacterial activity.

Production of chlorine gas and hypochlorous acid by electrolysis of salt water is theoretically understood. Under the conditions we prepared for electrolysis, chlorine concentration of 40 to 50 ppm was detected. Chlorine gas, hypochlorous acid and hydroxyl radicals were also found under the same conditions.

On the other hand, cells of E.coli that was treated by EW had their DNA, RNA and proteins almost completely decomposed. We also verified indirectly that the cell membranes are damaged.

The same phenomenon were observed in the solution of sodium hypochlorite (antihormine) acidified by hydrochloric acid to keep the same level of chlorine concentration as EW.

In conclusion, hypochlorous acid is produced and exists in EW in the super-active state, and the antibacterial system is structured by produced chlorine gas and hydroxyl radicals. As to low pH and high ORP that have been considered to be the main reason for the antibacterial activity, the former is indirectly important for the activity but the latter is not well known yet.

## 37. Studies on Electrolyzed Oxidized Water (Function Water) for Bactericidal Effects

by Teruaki Sumioka, Hai Tao Xu, Kanji Yoshida, Japan Structural Medical Science Institute

### A. Summary

In this study, we investigated microbicidal mechanism of electrolyzed oxidizing water (function water) by an interference microscope (Olympus IX70). Three stains of bacteria were used: Staphylococcus aureus (gram-positive), Escherichia coli and Pseudomonas aeruginosa (both gram-negative). For all of them, the cell membranes were broken and the plasma inside has burst out. This happened in totally different way from denaturation triggered by chemical mediators such as iodopher and oxydol.

Similar phenomena have been observed when direct current (ca 0.5mA, 12V) was passed through water containing bacteria. Their cell membranes consist of insular lipid bylayer. Since a cell has several electrolytes, bacteria act as a biological condenser. Bacteria are so small compared to animals and plants that these capacitances are also very small. On the other hand, molecular clusters of function water, having obtained electric energy, retain positive charge and keep moving randomly. It is as though many batteries are disseminated in the water. These electric charges in the clusters result in overloading.

In conclusion, we believe that the microbicidal effects of function water come from electric energy stored up in it.

### B. Preface

Organisms consist of cells as their basic unit. Those cells are separated by septa made of lipid bylayer, and have electric potential between inside and outside. Organisms function by maintaining these conditions actively.

Meanwhile, if we take a look at living creatures as a whole, the electrolyte held by each organism make itself a conductor, whereas it functions as a nonconductor against the outer world.

Considering these conditions, each organism should have its own capacity of static electricity. Since microorganisms are so small compared to animals and plants that their capacity of static electricity is extremely small. Therefore, their condensers are vulnerable to disruption by a slight electric charge.

On the other hand, water as a whole is nonconductor, although it is known to work as molecular clusters depending on the energy state when it is liquid. Function water is produced and disappears in the water as nonconductor maintaining those clusters with electric charge. In other words, it is as if micro-batteries are scattered in the water and move around randomly. They are supposed to hit microorganisms and destroy them by giving electric charges. We tested bacterial solution by dripping function water and disinfectant as well as by electric charge and compared the results.

### C. Study Materials and Method

We used Staphylococcus aureus (gram-positive), Escherichia coli and Psudomonas aeruginosa (both gram-negative) for bacteria samples.

(1) Observation was done by a reverse-system microscope made by Olympus with a differential interference adjuster. The magnifying power is 4,500 times on the monitor. The results were recorded in the form of animation by 8 mm video.
(2) For the electrolysis, Panase (Remodeling 21 Co., Ltd.) was used.
(3) Bactericidal activities were recorded by the video after dripping hipiten, cresol and oxydol in the bacteria broth. Then a slide was created by attaching platinum electrode at every 3 mm and concocted to a specially-made non-surge power source. Bacteria broth was filled between the electrodes to make an electrochemical device.

**D. Study Results**

(1) Cell disruption by dripping function water

1. The cells stop moving right after the dripping of function water.
2. Debris will appear when large amount of function water is applied. By dripping function water, the plasma was found to burst out at the time of membrane destruction. We also observed a similar phenomenon where the plasma flew out as the membrane was destroyed by drips of hipiten.

2) Cell disruption by electric charge

1. When electric charge was given to the solution, we saw the bacteria disintegrate bursting out the plasma in the source of being attracted to the anode between the electrodes (non-electrode contact disruption).
2. In case of large amount of bacteria, debris is attached to the surface of electrodes where some bacteria are still alive. It was found that proteins such as plasma would form an electric shield.

**E. Analysis**

By these experiments, we proved that function water can intrinsically have mechanism of destroying microorganism (bacteria) by electric charge. It was found that function water shows high level of effectiveness against microorganisms that have septa such as bacteria. Judging from the destroyed bacteria, it was considered almost impossible that resistant bacteria would generate.

Function water is basically water. It is harmless for large organisms that have large electric capacity. In this respect, function water is totally different from other disinfectants. While previous studies on disinfectants have been based on inference from the results of experiments using bacteria culture, we observed the dynamics of bactericidal activities and deduced our conclusion.

## 38. Antimicrobial Effects of Electrolyzed Acidic Water

by Atsuo Iwasawa, Yoshiko Nakamura, Dept. of Clinical Pathology, Showa University Fujigaoka Hospital

Electrolyzed acidic water (EA-water) contains considerably high concentration of chloride and oxygen. It also shows high oxidation-reduction potential over 1,100 mV and low pH below 2.7. The most distinct characteristic of EA-water is antimicrobial effects against bacteria and virus. In this report, we would like to present the mechanism of water in the context of high concentration of chloride and ozone.

To establish an effective method of applying EA-water to prevent infections in the hospital, we have been studying antimicrobial effects of EA-water, microscopic form of bacteria, and endotoxin tests.

Antimicrobial effects of EA-water in vitro have a wide range of spectrum from anti-MRSA to anti-virus. They also showed quick effects. Against most microorganisms, the effects were shown immediately after the application of EA-water. However, it took 5 minutes to kill blast cell bacteria, 2.5 minutes for some of anti-acid bacteria, and 1 minute for some eumycetes. EA-water needs to be applied above 20°C because its bactericidal effects dropped down under the temperature. Since the effects were also reduced by foreign materials such as serum, EA-water should be applied as running, or if it used for soaking, the solution has to be renewed quite often.

From the experiment using both EA-water and conventional disinfectants, we found that reagents such as benzalkonium chloride and povidin iodine did not reduce the effects of EA-water. It was considered that the mixed use would reduce the amount of conventional disinfectants to minimum.

In the cell growth inhibition test, cell adherence inhibition test, and lymphocyte toxicity test, EA-water had significantly lower toxicity than other disinfectants. Therefore, it is applicable for prevention of infections at the lesion after operation. Through observation of changes in bacteria form by an electron microscope and also in vitro tests, we found bactericidal mechanism of EA-water as follows: Ozone is produced by electrolyzing water. These ozone and chlorine ions caused by adding salt seem to react to produce chlorides. EA-water we have been studying contains mainly chlorides, whereas other EA-water has ozone in it. The nonspecific oxidation reaction between the ozone and chlorides is considered to be a contributing factor of the bactericidal effects.

In the future, it is desirable to clarity the amount of ozone and chlorine included in EA-water produced by electrolyzers manufactured by each company. Development and usage of the equipment are also expected to fit the concentration of these elements.

* See the original text for the bibliography

# 39. Bactericidal Effects of Bacterio-Killer Water

by Takamitsu Imanishi, Yuji Nakamachi, Shouhiro Kinoshita, Masahiko Mu
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We studied bactericidal effects of Bacterio-Killer water (hereinafter balled BKW) for several species of bacteria. Then we deduced its mechanism of sterilization. First of all, we determined concentration of BKW and the duration of its effects. Then bacterial broth was cultured on a particular agar plate.

We also investigated the impact of vitamin E or human albumin on the bactericidal effects. As a result, contacting more than equal amount of BKW over 10 seconds completely repressed bacterial growth except mycobacterium. Bactericidal effects were still observed in preserved BKW for as long as 35 days. From the fact that antioxidant such as vitamin E and human albumin deprived BKW of the bactericidal effects, we speculated that active oxygen and its metabolism are related to the mechanism of sterilization.

We examined bactericidal effects of both standard and clinically separated bacteria colonies as well as the conditions that reduces them by BKW which is one type of function water. From there, we deduced the mechanism of its sterilization. In addition, considering the environment where BKW is used, we examined effects of sterilizing the hand, and especially when an endoscope is used. Here is the report of our studies.

## A. Study Subject

BKW was produced by a flow-type device to produce water for cleaning and disinfecting manufactured by TRP Co. The original solution (salt water) with adjusted pH of about 6.5 is sent to this electrolysis device at the rate of 30 ml/min. The produced electrolyte that include hypochlorous acid generated by the electrolysis is mixed with tap water flowing at 6 liter/min. This is how BKW, water with bactericidal effects with concentration of free residual chlorine at 20 ppm, is produced. Electrolyzed acid water (EAW) was also produced by the Electrolyzer AITEC manufactured by Aiken Industries Co. Its pH was measured by pH Meter 150 made by Corning and Co., ORP was measured by ORP-Meter RM-10P manufactured by Toa and Co.

The following 12 species of bacteria were used for this experiment:

| | | |
|---|---|---|
| ATCC colony: | Staphylococcus aureus | (ATCC25923) |
| | Escherichia coli | (ATCC25922) |
| | Klebsiella pneumoniae | (ATCC27853) |
| Clinically separated colony: | Candida albicans | |
| | Cryptococcus neoformans | |
| | Helicobacter pylori | |
| ATCC-derived colony: | Mycobacterium tuberculosis | ($H_{37}$ RV) |
| | Mycobacterium kansasii | (ATTC12478) |
| | Mycobacterium scrofulaceum | (ATTC19981) |
| | Mycobacterium avium complex | (RIMD1312004) |
| | Mycobacterium fortuitum | (ATTC14407) |

## B. Study Method

(1) Step 1:
Immediately after producing BKW, its pH and ORP were measured. Changes by time were examined for 2 hours.

(2) Step 2:
Bacteria colonies were adjusted by disinfectant to become McF1. This bacteria broth was made to react with BKW with volumes of 20 times, 10 times, 1 time, 1/10 and 1/20 of the solution. In addition, bacteria broth was made to react with the same volume of BKW for 10 seconds, 30 seconds and 1, 5 and 10 minutes. In case of general bacteria, 20 μ liter of produced solution was inoculated into 5% sheep-blood agar medium (BBL) and cultivated for 24 hours at 35°C. 20 μ liter of Helicobacter pylori broth was inoculated into Skirrow agar medium (adjusted in-house), and aerobically cultured for 96 hours at 35°C. 20 μ liter of eumycetes broth was inoculated into Sabouraud agar medium (made by Eiken) and cultured for 72 hours at 25°C. Anti-acid bacteria broth was inoculated for 100 μ liter into VITE medium (made by Kyokuto Pharmaceutical), and cultured for 4 weeks at 35°C. Bactericidal effects were visually evaluated by bacterial growth.

(3) Step 3:
S.aureus, E.coli, K.pneumoniae, C.albicans, C.neoformans were made to react with BKW preserved for 1, 7, 14, 21, 28 and 35 days at the room temperature to examine each of its effects by preservation.

(4) Step 4:
Using the same species as Step 3, influence of organic material for each of 1) salt water (control sample), 2) salt water with 0.1, 0.2, 0.3, 0.5, 1 and 2% of human albumin, and 3) salt water with vitamin E that was adjusted to reach the final concentration of 0.1, 1, 10, 100 mg/dl were examined.

(5) Step 5:
To investigate hand-washing effects, 15 inspectors were divided into 3 groups and made to wash their hands once a day in the morning or after work with 1) tap water, 2) EAW, or 3) BKW by flashing it for 15 seconds. Then the water was wiped out by paper towel and a hand of each participant contacted 5% sheep-blood agar medium (BBL), which was cultured for 24 hours at 35°C to measure the bacteria colony counts.

(6) Step 6:
Waste solution was made as a control sample by pouring 20 ml of disinfectant into a fiberscope after use. Then, 20 ml of 1) disinfectant, 2) acid water, or 3) BKW was poured into the fiberscope to generate waste solution, which in turn was sucked by a membrane filter and cultured by Tryptic soy broth (made by TSB, Difco) for 24 hours at 35°C. We compared the growth of bacteria.

**C. Study Results**

(1) Right after the production of BKW, its pH was about 7.0 and ORP about 715 mV. pH did not change much but ORP has increased since then.
(2) Although the situations vary depending on the species, bactericidal effects were recognized when more than equal amount of BKW is added to the bacterial solution (Table 1). According to the contact time test, all bacteria have perished by contacting BKW for 10 seconds except for anti-acid bacteria. The number of anti-acid bacteria colonies has been reduced by 30 minute contact with BKW as compared to the control sample (Table 2).
(3) Period of preservation that maintains bactericidal effects was 35 days for general bacteria, 1 day for C.albicans, and 13 days for C.neoformans.
(4) When vitamin E was added, P.aerugimosa and C.albicans lost bactericidal effects at above 1 mg/dl as compared to the control group. For other species, it took 10 mg/dl or more to nullify the effects. Regarding the addition of human albumin, BKW was deprived of its bactericidal effects against all the species at 0.2% or more (Table 3).
(5) Comparing before and after hand-washing, bacteria colony counts have generally decreased but not completely perished. In 2 cases, bacteria grew back after hand-washing. This may be caused by personal manner of hand-washing (Table 4).
(6) BKW's bactericidal effects on the fiberoptic endoscope were clearly recognized as opposed to disinfectant (Table 5).

**D. Analysis**

BKW has low concentration of residual chlorine and is nearly neutral. Only 10 second exposure to BKW was enough to kill bacteria except for anti-acid bacteria. Even after preserving for a long time, BKW showed stable bactericidal effects. However, it was found the effects are impaired by organic material such as vitamin E and albumin just like the case of acid water and function water.

Mechanism of bactericidal effects of BKW is not clear yet, but antioxidant such as vitamin E and albumin are suspected to be involved in the loss of the effects. Bactericidal effects of BKW in general use such as hand-washing and equipment cleaning have been found more distinct than those of tap water. However, since there is individual difference in the manner of hand-washing, and the collecting method of bacteria is not repeatable consistently, we are planning to apply glove method and others combined with the use of membrane filter.

* Refer to the original document for the following tables:
Table 1: Bactericidal effects by the amount of BKW
Table 2: Bactericidal effects by reaction time
Table 3: Influence of vitamin E and albumin
Table 4: Comparison of bactericidal effects in hand-washing (n=5)
Table 5: Bactericidal effects on a fiberoptic endoscope (n=1)

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