034. Dutta S, De SP, Bhattacharya SK. In vitro antimicrobial activity of potash alum. Indian J Med Res 1996 Jul;104:157-9. 6 ref, Eng. National Institute of Cholera & Enteric Diseases, P-33, CIT Road, Scheme XM, Beliaghata, Calcutta 700010, India

"This study reports the bactericidal activity of potash alum when added to water, against various epidemic causing enteric pathogens like Vibrio cholerae O1, V. cholerae 0139 and Shigella dysenteriae 1 by lowering the pH of water (from 6.0 to 4.0). Growth of the enteric pathogens was monitored in vitro by inoculating broth cultures of the different organisms in distilled water containing increasing concentrations of potash alum and quantitatively determining the concentration of viable organisms over a 48 h period by the standard plate count method. Controls constituted cultures of each organism grown in the absence of potash alum. The pH of alum administered water was measured in each test tube before inoculation of organisms. Potash alum was found to inhibit growth (105 viable count per ml) of most of the organisms examined, particularly V. cholerae 01 and V. cholerae 0139 in a dose dependent fashion. Reduction of colony forming units was observed in presence of 0.25 g/dl of alum after 5 h and no growth was noticed after 24 h."

Decontamination of drinking water by alum for the preparation of oral rehydration solution

K. Ahmad and K. Jahan, institute of Nutrition, Dhaka University, Dhaka, Bangladesh
I. Huq, International Centre for Diarrhoeal Disease Research - B, Dhaka, Bangladesh

Oral rehydration has proved to be an effective and inexpensive therapy in diarrhoea and is being recommended for use in developing countries 11, 2). Though the principle and methods for the preparation of oral rehydration salt (ORS) are simple and easily taught to paraprofessionals and mothers of children with diarrhoea, the question always arises as to the use of available water to make the solution. The use of contaminated drinking water in rural areas may allow bacterial proliferation in the ORS because sucrose is one of its ingredients, and this has led to the assumption that only boiled water can be used for the preparation of ORS.

Although boiling water before use is an effective method of decontamination, it has disadvantages, such as the difficulty of obtaining costly fuel in rural areas as well as the time required for boiling and cooling that delays therapy. Extensive studies have been done on the use of locally available drinking water for preparation of oral rehydration salt solution and also on the survival of bacterial flora (both pathogenic and nonpathogenic) in the oral rehydration solution prepared with those drinking water samples (3; 4; and 1. Huq, unpublished data, 1981). In spite of this research, the question remains as to the safety of ORS prepared with contaminated water for paediatric patients. Studies by Black et al. (3) have shown that pathogens like Vibrio cholerae and enterotoxigenic Escherichia cold reached concentrations of 103 to 104 per millilitre by 12 hours and 104 to 106 by 24 hours after inoculation of solution made with river water, with a somewhat lower concentration in distilled water.

We made ORS solution with tank and pond water used by the village people and observed a 2 to 3 log increase in the growth of coliform and E. cold in 12 to 24 hours. Watkinson et al. (4) also found significantly greater bacterial multiplication in solution made with contaminated well water in a West African village. From all these studies it is clear that ORS solution prepared with untreated water having a high bacterial count and organic matter can support the growth of enteric bacteria at ambient temperature.

Although the study by Watkinson et al. did not show any difference in the isolation rates of pathogenic organisms in 97 stool specimens obtained from children who received ORS solution made with "clean" water and 87 stool specimens from children who received ORS solution made with well water, there was strong support for using either sterile distilled water or water decontaminated by natural or chemical means. Acra et al. (5) used the viricidal activity of sunlight and clearly demonstrated the efficacy of this simple technique for sterilizing water for the preparation of ORS.

No studies have been done so far on the use of chemical agents in decontaminating potable water for the preparation of ORS. As ORS is now being widely used for the treatment of diarrhoea in Bangladesh as well as other developing countries, and in view of the non-acceptability of using well or hand-pump drinking water in rural areas, we undertook a study to determine whether aluminium potassium sulphate (potash alum), commonly known in Bangladesh as fitkiri, would have an antibacterial effect on the total bacterial count in oral rehydration solution made with tank water and with pond water having a high bacterial count.

MATERIALS AND METHODS

Water samples for the study were collected from a well used for drinking water in the village of Kabrul on the outskirts of Dhaka, from a tank adjacent to the International Centre for Diarrhoeal Disease Research that contained water used mainly for bathing and washing purposes, and from tap water supplied to the laboratory from central supplies. Both the well and tap water are used for drinking. The water samples were collected at 8:00 a.m., and the experiment was started within one hour.

The water was not kept under refrigeration. Two sets of experiments were done. In the first set, V. cholerae inaba and E. coli in concentrations of 103 and 104 per millilitre were inoculated into tap water containing varying concentrations of potash alum (500 mg/ml, 250 mg/ml, and 1 mg/ml), with one control consisting of tap water only. Two loopfuls of the samples were plated onto gelatin agar every hour for eight hours to measure the viable bacteria.

In another set of experiments, 500 mg of powdered potash alum was incorporated in a packet of ORS and dissolved in one litre of pond water and one litre of well water in separate bottles. The solutions thus made were left at room temperature (24°C to 28°C) for 24 hours. At 0, 2, 4, 8, and 24 hours, 0.1 ml of each of the solutions was removed. Serial tenfold dilutions were made, and 0.1 ml was spread onto nutrient agar plates and McConkey plates. The differential bacterial count was obtained after overnight incubation of the plates.

RESULTS

In the experiments involving different concentrations of potash alum (table 1), V. cholerae at concentrations 103 and 104 per millilitre was killed in between one and two hours in 500 mg/ml of potash alum per litre. One milligram of potash alum per millilitre killed V. cholerae in water in under one hour. E. cold from stool at concentrations of 103 and 104 per millilitre survived almost the same length of time in the presence of 500 mg/ml of potash alum. The pH of the ORS fortified with 500 mg/ml of alum remained at 6.4.

When oral rehydration solution was made with well water or pond water with or without potash alum, not much change occurred in the ionic concentration of the solution. Table 2 shows the electrolyte concentration of the different ORS made with alum-containing water from different sources. It is interesting to note that changes in sodium bicarbonate level were within allowable limits.

TABLE 1. Survival of Vibrio cholerae and Escherichia coli in Solutions Containing Different Concentrations of Potash Alum*

Time (hours)	10^3 Organisms/ml			10^3 Organisms/ml		104 Organisms/ml	
No alum	250 µg/ml	500 µg/ml	1 mg/ml	250 mg/ml	500 µg/ml	1 mg/ml	
V. cholerae							
0	+++	+++	++	+	+++	++	++
1	+++	+++	+	0	+++	+	0
2	+++	++	0	0	+++	0	0
4	+++	+	0	0	++	0	0
6	+++	+	0	0	++	0	0
8	+++	0	0	0	++	0	0
E. coli							
0	+++	+	++	++	+	+	++
1	+++	0	+	+	0	+	±
2	+++	0	+	0	0	+	0
4	+++	0	+	0	0	+	0
6	+++	0	0	0	0	+	0
8	+++	0	0	0	0	0	0

* Locally available commercial grade
** Slightly sour taste, but drinkable

There were sharp decreases in the total bacterial counts in ORS solutions made with water from different sources and fortified with 500 mg/ml of potash alum. Table 3 shows that, though the total count decreased a little in all cases when oral rehydration salts were added, there was a steady increase in the count in solutions without potash alum, and by eight hours there was a 2 log difference in total bacterial count between the two groups in both types of water used.

DISCUSSION

Potash alum is available in Bangladesh even in villages and has traditionally been used for clearing river, pond, or tank water. The water thus treated is used for drinking, cooking, and other purposes. Potash alum has been found to be nontoxic at 0.1 to 5 per cent, though ingestion of a concentrated solution as high as 20 per cent may cause a burning sensation in the mouth and may also cause diarrhoea. In our experiments we have observed that 0.01 per cent solution, i.e., 1 9 per litre, kills V. cholerae and E. coli within one hour, and 0.005 per cent, or 500 mg per litre, kills bacteria within one to two hours. Alum precipitates the insoluble materials in the water and kills or lowers the total bacterial content of the water.

TABLE 2. Electrolyte Concentration in mmol/litre of ORS Made with Water from Different Sources

	Na+	K+	Cl	HCO-3
Well water	100	21.8	90	29.8
Well water + 500 µg potash alum	96	21.1	89	26.5
Pond water	97	22.7	85	30.8
Pond water + 500 µg potash alum	100	23.4	86	29.7

It has been seen that, in remote areas of developing countries where food hygiene is poor and where most of the people are in the lower income group, oral rehydration

solution is usually made with water from available drinking water sources such as untreated pond and well water. The adverse effect of prolonged exposure to the high number of bacteria present in ORS solutions prepared with untreated water is well recognized. Though the use of boiling water for making ORS has been suggested by many and is practiced in some places, the cost of doing so is beyond the economic reach of people in rural areas. Even oral rehydration solution made with boiled water is capable of supporting growth of bacterial enteric pathogens.

There is not much epidemiological information on the use of traditional drinking water (e.g. from a pond, river, or well) for preparing ORS solution and its association with diarrhoea of increased severity or renewed attacks of enteric infection. It is recommended that ORS solutions be prepared as far as practicable with water made substantially bacteria-free by physical or chemical means. We have seen that ORS containing potash alum at a concentration of 500 /la/ml lowers the total bacterial content of the pond or well water by 2 logs. Oral rehydration solution made with water previously treated with potash alum has also been found to bring down the total bacterial count by approximately 2 logs. Though addition of potash alum lowered the pH of the ORS a little (from 8.3 to 6.4), the concentrations of bicarbonate, sodium, potassium, and chloride were found to be within acceptable limits.

The results of this study clearly indicate the efficacy of potash alum in lowering the total bacterial content of the"usual" pond or well water contaminated by Gram-negative bacteria to make it suitable for drinking purposes as well as preparing oral rehydration solution for patients with diarrhoea. As diarrhoeal diseases are often caused by contaminated water, treating water with potash alum before drinking or making oral salt solutions with potash alum during epidemics in rural or urban areas should bring down the incidence of disease as well as deaths caused by diarrhoeal disease. Epidemiological studies are currently under way in the field to see whether potash alum can act as a preventive measure in diarrhoeal disease.

TABLE 3. Survival of Bacteria in ORS Made with Water from Different Sources with or without Potash Alum

	Total Bacterial Count				
	0 Hr	1 Hr	2 Hr	4 Hr	8 Hr
Well water	1.4 x 10^2	7.6x 10^2	8.2x 10^2	8.0x 10^3	7.4 x 10^3
Well water + 500 µg/ml alum	1.2 x 10^3	1.6 x 10^2	7.0 x 10^2	2.0 x 10^2	7.2 x 10^2
Pond water	2.0 x 10^3	1.8 x 10^2	2.0 x 10^3	6.0 x 10^3	2.4 x 10-4
Pond water + 500 µg/ml alum	1.0x102	1.2x102	1.0x102	4.0x102	4.1 X102

REFERENCES

1. D. R. Nalin, R. R. Cash, R. Islam, M. Molla, and R. A. Phillips,

"Oral Maintenance Therapy for Cholera in Adults," Lancet, ii: 370 (1 968).

2, N. Hirschhorn, "The Treatment of Acute Diarrhea in Children: An Historical and Physiological Perspective," Amer. J. Clin. Nutr., 33:637 (1980).

3. R, E. Black, M. M. Levine, M, L. Clements, P. Angle, and R. Robins-Browne, "Proliferation of Enteropathogens in Oral Rehydration Solutions Prepared with River Water from Honduras and Surinam," J. Trop. Med. Hyg., 84 (5): 195 11981).

4. M, Watkinson, N. L. Lloyd-Evans, and A. M. Watkinson, "The Use of Oral Glucose Electrolyte Solution Prepared with Untreated Well Water in Acute Non-specific Childhood Diarrhoea," Trans. Roy. Soc. Trop. Med. Hyg., 74 (5): 657 (1980).

5. A, Acra, Y. Karahagopian, Z. Raffoul, and R. Dajani, "Disinfection of Oral Rehydration Solutions by Sunlight," Lancet, ii: 1257 (1 980).

6. M. G. M. Rowland and J. P. K. McCollum, "Malnutrition and Gastroenteritis in the Gambia," Trans. Hyg. Soc. Trop. Med. Hyg., 71: 199 (1977).