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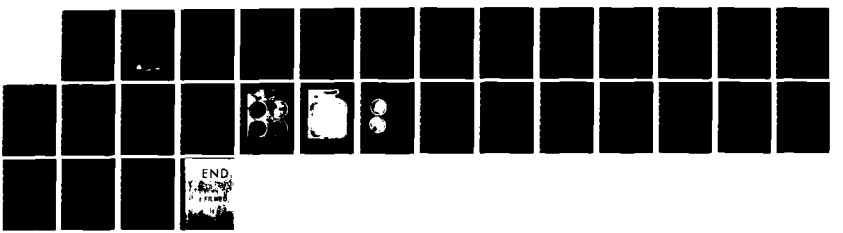

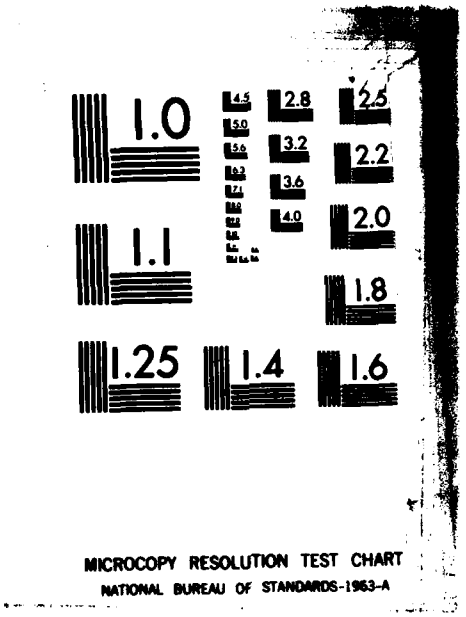

INSTITUTE REPORT NO. 85

EVALUATION OF A REVERSE OSMOSIS APPARATUS FOR FIELD PRODUCTION OF USP GRADE INJECTABLE WATER FROM SEA WATER, POND WATER, AND HUMAN URINE

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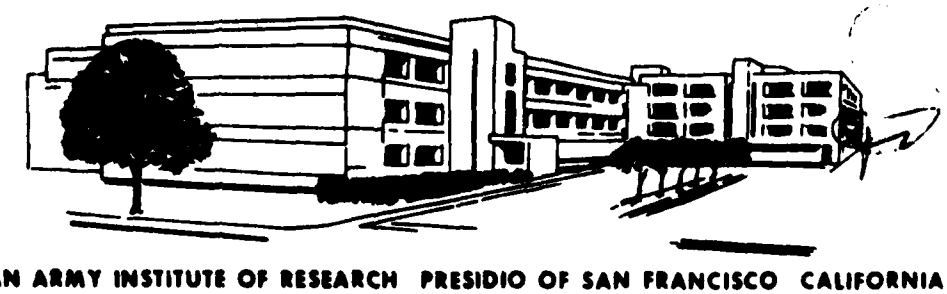
JULY 1980



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A compact, portable, single unit apparatus has been evaluated for purification of sea water, pond water, and human urine. The process is based on the reverse osmosis procedure with a combination of pure bleached cotton, cellulose, and activated carbon filters. The results indicate that brackish, polluted water can be purified by a single passage through this system, as demonstrated by considerable reduction or complete elimination of ions, metal content, and organic

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matter present in the source of water. The water obtained is clear, colorless, odorless, sterile, non-pyrogenic, does not contain antimicrobial agents or other added substances, and appears to satisfy the criteria for USP grade water for injection except for the limits on total solids. The remaining solids are sodium and chloride ions which are commonly added to injectable water prior to clinical use. The applications of this water purification process are discussed.

ABSTRACT

A compact, portable, single unit apparatus has been evaluated for purification of sea water, pond water, and human urine. The process is based on the reverse osmosis procedure with a combination of pure bleached cotton, cellulose, and activated carbon filters. The results indicate that brackish, polluted water can be purified by a single passage through this system, as demonstrated by considerable reduction or complete elimination of ions, metal content, and organic matter present in the source of water. The water obtained is clear, colorless, odorless, sterile, non-pyrogenic, does not contain antimicrobial agents or other added substances, and appears to satisfy the criteria for USP grade water for injection except for the limits on total solids. The remaining solids are sodium and chloride ions which are commonly added to injectable water prior to clinical use. This water purification process could be useful in many non-military situations, such as at disaster sites, on off-shore drilling platforms, on commercial and pleasure boats, and at other locations where pure water is needed but not available.

PREFACE

The authors wish to thank David B. Milne, Ph.D., for the metal content determinations, Mr. Donald A. Jess for the gas chromatographic assays, SP 5 Dan J. Smith for the bacterial analysis, and James H. Skala, Ph.D., for his assistance in several analytical procedures.

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In the usual urban environment, sterile water for injection is readily available; however, in field situations or in areas remote from supply sources, the procurement of purified water could present logistic difficulties. The problems associated with transportation, storage, and supply of large quantities of pure sterile water can be overcome by its production in situ by using any available source of water which is purified by a simple inexpensive process. Purified, injectable water may be needed in military field situations as a diluent for pharmaceutical formulations, for preparation of sterile saline solutions, reconstitution of lyophilized hemoglobin for fluid therapy (1-3) and for other purposes.

According to the criteria of the United States Pharmacopeia (USP) (4), sterile water for injection is a clear, colorless, odorless liquid; it is sterile, contains no antimicrobial agent or other added substances, is pyrogen-free, and has a total solids content of 2 to 4 mg%. The present report details the evaluation of a portable, compact, single unit apparatus used for the purification of sea water, pond water, and human urine, and the production of purified water that appears to satisfy the criteria for USP grade water for injection except for slightly higher content of total solids. Human urine was used in these studies to evaluate the potential of the purification process in extreme situations, such as a desert environment, where urine might be the only source of water.

MATERIALS AND METHODS

Water Purification

Three different sources of water were used for purification. Sea water was collected from the marina at Fort Baker, California, in the San Francisco Bay area. Pond water was taken from the lagoon in front of the Palace of Fine Arts in San Francisco. Pooled human urine was collected during a 24-hr period from several male laboratory workers. The apparatus used for the purification of water from the three different sources is manufactured by Allied Water Corporation, San Francisco, California. This portable SweetWater™ system, Model 200, enclosed in a fiberglass case, measures 45.7x48.3x78.7 cm, has a weight of 62 kg, and an output of 757 liters of water per day. The system is equipped with a water pump which, in the set-up used for these experiments, pumps the source water through three serial filters made of pure bleached cotton, cellulose, and activated carbon, then through a reverse osmosis purifier consisting of an acetate micropore filter of activated carbon, a source of ultraviolet light, and finally, through an outlet provided with a sampling device. In some experiments, a millipore Twin-90 sterile 0.22 μ filter was connected to the outlet system prior to collection of purified water to remove bacterial contamination.

followed by a filter

Water Assays

Samples of water were analyzed before and after purification and, when appropriate, samples of USP water, prepared by Cutter Laboratories, Berkeley, California, were used as controls. All determinations were done at least in duplicate. Sodium and potassium (flame photometry), calcium, magnesium, chlorine, phosphorus, pH, and osmolality (freezing point) were determined by standard methods. Metal content, such as copper, iron, manganese, and zinc were measured by atomic absorption procedures. Electrical resistance and conductance were assayed with the Yellow Springs Instrument Co. Model 31 conductivity bridge instrument. Absorbing curves between 220 and 650 cm were obtained with a Cary Model 14 recording spectrophotometer. Fluorescence was measured with a Farrand ratio fluorimeter using a primary filter No. 7-37 and secondary filters No. 3-74 and 5-60. Total protein was determined by the biuret reaction procedure and total matter by partial lyophilization of 50 ml water samples and final drying of the residues in Petri dishes. Bacterial analysis was done by inoculation of water samples in fluid thioglycolate medium, M-Endo broth, brain-heart infusion broth and blood agar, followed by aerobic and anaerobic incubation at 23 and 37 C. Differential identification of bacteria was obtained by the screening procedures represented by the biochemical test designed for the "Enterotube" (5) and the "API 20 E" systems (6). The detection of pyrogenic bacterial endotoxin was carried out by the Limulus Amebocyte Lysate procedure (Bulletin, Mallinkrodt, Inc., St. Louis, Missouri).

Gas chromatographic analysis was accomplished by injecting 2.5 µl of water sample through a gas chromatograph (Perkin-Elmer Model 900B) equipped with a column 1.8 m x 0.32 cm outside diameter, packed with 3% OV-17 on chromosorb WHP, 100 to 120 mesh, and a flame ionization detector and glass injector at 250 C; column temperature was 110 C. Nitrogen carrier gas flow was set at 50 ml per minute.

RESULTS

The ion content of the water samples from sea water, fresh pond water, and human urine before and after purification is shown in Table I. The analysis of a sample of USP water is also included in this Table for comparison. Sea water with a high Na and Cl content, as expected, was purified to the extent that, with a single passage through the purification system, more than 98% of Na and more than 99% of Cl were removed. Na, K, and P, which are present in elevated amounts in human urine, were also reduced by the purification procedure to 1 to 2% of the initial value. All other ions indicated in the Table were reduced considerably independently of the source of water used. The detection limits of the methods used are indicated in Table I.

Table II shows the metal content in the water samples before and after purification. In some samples the metal content before purification was below the limit of sensitivity of the assay (Table II). However, in those instances when significant amounts were present, removal was achieved by the purification procedure, except for zinc in the urine sample where a decrease of 87.5% was observed.

Conductivity, electrical resistance, osmolality, and pH of the water samples before and after purification are shown in Table III. With a considerable decrease of ion content, as observed in Table I, a corresponding decrease in electrical conductance and parallel increase in electrical resistance were obtained as expected. The decrease in osmolality after purification also reflected the removal of osmotic materal from the water of different sources. The difference observed in the pH of pond water before and after purification may indicate loss of CO_2 dissolved in fresh pond water and/or removal of other alkaline material.

In Table IV, the spectrophotometric absorbance between 220 and 650 nm is indicative of the presence of organic or pigmented material with light absorbing characteristics in the ultraviolet or visible region. Pond water showed absorbance in the 220 to 280 nm region and urine at 280 nm before purification. After purification, the light absorbing material was removed since no absorbance was observed. Significant fluorescence was measured in sea water and to a greater extent in pond water and urine before purification. However, this fluorescence was not present in samples of purified water. No significant amounts of protein could be detected in the sea or pond

water; the protein content determined in the urine sample was absent after the process of purification. The residue remaining after evaporation of 50 ml of water from the three different sources before and after purification is shown quantitatively in Table IV and is illustrated in Figure 1 (for sea water), Figure 2 (for pond water), and Figure 3 (for human urine).

Figure 4 depicts the results of the gas chromatographic analysis of samples of sea water and urine before and after purification and of a sample of USP water. Sea water or urine, prior to purification, showed patterns indicative of the presence of several impurities, but after purification the pattern was similar to that obtained with USP water. Purified water obtained from the three different sources appeared as a clear, colorless, odorless liquid.

All the data obtained in Tables I to IV and Figures 1 to 4 are essentially the same with or without addition of the Twin-90, 0.22 μ millipore filter before the purified samples were collected. The addition of this sterile filter, however, had a remarkable effect on the bacteriological assays. The results of these tests are shown in Table V. Water obtained from the three different sources was contaminated by different bacteria with heavy contamination observed in pond water and urine. Purification without addition of the sterile filter showed little or no effect on the elimination of bacterial contamination from sea or pond water, although bacteria present in human urine were considerably reduced. However, the addition of the sterile filter before the water was collected assured absence of bacterial contamination in the purified water.

Table VI shows the results of the detection of pyrogenic bacterial endotoxin in samples of water before and after purification. The limit of sensitivity of the test is indicated by data of the positive control, represented by Escherichia coli endotoxin; this limit was reached at a concentration of 0.05 ng/ml. Based on earlier assays, a pyrogenic response was obtained in rabbits at a concentration of 0.5 ng/ml. Before purification, water from the three different sources gave a positive reaction which was present also in purified water samples obtained without addition of a sterile filter. However, all the water samples collected after filtration through the sterile filter showed a negative reaction, indicating removal of pyrogenic bacterial endotoxin. A negative reaction was observed also in the USP control water sample.

DISCUSSION

The development of portable equipment for the production of pure water from fresh pond, sea water, or other sources is important for military requirements in field situations. Also, it is necessary for many civilian activities whenever pure water is not available. The criteria for purity are influenced by the intended use of the water.

Lake water which could be considered pure for swimming may not be suitable for drinking, and municipal water found pure for drinking is not considered pure for pharmaceutical preparations or for clinical use. The American Chemical Society, the American Society for Testing Materials, and the College of American Pathologists have specified various parameters for the purity of water. However, each of these agencies has proposed different standards (7), which apply for different purposes, and therefore, do not represent a uniform guide.

The criteria established by the United States Pharmacopeia for sterile water for injection have been outlined in the introductory section of this report. According to the data obtained in our studies, the purification system used satisfies all criteria for USP grade injectable water, except one: the limits of total solids. However, the purified water was non-pyrogenic, clear, colorless, odorless, and it was sterilized without addition of antimicrobial agents or other substances. The ion, metal content, and organic material present in human urine, pond water, and sea water are reduced considerably or completely removed by a single passage through the system. Although the total solids in the purified water exceed the limit of 2 to 4 mg% established for USP water (Table IV), sodium and chlorine ions represent 96% and 64% of the total solids present in water purified from sea water and urine, respectively (Table I). However, these ions are generally added (900 mg sodium chloride per 100) in order to make isotonic saline for injection. The results in Table IV indicate that organic substances, with spectrophotometric absorption in the ultraviolet and visible regions, fluorescent compounds and protein material present in the water source are removed by the purification process.

The acetate micropore filter incorporated in the reverse osmosis purifier lasts for two years or longer if properly maintained. It is continously rinsed by water pumped into the apparatus, thus preventing the accumulation of particulate matter on the filter. The three serial filters placed before the reverse osmosis purifier and the activated carbon filter which follows remain effective for a period of time dependent on the impurities present in the water source used. Purification of sea water in a continuous operation requires replacement or regeneration after one week for the first-in-line filter and after two weeks for the other filters in order to obtain purified water of consistent high quality. It is important to emphasize that this purification system, as presently available, will not manufacture water completely sterile and free of pyrogenic bacterial endotoxins, but requires the addition of a sterile in-line filter with small pores to produce water without bacterial contamination.

It appears that the reverse osmosis process is efficient in the production of purified water and it has been utilized for the preparation of drinking water in a desalinization project (8). The system evaluated in these studies is based on the reverse osmosis

process, is portable, and can be used anywhere water is needed as long as a water supply of some source is available. It can be modified to use different power sources such as a combustion engine or even manpower. It represents a multipurpose water purification process which could be useful in many non-military applications, such as, at disaster sites where sources of water are contaminated, at construction sites, on off-shore drilling platforms, on commercial and pleasure boats, and at other locations where pure water is needed but not available.

CONCLUSIONS AND RECOMMENDATIONS

The results indicate that brackish, polluted water can be purified and that the system described can provide clear, colorless, odorless, sterile, nonpyrogenic water which appears to satisfy the criteria of USP grade water for injection. It is recommended that further evaluation be made for elimination of eventual viruses or other specific contaminants which might be present in the source of water used. Futhermore, complete pathophysiological studies on animals injected with the purified water should be accomplished.

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LEGENDS TO FIGURES

Appendix A

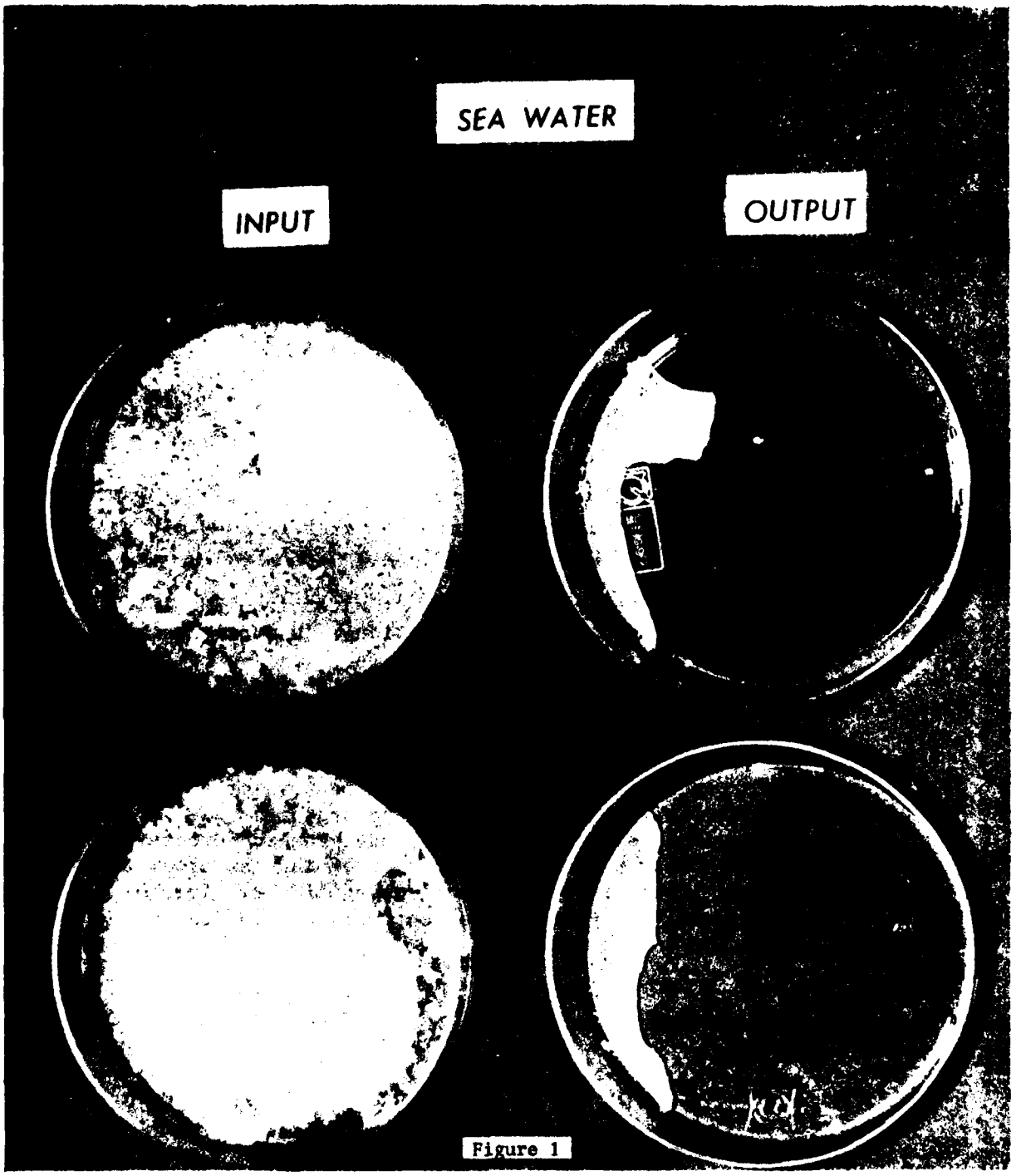

Figure 1

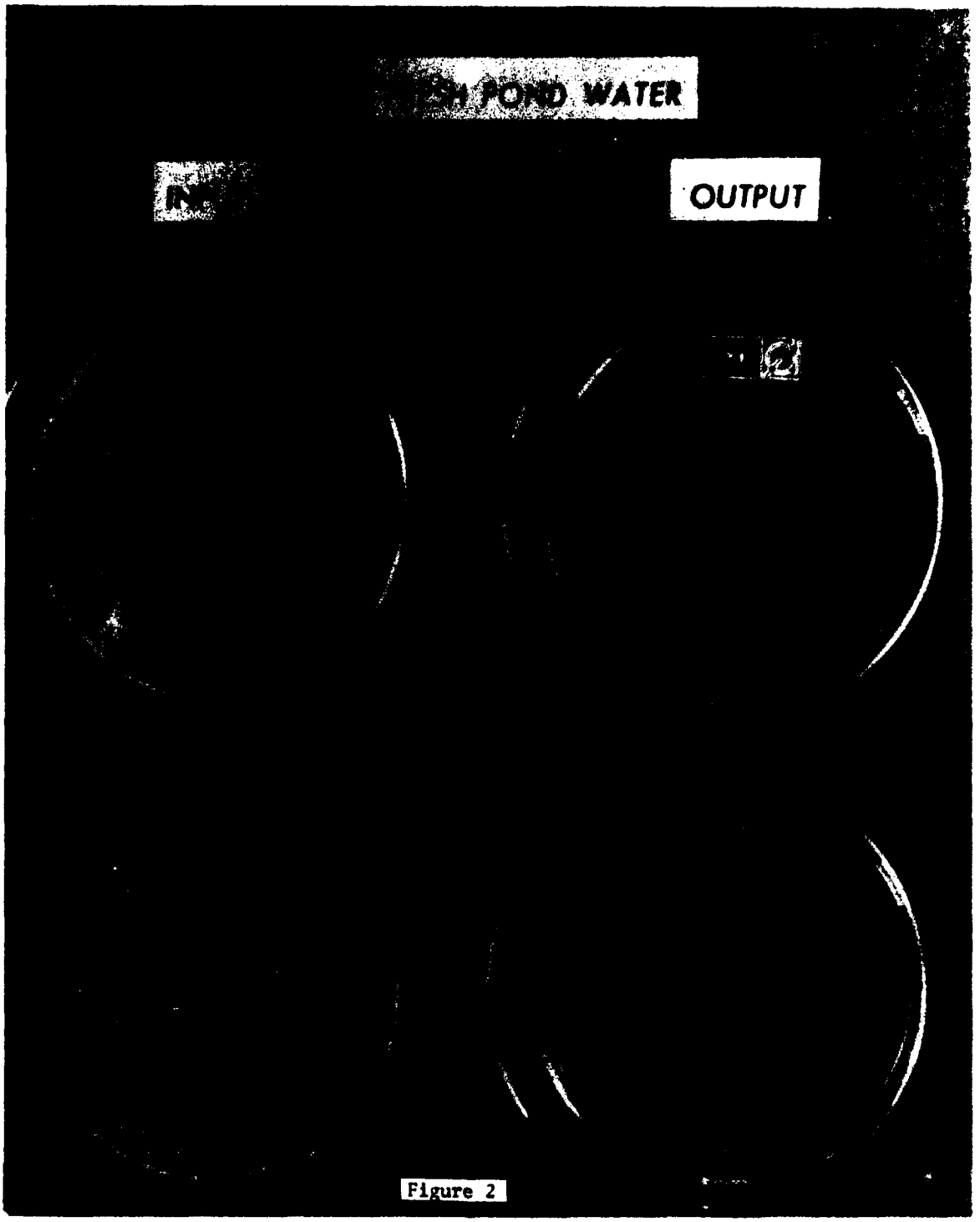

Figure 2

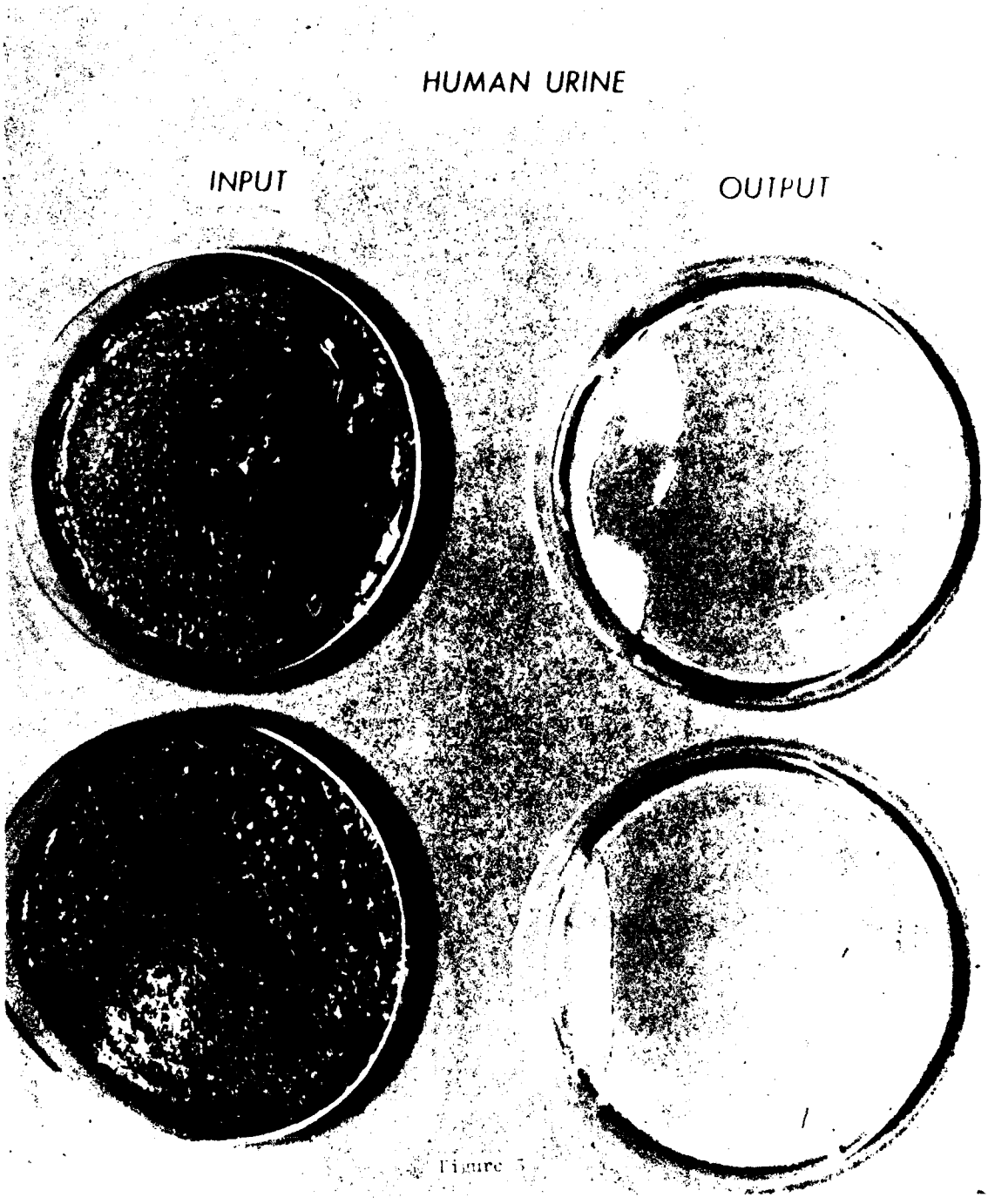

Figure 3

GAS CHROMATOGRAPHY

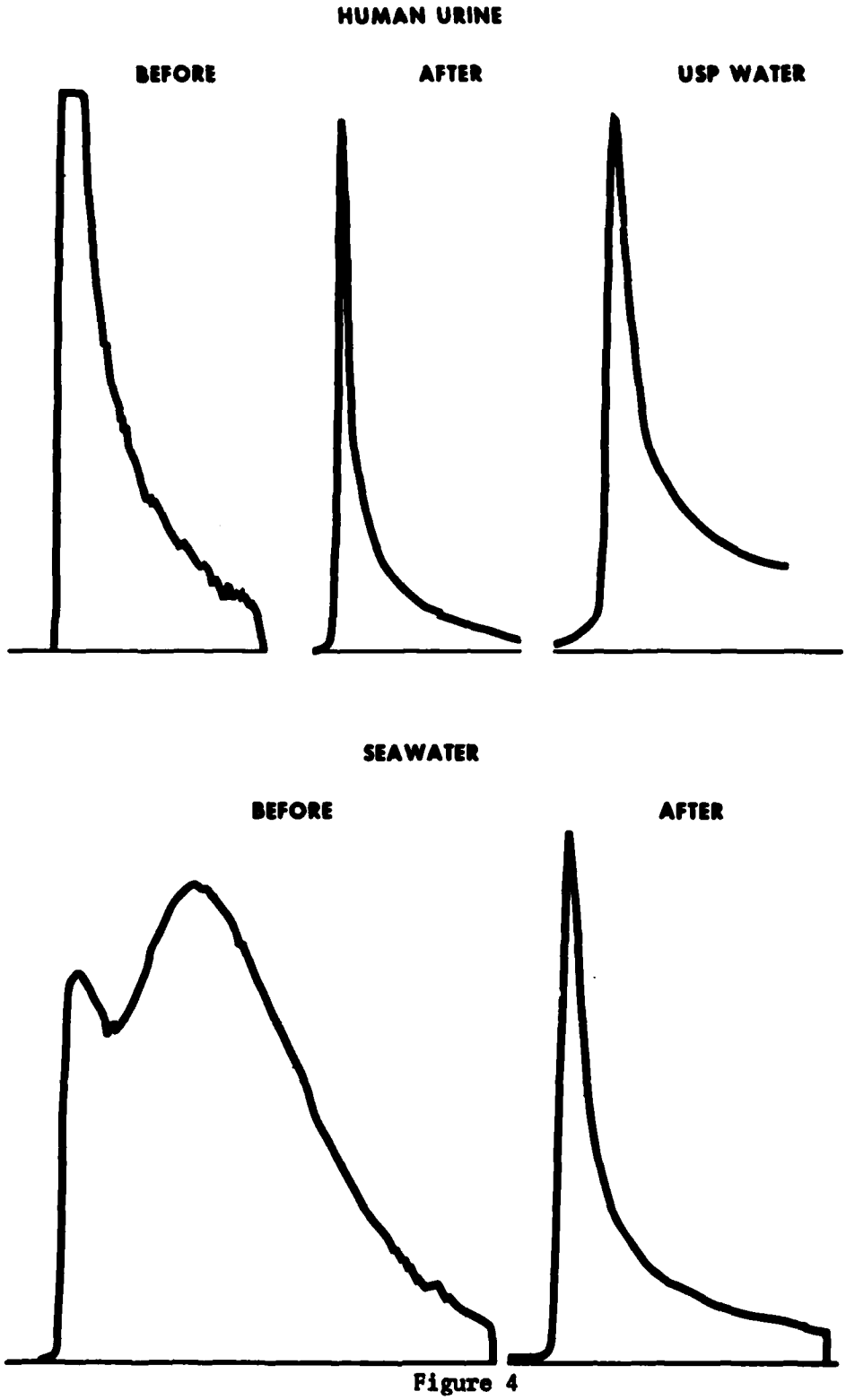

Figure 4

LEGENDS TO TABLES

Appendix B

TABLE I - ION CONTENT* BEFORE AND AFTER WATER PURIFICATION

		USP	SEA		POND		URINE	
			BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER
Na	(mg/dl)†	0.0	1,023.05	18.39	19.54	1.15	188.52	2.30
K	"	0.0	39.10	0.78	2.35	0.39	174.00	1.17
Ca	"	0.2	22.80	0.31	2.65	0.05	8.30	0.30
Mg	"	0.59	14.70	0.28	1.12	0.00	4.52	0.35
Cl	"	0.0	1,730.11	13.26	9.08	0.00	5.67	1.06
P	"	0.0	0.0	0.0	0.95	0.20	105.20	0.30

*Values of 0.0 represent levels below the limits of detection, which, in mg/dl are: 1.1 for Na, 0.08 for K, 0.9 for Cl. and 0.05 for Ca Mg, and P.

†ppm = data in table x 10

TABLE II - METAL CONTENT BEFORE AND AFTER WATER PURIFICATION

	USP	SEA		POND		URINE	
		BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER
Cu (ppm)	<0.06	<0.06	<0.06	<0.06	<0.06	0.71	<0.06
Fe "	<0.11	0.38	<0.11	<0.11	<0.11	0.34	<0.11
Mn "	<0.06	0.07	<0.06	<0.06	<0.06	<0.06	<0.06
Zn "	0.024	0.059	0.02	0.043	<0.011	0.526	0.066

TABLE III - CONDUCTIVITY, ELECTRICAL RESISTANCE, OSMOLALITY, AND pH BEFORE AND AFTER WATER PURIFICATION

	USP	SEA		POND		URINE	
		BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER
CONDUCTIVITY (μmhos)	2.8	37,000	750	1,000	29	11,000	200
ELECTRICAL RESISTANCE (ohms)	400,000	30	1,400	1,000	37,000	95	5,200
OSMOLALITY (mOsm/kg)	1	925	15	18	3	426	33
pH	7.45	7.20	7.60	9.2	6.30	6.45	6.20

TABLE IV - ABSORBANCE, FLUORESCENCE, TOTAL PROTEIN, AND TOTAL MATTER BEFORE AND AFTER WATER PURIFICATION

	USP	SEA		POND		URINE	
		BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER
ABSORBANCE (220-650nm)	0.0	0.0	0.0	220-280nm	0.0	280nm	0.0
FLUORESCENCE (IU 340/465)*	0.0	16.5	0.0	>100	0.0	>100	0.0
TOTAL PROTEIN (g/dl)	—	—	—	—	—	0.15	0.0
TOTAL MATTER (g/dl)	0.003	4.05	0.034	0.17	0.007	3.02	0.20

*Intensity units at 340 nm excitation and 465 nm emission.

TABLE V - BACTERIAL ANALYSIS. COLONY FORMING UNITS PER ML.

	BEFORE PURIFICATION	AFTER PURIFICATION	
		WITHOUT TWIN-90	WITH TWIN-90
SEA WATER	1.1*	1.2**	0.0
POND WATER	4.5×10^3***	3.1×10^3***	0.0
URINE	3.0×10^4†	1.2‡	0.0

BACTERIA IDENTIFIED

*Klebsiella pneumoniae, Enterobacter agglomerans, Serratia liquefaciens

**Enterobacter agglomerans and Serratia liquefaciens

***Proteus

†Bacillus, Escherichia coli, Pseudomonas aeruginosa, Pseudomonas fluorescans, Proteus mirabalis, Enterobacter agglomerans

‡Pseudomonas

TABLE VI - DETECTION OF PYROGENIC BACTERIAL ENDOTOXIN BEFORE AND AFTER WATER PURIFICATION

		REACTION DUPLICATE		RESULT
positive control	50.0 ng/ml	+	+	positive
(E.coli endotoxin)	0.5	+	+	positive
	0.1	+	+	positive
	0.05	±	±	positive
	0.025	-	-	negative
	0.0125	-	-	negative
	0.006	-	-	negative
Control (USP water)		-	-	negative
Sea water (before)		±	±	positive
(after, without Twin 90)		±	±	positive
(after, with Twin 90)		-	-	negative
Pond water (before)		+	+	positive
(after, without Twin 90)		±	±	positive
(after, with Twin 90)		-	-	negative
Urine (before)		+	+	positive
(after, without Twin 90)		±	±	positive
(after, with Twin 90)		-	-	negative

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