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THE EFFECTS OF PROLONGED WATER EXPOSURE ON HUMAN SKIN*

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ABSTRACT

To gain insight into the pathogenesis of warm-water immersion dermatoses, an experimental technique was developed whereby skin sites on human subjects could be continuously exposed to water for varying time periods simulating actual exposures received by affected patients. Clinical and histologic observations of test sites exposed to water for 72 to 144 hours revealed intense subacute dermatitis. Contrary to established beliefs, the induction and intensity of responses were not dependent upon increases in skin surface pH or bacterial population. Moreover, neither posthydration anhidrosis nor miliaria were found, although stratum corneum hydration and maceration were pronounced. It is proposed that either water itself or some unrecognized potentially toxic natural surface substance is responsible for the dermatitis.

The nature of certain occupations and personal responsibilities require certain individuals to be exposed to warm water for prolonged periods of time. Housewives, bartenders, farmers who work in flooded fields, certain industrial workers, and foot soldiers operating under inundated ground conditions are among those who are most often exposed.

Dermatoses associated with prolonged water exposure are not usually attributed to a direct effect of water on living skin, but to alterations in the horny layer that lead to increases in bacterial, yeast, and fungal growth, interference with sweating, a decrease in "barrier" function, and increased penetrability of potentially irritating and sensitizing chemicals and agents into the skin [1–7]. Except for instances in which a specific chemical, agent, or organism is proved responsible for a given disorder, the majority of such dermatoses are diagnosed as housewives' eczema, warm-water immersion syndrome, paddy foot, or nonspecific dermatitis.

In an attempt to elucidate the roles of water in the pathogenesis of such dermatoses, an experimental method for induction of warm-water dermatitis was developed so that controlled studies of evolutionary changes in the skin and suspected pathogenic variables could be undertaken. In this communication new experimental findings of skin changes induced by continuous warm-water exposure are presented and a relationship between the pathogenesis of the experimentally induced dermatitis and the above clinical disorders is proposed.

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MATERIALS AND METHODS

Design of a Method for Continuous Water Exposure

Cups of 5-ml capacity that covered a surface area of 3.97 cm^2 (Fig. 1) were made from clear translucent Plexiglass® (Acrylic "G" sheet and Acrylic extruding tubing, Cadillac Plastic and Chemical Co., San Francisco, California). Each cup contained a flexible plastic tube (approximately .16 guage) connected from its side for introduction and removal of water samples while the cup was affixed to the skin. This flexible tube could be bent upon itself or plugged to seal off the cup and its contents. Following ethylene oxide gas sterilization of the cups, their ends were first affixed to sterile 2 × 2-inch strips of pliable cellophane tape (Mystik Tape®, Borden Co.) using plastic rubber (Duro-Plastic Rubber®, Woodhill Chemical Co.). The rubber was allowed to dry for 24 hours and the cellophane tape covering the open end of the cup was cut away. Two coatings of a nonirritating aerosol surgical adhesive (Medical Adhesive B®, Dow-Corning) were sprayed on the skin around the test site and 30 seconds later the test cup, affixed to its cellophane tape, was placed in position. An elastic adhesive bandage tape (Elastoplast®, Duke) was used to cover and support the entire base and edges of the test site.

Method of Testing

Subjects. Thirty normal young adult male volunteers were utilized. The legs and backs served as test sites. A maximum of either three sites on each leg or 10 sites on the back could be tested simultaneously.

Water samples. These consisted of sterile water for injection, USP (containing no preservative) and sterile buffered solutions of 0.1 M N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) at pH 3.5 and pH 7.5 [8].

Preparation of subjects and application of test materials. A 14-day period during which no antibacterial soaps or cosmetics were used preceded the studies. Volunteers used only Ivory soap during this time and discontinued all bathing for 24 hours immediately prior to testing. Water cups were then affixed to the skin (Fig. 2). Four-ml sterile water samples were delivered via syringe and Millipore filters (Swinnex 25, Millipore

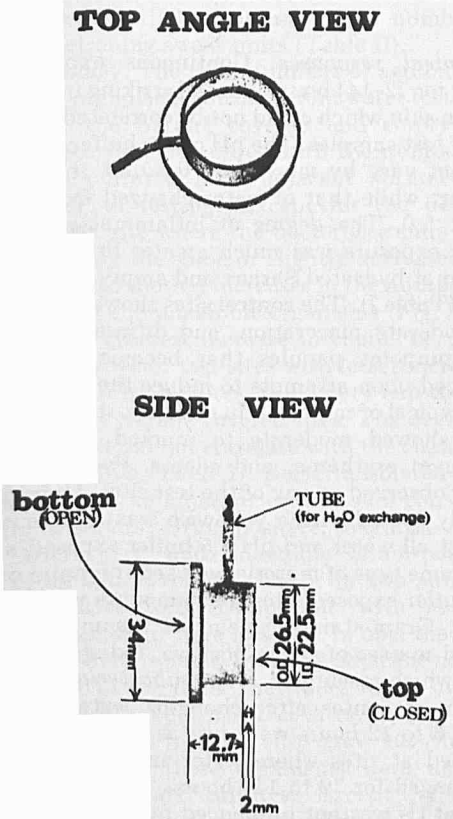

FIG. 1. Top angle and side views of water cup used to expose skin to water and buffer solutions for prolonged periods of time.

Co.) to the test cups, leaving a 1-ml air space inside the cup to allow water movement. Water samples delivered in the same manner were inoculated into culture media to detect bacterial contamination. The tube used for delivering water to each cup was bent upon itself and secured in position using tiny winding strips of adhesive tape. Test sites were randomized and each sample was tested in duplicate. One of each duplicate sample was changed every 6 to 12 hours while others remained unchanged for the duration of the test period (72–144 hr).

Controls. These consisted of an empty water test cup affixed and sealed as above and a 3.97-cm^2 site occluded with Saran® wrap and fixed to the skin in the same manner as the cup.

Temperature of water. Water temperature, which was monitored with a needle thermistor at varying times beginning 1 hour after cups had been filled, did not vary by more than 1.0° F from that of the underlying skin at any time during the experiments.

Methods of Evaluating Test Responses

Clinical. Preliminary observations for erythema were made through the transparent plastic at 12-hr intervals. Edema could not be adequately evaluated until the cups were removed. Final test responses recorded at 2 hours after removal of test materials were graded on a 5-point scale:

0 no reaction
1+ mild, diffuse or moderate, spotty erythema
2+ moderately intense, confluent erythema
3+ moderately intense erythema plus moderate edema
4+–erythema, intense edema, and vesiculation

Histologic. Biopsy specimens obtained from 72- and 144-hr test and control sites were formalin fixed, and prepared by routine hematoxylin-eosin staining for light microscopic examination.

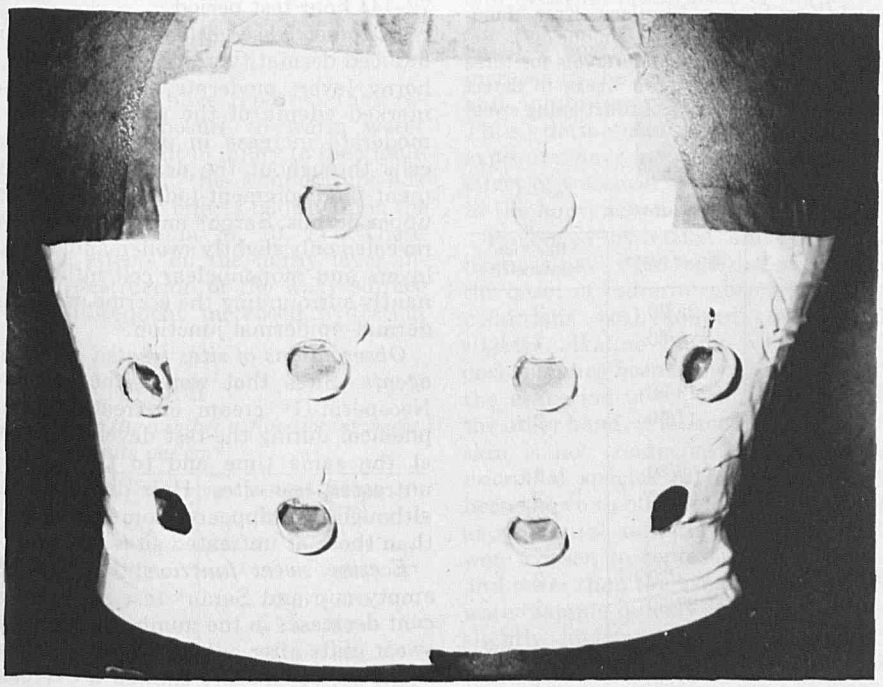

FIG. 2. The back of a volunteer with water cups, empty cup, and Saran® being tested in duplicate.

Bacterial. Baseline bacterial samples were obtained immediately prior to application of cups in 18 subjects from four different sites located between areas demarcated for testing. In one-half of these subjects cultures from control sites, test sites, and the cup water were obtained at 72 hours. The other half were cultured at 144 hours.

Samples were collected using a mechanical scraping technique (to be published) in which phosphate-buffered saline, pH 6.9, containing 0.1% Triton X-100 served as the collecting medium. Total bacteria counts were calculated from diluted samples incubated aerobically and anaerobically for 5 days on Casman agar. MacConkey agar was used to isolate Gram-negative bacteria, and Baird-Parker agar to isolate staphylococci and micrococci.

Method of Assaying the Effects of Antibacterial Agents

Ten subjects were utilized. One of each duplicate test site received 0.1 ml of syringe-delivered Neosporin-G® cream (Burroughs Wellcome & Co.) 12 hours before testing and again immediately preceding testing. The cream was spread evenly over each test site using sterile wooden applicator sticks. In 10 other subjects, crystalline chloramphenicol (Parke, Davis & Co.) was added (0.5 mg/ml) to one of each duplicate set of water samples. Observations were recorded as in the previous studies.

Method for Demonstrating Changes in Eccrine Sweating

The concentration of freely functioning eccrine sweat units was determined at each water test, control, and adjacent normal untested skin site. Within 15 minutes after removal of test materials subjects were placed in an environmental chamber at a temperature of 110° F and 50% relative humidity. Fifteen minutes later, functioning sweat units were recorded using a silicone impression material (Kerr Syringe Elasticon, Kerr Manufacturing Co., Detroit, Michigan) technique [9]. Repeated imprints were taken at 24-hr intervals for three days and at weekly intervals for two weeks to detect any delayed change in the number of functioning sweat units.

TABLE I

Test responses to hydration

Test Site	No. of Sites	Reaction Intensity
Sterile Water	25/30	3+
	5/30	2+
Buffer (pH 3.5)	19/30	3+
	11/30	2+
Buffer (pH 7.5)	6/30	4+
	20/30	3+
	4/40	2+
Empty Cup Occlusion	3/30	2+
	27/30	1+
Saran® Wrap Occlusion	6/30	2+
	24/30	1+

RESULTS

Clinical responses. Continuous exposure to water for 72–144 hours induced striking inflammation in skin which could not be correlated with the pH of test samples. The pH of the buffer solutions did not vary by more than 0.5 unit throughout testing, while that of water changed from 6.7 to about 5.0. The degree of inflammation due to water exposure was much greater than that observed at hydrated Saran® and empty cup control sites (Table I). The control sites showed only mild to moderate maceration, and diffuse inflammatory pinpoint papules that became more pronounced upon attempts to induce thermal sweating (typical of miliaria). In contrast, the water test sites showed moderate to marked maceration, confluent erythema, and edema. Pyoderma was never observed at any of the test sites. Hairs were thickly coated with a yellowish waxy material at almost all water and pH 7.5 buffer exposed sites. The same type of material was seen on hairs of pH 3.5 buffer exposed sites, but amounts were much lower. Gram-stained smears of the material revealed masses of Gram-positive, rod-shaped bacteria which resembled *Propionibacterium acnes*.

Skin responses after changing water samples every 6 to 12 hours were just as intense as those induced at sites where water samples remained unchanged for 72 to 144 hours. The induction of dermatitis was not influenced by location (leg vs back). However, the back proved to be a more suitable test area because of more available testing space and less chance of trauma to the cups. Less than 15 percent of the cups leaked during the 72–144 hour test period.

Histologic observations. Specimens from water-induced dermatitis revealed: a swollen, thickened horny layer; moderate acanthosis; moderate to marked edema of the upper dermis; a mild to moderate increase in perivascular mononuclear cells throughout the dermis; and scattered pigment and pigment-laden macrophages in the upper dermis. Saran® and empty cup control sites revealed only slightly swollen and thickened horny layers and mononuclear cell infiltrates predominantly surrounding the eccrine sweat ducts at the dermal–epidermal junction.

Observations of sites treated with antibacterial agents. Sites that were either pretreated with Neosporin-G® cream or treated with chloramphenicol during the test developed inflammation at the same time and to the same degree as untreated test sites. Hair deposits were present although they appeared somewhat less prominent than those at untreated sites.

Eccrine sweat function. Although the control empty cup and Saran® test sites showed significant decreases in the number of freely functioning sweat units after only 72 hours of occlusion, not a single water test site showed a decrease even after 144 hours of exposure. In fact, most water test

sites showed a slight increase in the number of freely functioning sweat units (Table II).

Bacteriology. The total numbers of aerobic and anaerobic organisms cultured from water test sites and from the Saran® covered and empty cup control sites were compared with the numbers of organisms cultured from adjacent normal skin areas prior to testing. Except for the pH 3.5 buffered test site where the bacterial counts were approximately the same or slightly decreased, all other test sites showed increases in the numbers of both aerobic and anaerobic organisms (Fig. 3). In general, the greatest increase in counts occurred at the sterile water test sites with lesser increases at the pH 7.5 buffer sites, the empty cup control sites, and the Saran® covered sites. The degree of inflammation did not correlate with the change in bacterial counts (Table I). Bacteria isolated from test sites and the adjacent control skin consisted mainly of aerobic, Gram-positive, coagulase-negative *Micrococci*, and anaerobic Gram-positive rods (*Propionibacterium acnes*). In two subjects Gram-negative rods (compatible with *Pseudomonas aeruginosa*) were isolated. In one, the same organisms were isolated from the adjacent normal skin, while in the other they were found only at one of his water (pH 3.5) test sites. In two other subjects, cultures from all sites grew out *Staphylococcus aureus*; these organisms were not obtained in prestudy cultures. Except for these four subjects, no suspected pathogenic organisms were found.

Samples from water cups grew out 1×10^1 to 1×10^7 organisms per cc. No particular type of sample consistently grew out more organisms than another.

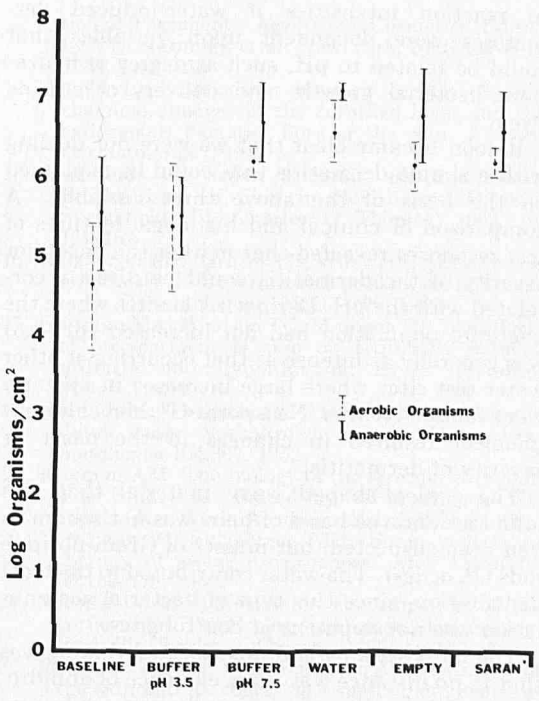

FIG. 3. Bacterial counts before testing (baseline) and after 72 hours of continuous testing. Note that except for the pH 3.5 buffer (bacteriostatic) test site where the counts remain essentially unchanged from the baseline, increases in bacteria occurring at water and control hydrated skin sites were very similar. Counts after 144 hours of testing were not significantly different from those shown.

DISCUSSION

Previous investigators have reported that repeated or prolonged exposure to warm water (85°–105° F) does not result in injury to the viable portions of skin. Water at this temperature has been said to exert adverse effects mainly upon the nonviable stratum corneum [6, 10, 11]. These proposed adverse effects on the horny layer include or lead to destruction of the waxy surface "barrier" with subsequent increased hydration and eventual maceration of the horny layer [10], an increase in permeability of skin to potentially adverse environmental substances [11–13], an increase in the growth of microorganisms [3, 14], and inhibition of eccrine sweating [4, 15–17]. Thus, dermatoses associated with warm-water exposure have not been attributed to a direct effect of water on skin, but secondary alterations in the horny layer [3, 4, 10–19].

Prolonged hydration and an increase in skin bacteria have been regarded as essential factors in the onset of miliaria rubra and other dyshidrotic conditions [20]. Indeed, exposure of skin to slightly alkaline buffer solutions under plastic occlusion has been shown to significantly enhance the induction of experimental miliaria [17]. On the other hand, it is accepted that the acidic pH of skin is not conducive to proliferation of many microbial species (21), and acidic buffers have been shown to offer some slight protection against experimental miliaria [17]. The buffer samples were chosen to represent pH's of 2 units higher and lower than the average skin pH. The distilled water sample quickly shifted to a pH close to but slightly higher than that of the normal skin surface. It was felt that these three samples represented a range sufficient to show differences

TABLE II

Effects of hydration on the number of functioning sweat units per cm²

Test Site	Percent Change from Control (Range)
H_2O for Injection, USP	+1 to +10%
Buffer at pH 3.5	+3 to +10%
Buffer at pH 7.5	−2 to +8%
Empty Cup	−30 to −80%
Saran®	−75 to −100%

in reaction intensities if water-induced dermatoses were dependent upon variables that could be related to pH, such as degree of hydration, bacterial growth, and delivery of eccrine sweat.

It soon became clear that we were not dealing with a simple dermatitis that could be explained on the basis of the above three variables. A comparison of clinical and histologic features of test responses revealed that neither the onset nor severity of the dermatitis could be directly correlated with the pH. Dermatitis at sites where the bacterial population had not increased (pH 3.5) was generally as intense as that occurring at other water test sites where large increases in bacteria were found. Neither Neosporin-G® nor chloramphenicol resulted in changes in the onset or severity of dermatitis.

The conical-shaped waxy material that was found around the bases of hairs was not sebum as had been suspected, but masses of Gram-positive rods (*P. acnes*). The water contributed to the focal distribution, since this type of bacterial accumulation was not apparent at control sites.

The most striking and unsuspected finding was that in no instance was there evidence of anhidrosis or miliaria at water-exposed sites, while profound anhidrosis and clinical miliaria rubra developed in every Saran® occluded and empty cup hydrated control site. This finding is unusual since anhidrosis is known to develop rapidly in hydrated, thickly keratinized areas (palms and soles) [22, 23], and since the three major requirements for induction of anhidrosis and miliaria on glabrous skin were maximized (prolonged marked hydration under warm conditions, an increase in bacterial population, and an increase in pH). These extremes in sweat activity seen at sites after prolonged water hydration on the one hand and hydration by Saran® and empty cup on the other indicate that factors other than simple hydration, maceration, and warmth account for the miliaria that develops under Saran® and empty cup occlusion. This difference is probably not due to variations in skin surface salt concentration since previous studies have shown that saturated or highly concentrated sodium chloride solutions, as might occur under Saran® or an empty cup, actually prevent anhidrosis [22, 23]. The difference is more probably due to some other surface substance such as urea, to an alteration in the distribution of bacteria, to an accumulation of debris, lipids, and sebum, or to the leaching-out of water-binding substances in the horny layer.

The horny layers at our water test sites were so macerated after 72 hours that only moderate lateral friction was needed to give the appearance of abraded skin as contrasted with the more compact and less macerated horny layer of control Saran® and empty cup sites. This observation suggests that the more severe confluent inflammation occurring at a water test site could be due to penetration of potentially irritating, naturally occurring substances into the skin from its surface. One is reminded of the dictum of Kligman that "almost any substance can be an irritant under some circumstances" [24]. This may be the most suitable explanation of our water-induced reactions. However, when surface debris and superficial stratum corneum were removed by 8 to 12 cellophane tape strippings, the inflammatory-inducing potentiality of water was not altered.

Another possible mechanism exists. Water is cytotoxic to cells in tissue culture [25] as well as to eroded skin in vivo [26], and it causes inflammation when injected intradermally. Perhaps no damage of this type was observed in previous studies because of the relatively short, interrupted periods of immersion employed. Indeed, damage to both epidermis and horny layers may have been reversed between exposures. Prolonged exposure in this study presented a constant stress that could result in cumulative damage to the horny layer and loss of its barrier function. This state could allow enough water to enter the viable skin to exert cytotoxic effects. An analogy may be drawn with the clinical disorder called "paddy foot" seen in rice paddy farmers and soldiers who remain in warm inundated areas for long periods of time [7, †]. This acute inflammatory dermatitis which affects the dorsa of the feet and lower legs occurs within 72 hours following continuous immersion, but can be prevented if the soldier allows his extremities to dry out for several hours every day. Apparently the drying period allows epidermal repair and/or protection to be restored in the horny layer, while continuous immersion leads to cumulative damage and inflammation similar to that seen in this study.

Although an exact mechanism cannot be offered to explain the water-induced inflammation in this experiment, the histologic and clinical features are typical of a subacute contact dermatitis. The results tend to implicate water alone as a direct pathogenetic factor and do not implicate the usually suspected factors of elevated pH with increased growth of cutaneous microorganisms, simple horny layer hydration and increased penetrability to potential irritants, and interference with eccrine sweating that might lead to miliaria. Undoubtedly, these and other external factors such as friction contribute to water-induced clinical dermatitis although water itself may be considered the most important direct factor.

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† Akers WA: Paddy foot: a warm water immersion foot syndrome variant. (Unpublished)

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