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THE RELATIONSHIP OF 'PSEUDOMONAS AERUGINOSA' TO OTITIS EXTERNA AND ATYPICAL PNEUMONIA IN DIVERS AND SWIMMERS

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The Relationship of Pseudomonas aeruginosa to Otitis Externa and Atypical Pneumonia in Divers and Swimmers

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13. ABSTRACT

1. Clinical otitis externa, swimmers ear, has a multiplex etiology predisposed by water, trauma or an inbalance in the microbial flora of the ear canal.

2. The most common microbial pathogen causing swimmers ear is Pseudomonas aeruginosa, although other Gram negative bacteria may produce this disease.

3. Swimmer's ear is preventable through proper application of silicone. (Dow corning 360 medical fluid, 350 cstk.)

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- INTRODUCTION -

Diving personnel of the United States Navy have been concerned with an occupational condition commonly known as acute otitis externa, or "swimmers ear." The continuing occurance of this disease led to the submission of a research proposal in 1969. It was the objective of the work proposed to do the following:

1. Determine the nature of the microbial flora responsible for the disease.
2. Establish a laboratory model, useful in study of otitis externa.
3. Determine what environmental or occupational characteristics predisposed the infection.
4. Define the pathology associated with disease initiation and continuation.
5. Development of a prophylactic regimine useful in the prevention of "swimmers ear."

Much of the work conducted in an effort to meet the objectives of this proposal has been reported in the open literature or as technical reports submitted in accordance with the awarded contract. However, a synopsis of this work is given under the heading, "Research Completed."

- RESEARCH COMPLETED -

With the cooperation of the officers and men of the Navy Experimental Diving Unit, the Brigham Young University swimming team and other non-swimming personnel, an extended study of the microflora of the ear canals of these individuals was completed. These data showed a direct correlation between the degree of exposure of the ears to water and the type of bacterial flora resident in the ear canal. Disease was associated with any factor which resulted in a disturbance of the normal Gram positive bacterial flora yielding to an overgrowth of Gram negative microorganisms. Technical Report 136-853-2 contains the details of these findings. Pseudomonas aeruginosa was the organism most frequently associated with otitis externa. Recovery from clinical otitis resulted when either of two events occured: 1) a return to normal bacterial flora or 2) removal of all microbial forms from the ear canal.

Efforts made to develop a laboratory model resulted in a technique which successfully adapted the Guinea pig to this study. (Technical report 136-853-1) Although other animal species were studied, the Guinea pig developed infection analogous to that in man. The clinical appearance was similar to that seen in divers and prolonged water exposure resulted in a similar shift in microbial flora.

Use of the Guinea pig model, and observations made on swimmers demonstrated the disease predisposing effects of water, trauma or removal of normal lipid (cerumen) from the ear canal. These observations, coupled with the shift in microbial flora supported the concept of a multiplex etiology for otitis externa.

Definition of the pathology associated with disease initiation is not yet complete. This work has not been reported in detail but will be published upon completion. The study has been conducted in the Guinea pig and histological section of the meatal tissues from both normal and infected animals have been prepared for study.

Efforts to develop a prophylactic regimine for prevention of external otitis have resulted in the recommended use of a silicone agent sold by Dow-Corning. No technical report has been submitted covering this work. The technical details of these studies are submitted as appendix A to this report. This data has been submitted for publication.

Additional studies on the organism Pseudomonas aeruginosa, designed to facilitate our completion of the proposed objectives have resulted in the publication of three masters degree theses. The contents of these studies have not been submitted as technical reports as they are ancillary to the defined project.

- CONCLUSIONS -

1. Clinical otitis externa, swimmers ear, has a multiplex etiology predisposed by water, trauma, or an inbalance in the microbial flora of the ear canal.
2. The most common microbial pathogen causing swimmers ear is Pseudomonas aeruginosa, although other Gram negative bacteria may produce this disease.
3. Swimmers ear is preventable through proper application of silicone. (Dow-Corning 360 medical fluid, 350 cstk.)

- ACCOMPLISHMENTS -

Each of the objectives contained in the original proposal have been met. The most significant practical result of the work has been the development of the prophylactic procedure which can be used to prevent otitis externa.

INDEX OF TECHNICAL REPORTS AND PUBLICATIONS

Technical Report: 136-853-1 An Experimental Model for the Study of Infectious Otitis Externa. June 1971.

Technical Report: 136-853-2 Effect of Water on the Bacterial Flora of Swimmers Ears. September 1972.

Publications:

Wright, D. N. and M. Dineen. 1972. A Model for the Study of Infectious Otitis Externa. Arch. Otol. 95:243-247.

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Seaman, J. P. Fr. 1972. Application of a Multipoint Inoculator System to Proficiency Testing in the Clinical Microbiology Laboratory. A Thesis.

Wong, G. 1973. Ultrastructural Changes in Pseudomonas aeruginosa following Phagocytosis by Mouse Peritoneal Macrophages. A Thesis.

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APPENDIX A

Prophylaxis of Predisposed Otitis Externa

INTRODUCTION

Diving personnel of the United States Navy have been concerned with an occupational condition commonly known as acute otitis externa, or "swimmers ear."[1,2]

A number of preparations have been recommended for prophylactic use in preventing acute otitis externa.[1,3,4] However, most of these recommended compounds are relatively ineffective under severe environmental conditions, and are frequently most useful as therapeutic rather than prophylactic preparations.[2,5] The purpose of this paper is to report the effectiveness of several trial prophylactic compounds chosen to counteract the predisposing action of water in otitis externa.[1,5] Such compounds may also lessen the environmental challenge faced by individuals whose residence or occupation make acute external otitis a relatively common disease. Also presented is supplementary data relating to the prophylactic effectiveness of two established commercial prophylactic otic solutions.

MATERIALS AND METHODS

Data presented by Wright and Dineen suggests that exposing the external meatus of the guinea pig (Cavis porcellus) to sterile physiological saline (PSS) or distilled water predisposed the ear canal to a symptomatic acute otitis externa with a clinical appearance similar to that seen in human divers or swimmers under natural conditions.[2] Therefore, this animal was used as a model for the studies reported here. The animals were kept in laboratory holding pens and were observed weekly for signs of general well-being. The guinea pig ear canals were regularly examined with an otoscope for two weeks prior to use in experimental procedures. Only animals which were apparently disease free were used in the study.

Six prophylactic preparations, selected from a total of eleven potentially useful compounds, were used in this study. The eleven compounds which were screened were similar in that they all possessed water repellent or antimicrobial characteristics. Those compounds screened and not used further were Fluo-Kem teflon (Dupont), Silicote (Silicote Corp.), Vaseline, and two commercial deodorant preparations. Data from studies of the six compounds selected (after screening) for additional investigation are reported in this paper. These six materials include the following: (1) artificial ear wax which has previously been used in human studies and is described by Senturia[2], (2) 0.015% sodium acetate in propylene glycol, and the following four silicone preparations supplied by Dow Corning Midland, Mich.) (1) 360 Medical Fluid, 350 cstk; (2) X7-2015 aerosol containing 6% silicone, 7% isopropyl alcohol, freon and fluorocarbon propellants; (3) MDX-4-4219, a silicone based antimicrobial ointment containing a 1% cationic silane as an active agent and, (4) the same ointment without the

antimicrobial silane. Additionally, two commercially available prophylactic therapeutic otic solutions were evaluated as to their effectiveness in limiting the occurrence of otitis in the guinea pig model. These compounds were Vōl Sol Otic solution (Wampole Laboratories, Stanford) and Domeboro Otic solution (Dome Laboratories, New York). Six guinea pigs were used in testing each prophylactic agent and both ears were used on each animal. Two control ear canals were chosen randomly among the six giunea pigs assigned to each study group, providing a total of 12 such controls.

The guinea pigs were anesthetized with ether to the level of surgical anesthesia in order to decrease the danger of mechanical injury to the ear canals during otoscopic examination, application of prophylactics, and insertion of cotton-tipped swabs.

The epithelial surface of the external ear canal was coated with the trial compounds using a procedure dictated by their chemical compositions. Volatile liquids were applied utilizing a DeVilbiss #40 nebulizer and emollient compounds were applied by introducing the material on a sterile cotton-tipped swab. Both the cartilaginous and bony portions of the ear canal were coated with the test compounds.

Following application of the prophylactic compounds, the ear canals were examined with an otoscope each day for 9 days and observed as to their clinical appearance. Each prophylactic was applied to the ear canals only for the first two days of the experiment. Then, on the third day, sterile physiological saline (PSS) was carefully placed in each ear canal and the auricle was taped down securely to prevent inadvertent removal of the saline. This procedure was repeated for five consecutive days, or until the development of clinical otitis was evident, at which time the experiments on the infected ears were terminated. Criteria used to indicate acute otitis externa were the same as those used by Wright and Dineen.[2]

Two groups of animal controls were maintained throughout the experiments; one without the test compounds in which PSS was placed in the ear canals of one guinea pig chosen at random from the study group, and the other consisting of two selected guinea pigs (four ear canals) in which the trial prophylactic agents were introduced into the ear canals every even-numbered day during the nine day test period.

Bacterial samples were taken on days one, three, five, seven, and nine. They were obtained by using the procedures described by Wright and Dineen.[2] Dilutions (1:1000) of the bacterial samples were placed on appropriate diagnostic bacteriological media for partial identification and semi-quantitation of the microbial flora. Biochemical analysis was employed to aid in the identification of bacteria to the genus level. Numerical data is reported on a semi-quantitative basis because of the difficulty in collecting uniform bacterial samples from the ear canals.

An experiment was performed to investigate previous published assertions[2] that water alone could predispose guinea pig ear canals to acute otitis externa within a period of 48 hours. Anesthesia, application of PSS, and bacteriological sampling were all done in the same manner as previously stated.

Additional experiments were performed on two commercially available compounds, Vōl Sol Otic solution and Domeboro Otic solution. Three guinea pigs were utilized along with one control for each compound. The procedures used were identical to those stated earlier in this section, except that bacterial counts were not employed and the period of exposure was reduced to 8 days.

Figure 1 shows the change in the bacterial flora of guinea pig ear canals during exposure to water for eight consecutive days. The ears remained normal for the first two days with classical signs of inflammation commencing on the third day. Inflammation continued to increase in severity until the experiments were terminated. The significance of this data is two-fold. First, it supports previous reports that water is indeed a consistent factor involved in the predisposition of acute otitis externa[1,2,3,6,7] and second, it reveals a profound shift in bacterial flora of the ear canals from a predominately gram positive to a gram negative population. It was observed that the onset of initial signs of inflammation correlated well with the point in time when the two bacterial populations approached and intersected as shown in Figure 1.

The results of the preliminary studies on all the compounds tested showed that only six had a positive prophylactic effect. Those agents which were ineffective were discarded and not examined further. The six remaining compounds, Dow Corning 360 Medical Fluid, artificial ear wax,[1] 0.015% sodium acetate in propylene glycol, aerosol compound X7-2015, and the two ointments were subjected to the further studies which are reported here.

The data in Table 1 shows the results of study done to examine the possibility that the trial prophylactic compounds alone contributed to acute otitis externa. The data indicates that three compounds (0.015% sodium acetate in propylene glycol and those containing the cationic agent and the ointment base) appear to cause some erythema after seven days of use during which four applications of the compounds were made. None of the

compounds, when used alone, produced a significant change in the bacterial flora of the ear although limited change is notable by the seventh day.

The data presented in figure 2 are representative of the effectiveness of the six compounds in limiting the predisposing action of water on guinea pig ear canals as a function of the microbial population in the ear.

Figure 2A shows the approximate bacterial counts of the nine genera of bacteria, grouped by Gram stain characteristics, isolated from ten ear canals treated with artificial ear wax[1] and subsequently challenged with water. It should be noted that, following water challenge (day 3), there was a 10-fold increase in the gram negative bacterial population and a like number decrease in the gram positive populations. Each of the bacterial genera are not independently represented on figure 2 because of the graphic confusion which results. However, tabular data showed that bacteria of the genera Mima, Neisseria, Escherichia, Flavobacterium, and Pseudomonas each behaved in a manner similar to that represented by the gram negative curves in figure 2, and bacteria of the genera Bacillus, Staphylococcus, Streptococcus, and Corynebacteria correlated with the gram positive curves.

Careful clinical observations during each challenge experiment were made and showed excellent correlation in the development of clinical signs of inflammation with the change in microbial flora. In all cases, inflammation was first observed within at least 12 hours following that day when the major gram positive and gram negative bacterial populations approached each other and intersected.

The data (in figure 2) for each of the other prophylactic compounds may be analyzed in a similar fashion as that for the artificial ear wax. The use of propylene glycol + 0.015% sodium acetate (figure 2B) or aerosol X7-2015 (figure 2C) resulted in data very similar to that obtained when

artificial ear wax was used. Inflammation was commonly observed on about the sixth day of the experiment (four days post water challenge). Use of the two ointment compounds (MDX-4-4219) with silane (figure 2D) and MDX-4-4219 without silane (figure 2E) were comparable, suggesting that the cationic silane had no additive prophylactic effect on the ear canal tissue. These two sets of experiments were terminated after seven days because of the severity of the infection which developed in the animals.

Figure 2F presents data which suggests that the shift in bacterial flora as seen when water alone or other prophylactic agents are used does not occur when Dow Corning 360 fluid is used as the protective agent. Instead, this agent appears to provide a stabilizing effect in the ear canal resulting in a significant deterrent to inflammation when subjected to water challenge. The clinical picture observed during the use of 360 fluid is characterized by an important delay in the development of otitis of approximately 6-7 days beyond that seen in the water challenge and 3-4 days beyond that seen in reference to the other trial prophylactic compounds. Upon termination of the experiments, 60% of the ears treated with 360 medical fluid remained normal.

The clinical picture presented after Vōl Sol Otic application followed by water challenge for an 8-day period was favorable in that inflammatory events were curtailed up to and including the fifth day of exposure to PSS. The clinical picture of Domeboro Otic solution, tested in a similar fashion as was Vōl Sol Otic solution, did not appear as encouraging in that the development of otitis externa became evident in the third day of exposure to PSS in 50% of the ears tested.

COMMENT

The data presented in this investigation lend support to the hypothesis that water is indeed one factor which may act to predispose the external meatus to acute otitis externa. Sataloff and Zapp[8] have observed that the delicate skin of the ear canal increases the susceptibility of this area to irritation when exposed to moisture and warmth. Therefore, the most common cause of untraumatized cases of external otitis is prolonged exposure of this area to water as commonly occurs during swimming and showering.[8] Diving, both recreational and occupational, can also be added as a common means of exposure.[5,9]

The goal of this study was to initiate serious efforts towards prophylaxis of acute otitis external via the application of a water repellent substance to the epithelial surface of the external meatus in hopes of curtailing the sequence of events leading to external otitis.

The data presented in this paper appears to indicate strongly that Dow Corning 360 Medical Fluid provides a good water repellent along with the characteristic of good coverage and adhesion to the epithelial surface. It should be noted that silicone 360 fluid extended the time significantly over that of the water controls before signs of inflammation ensued, and was significantly more effective than the other compounds tested. There was no apparent build up of this material in the ear canal even with repeated use and the compound caused no apparent irritation to the epithelial surface (Table 1). At least one clinical otology service prescribes 360 fluid for swimming and diving patients. Results of this work have suggested that fewer than 5% of the patients have recurring symptoms.[10]

The fact that the curves in figure 2F do not intersect appears to be

significant in that this was the only compound which provided "good" protection to the water challenge. Wright and Alexander[5] have stated that the shift from a Gram positive to a Gram negative bacterial flora is of primary importance in the development of otitis externa. The ability of Dow Corning 360 fluid to prevent this shift in microbial populations may account for its effective prophylactic action. This data also suggests that monitoring the bacterial flora of the external ear canal may provide a means for following the health of the canal in individuals who have occupational or environmental edispositions to the disease.

The five remaining compounds tested did not appear to be as effective as 360 fluid but did provide some prophylactic activity when compared to the water challenge data (figures 1 and 2). The initial selection of these compounds for study was primarily based on this extension of resistance.

The use of artificial ear wax in this study was prompted by reports that the loss of cerumen may be a major contributing factor to swimmers ear[1,5,11,12] and that a positive human trial had been made.[1] In our study the ear wax preparation seemed to be non-irritating but did not prevent microbial change and infection in the ear. The two ointment preparations provoked an immediate change in the microbial ear flora. Even though the ointments contained emollient base and silicone, which should have provided some protection, the infections in these ears were severe and often fatal.

The role of Vōl Sol Otic solution as a prophylactic agent was partially substantiated by these studies although the caution regarding external challenge[12] should be considered in its use. Domeboro Otic solution was not effective as a prophylactic agent under the condition reported here.

It should be noted that the application of all test substances was made for only two days prior to PSS challenge and that it was not extended periodically through the challenge period. Therefore, it can be implied

that if such extensions were part of a therapeutic or prophylactic procedure, more favorable results might be expected.

Based on the observations made during this investigation and work by others[3,5,8,12,13,14] it is suggested that direct, prolonged exposure of the epithelial surface of the external meatus to water results in uptake of water by the cells and in the interstitial spaces of the stratum corneum. This in turn may lead to an overall disruption of surface integrity such that the mechanical or physical adhesion of cerumen to the epithelial surface is lost. The removal of cerumen from the external meatus would provide conditions favorable for the initiation of infection by bacteria as the primary etiological agents of acute otitis externa.

This assumption receives partial support from observations made by Yassin, Mostafa and Moawad[11] that in cases of external otitis of bacterial origin, cerumen almost disappears from the ear due to mechanical washing by the discharge itself and the consumption of its nutritional constituents by microorganisms. It could also be added that mechanical washings by water itself can cause similar effects.[5]

Photographs of biopsies of the skin of the external meatus exposed to humid environment showing the dilated apocrine acini lined by flattened cells demonstrated by Seuturia and Liebmann[12] also lend support to this assumption. Further research in this area is being conducted by the authors.

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TABLE 1. BACTERIAL FLORA AND CLINICAL APPEARANCE OF GUINEA PIG EAR CANALS EXPOSED TO SIX PROPHYLACTIC COMPOUNDS IN THE ABSENCE OF WATER

		Day 1		Day 3		Day 5		Day 7		Day 9	
		G+*	G-	G+	G-	G+	G-	G+	G-	G+	G-
Artificial Ear Wax	Flora	+3**	-	+4	-	+4	+1	+4	+1	+4	+1
	Appearance	Normal		Normal		Normal		Normal		Normal	
Propylene glycol with 0.015% sodium acetate	Flora	+3	-	+4	-	+3	-	+4	-	+4	-
	Appearance	Normal		Normal		Normal		Normal		Erythema	
Dow Corning 360 fluid	Flora	+3	+1	+4	+1	+4	+1	+4	+2	+4	+2
	Appearance	Normal		Normal		Normal		Normal		Normal	
Dow Corning X7-2015	Flora	+4	-	+4	-	+3	-	+3	+2	+3	+2
	Appearance	Normal		Normal		Normal		Erythema		Erythema	
Dow Corning MDX-4-4219 with silane	Flora	+3	-	+3	-	+2	+1	+3	+1	+3	+1
	Appearance	Normal		Normal		Normal		Erythema		Erythema	
Dow Corning MDX-4-4219 without silane	Flora	+3	-	+4	-	+3	-	+3	+2	+3	+2
	Appearance	Normal		Normal		Normal		Erythema		Erythema	

*G+ = Gram positive bacteria, G- = Gram negative bacteria

**Semi-quantitation of bacteria based on a scale of 1 to 4 with 4 a maximum figure.

A-12

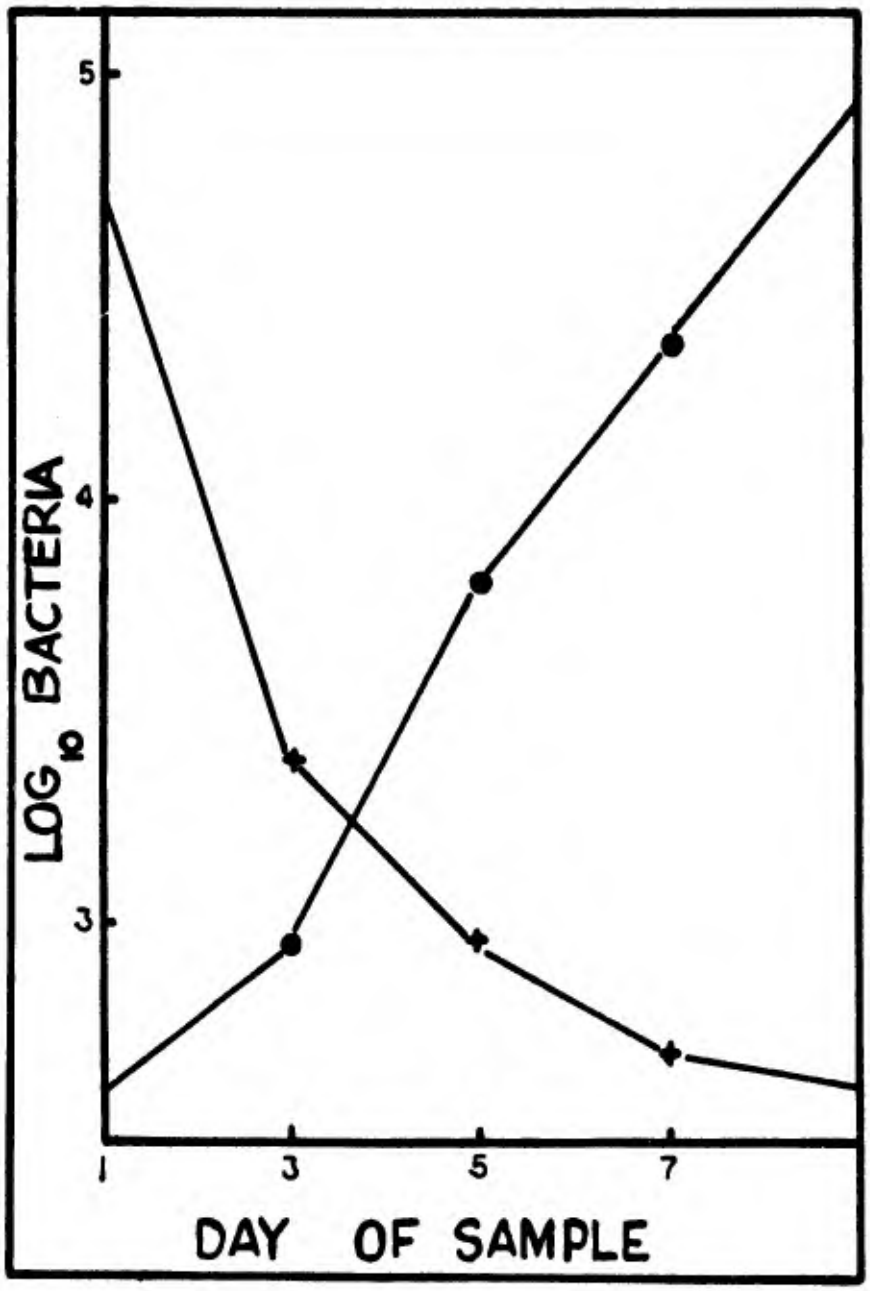

Figure I

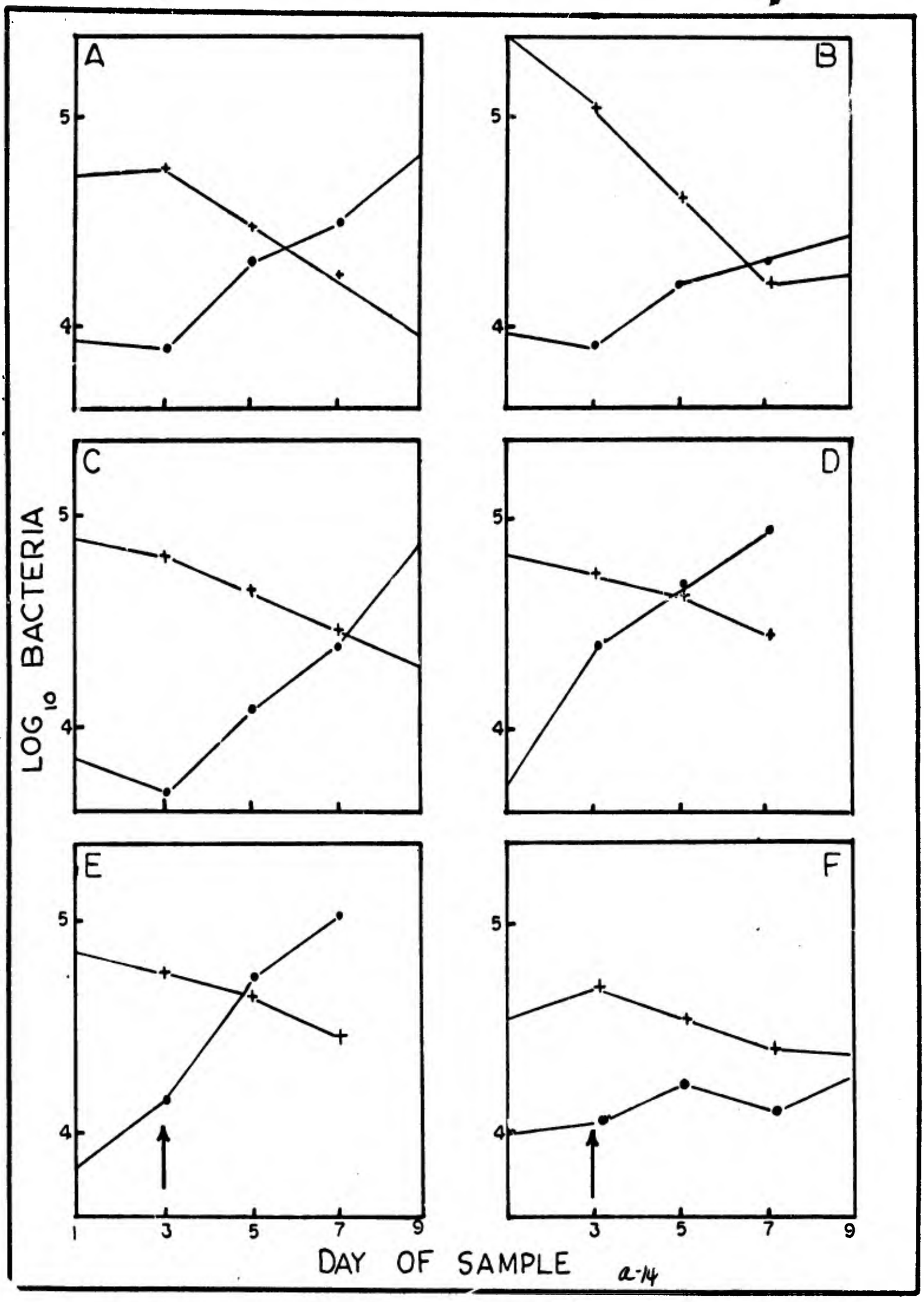

Figure 2

LEGENDS

Figure 1:

Bacterial flora of guinea pig ear canals during exposure to water for eight consecutive days. +——+ gram positive flora, •——• gram negative flora.

Figure 2:

Change, during water challenge, of bacterial flora in guinea pig ear canals protected by prophylactic compounds: A) artificial ear wax,[1] B) 0.015% sodium acetate in propylene glycol, C) Aerosol X7-2015, D) MDX-4-4219 with silane, E) MDX-4-4219, F) Dow Corning 360 Medical Fluid. +——+ represents gram positive bacteria, ●——● represents gram negative bacteria, arrows indicate first day of water challenge.