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FUNGOUS AND BACTERIAL SKIN INFECTIONS IN THE TROPICS (U)

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ANNUAL PROGRESS REPORT

by

David Taplin

January 1976

(For the period 1 June 1974 to 31 Dec. 1975)

Supported by

U.S. ARMY MEDICAL RESEARCH & DEVELOPMENT COMMAND
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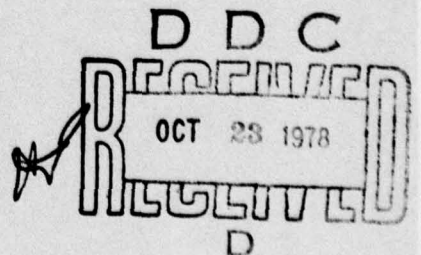
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field epidemiology teams	Costa Rica																	
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) <p>A bar soap containing Trichlorocarbanilide and Trifluorocarbanilide demonstrated no value in the prevention of common skin infections in a double blind study against plain soap bar, when used daily under supervision for two months. Lack of efficacy against <u>Streptococcus pyogenes</u> was confirmed by <u>in vitro</u> testing.</p> <p>Field studies in Costa Rica showed that most skin infections were of streptococcal etiology. Children below 10 years of age were most at risk, and children of all ages were at significantly higher risk below 300 M altitude.</p>																		

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20. Abstract (continued)

Sixty-eight (68%) of Staphylococcus aureus recovered from lesions were resistant to penicillin even in remote rural areas where penicillin was not available. Dermatophytosis and Tinea versicolor were confined to postpubertal age groups.

The hazards of bacterial and parasitic skin infections of military importance can be more accurately predicted by surveys of native children than native adults. Anthrophilic dermatophyte infections should be studied in postpubertal populations, but inflammatory zoophilic infections are best studied in children.

A mobile unit was shown to be a most effective way of conducting field epidemiology and research in rural populations. The unit also served well as a mobile clinical diagnostic laboratory, and in addition provided comfortable sleeping quarters, washing facilities and safe food and water for the field teams.

Populations suitable for further work in prevention and evaluation of current therapeutic agents have been identified, but the programme is suspended, pending approval and funding from USAMRDC.

The diagnostic method to enable untrained personnel to recover and identify Staphylococcus aureus from skin lesions has been developed. The method is 94% accurate without microbiological expertise. Additional simple instruction increases accuracy to 99%.

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INTRODUCTION

Since 1967, when it became clear that skin infections among US troops in Vietnam were a very significant cause of lost duty, we have pursued investigations along three major lines, following the advice of in service monitors at LAIR and our contract officers who were in a better position to evaluate military needs and ways in which our particular capabilities could be used to the best advantage.

- 1) The relationship of natural environments and occupation to the etiology, prevalence and severity of bacterial and fungal infections of the skin in order to predict hazards on a geographic basis.
- 2) Training and deployment of field epidemiology teams in the tropics to accurately delineate relative causes of disability, define priorities and evaluate new diagnostic field methods.
- 3) Development of mobile capabilities and new diagnostics for use by field teams and unskilled personnel.

In short, we hoped to provide the USA Medical R & D Command with a blueprint of what infectious diseases of the skin could be expected under a given set of climatic and occupational stresses, and to pin-point needs which required more research emphasis, or in which field epidemiology had uncovered new questions which might better be answered in the laboratory.

Throughout this period we have worked closely with scientists at the Walter Reed Army Institute of Research, and in later years with the Letterman Army Institute of Research. Much of the current research supported by USA Medical R & D, both within the service and in extramural contracts has evolved from the field studies conducted under this contract in collaboration with our in-service colleagues. We have largely confined our efforts to establishing the etiology of disease and the relative priorities in terms of military disability or more precisely man days lost, and have left the more basic laboratory studies to those more capable than ourselves. In any event, we felt that we could not tackle the entire spectrum of researches alone, although in the last two years we have incorporated several projects aimed at prevention or therapy, because we were approaching the end of the useful cost effectiveness of further point prevalence studies.

Summary of Projects Since 1967

- 1) Survey in Vietnam in 1967 to define the extent of skin infection and effect on combat effectiveness.
- 2) Training, deployment and assistance to WRAIR/LAIR team in Vietnam 1968-1969, to establish the epidemiology and generate scientific data relating to disability.
- 3) Development of Dermatophyte Test Medium as a field diagnostic tool for dermatophytes.
- 4) Development of field diagnostic methods for streptococcal infections.
- 5) Development of field diagnostic methods for staphylococcal infections.
- 6) Evaluation of hazards, development of diagnostic methods and animal models for Gram-negative wound infections in wet terrain, and in hospitalized burned patients.
- 7) Identification of new sources and new species of Gram-negative bacteria capable of infecting injured patients.
- 8) Evaluation of antibacterial soaps in the prevention of streptococcal and staphylococcal infections.
- 9) Evaluation of clotrimazole lotion and nystatin/tolnaftate cream for the treatment of superficial fungal infections.
- 10) Field survey of climatic and occupational stresses in populations at risk in Venezuela and Colombia and Costa Rica.
- 11) Training and logistical support of military field teams in the United States, Panama, and Colombia.
- 12) Design and testing of a multipurpose field unit capable of field epidemiology, laboratory diagnosis and limited primary health care.
- 13) Training of a field epidemiology team, University of Costa Rica.

Summary of Results

1. Vietnam: The training and in country support of a military field team in Vietnam resulted in a precise evaluation of the epidemiology and etiology of the principal causes of disability, and firmly established the value of trained research teams in the field adequately supported by laboratory facilities and a good supply system. The fact that the team was able to begin work within the first week of arrival and was never without fresh culture media and supplies was made possible by high level support in Vietnam and several years of experience and development of field methods under this contract.

2. Epidemiology of common skin infections in relation to environment: Our many studies in the tropics among populations at high risk as opposed to clinic populations have led us to conclusions which now form the basis for much of the on-going research supported by the USA Medical R & D. The principal findings are as follows:

- a) In the tropics the most common causes of disability related to the skin are infections.
- b) Streptococcal infections account for most cases of pyoderma and outweigh staphylococcal infections by a factor of at least 100:1.
- c) All streptococcal infections are preceded by some trauma to the skin, whether it be insect bites, abrasions, puncture wounds or chafing.
- d) The prevalence of streptococcal pyoderma is determined by environmental temperatures and humidity, level of hygiene, and exposure to trauma. Local terrain, vegetation, and agricultural practices influence the prevalence of biting or vector insects.
- e) Surveys of native children in endemic areas are a more useful guide to potential hazards than surveys in adult populations. Repeated streptococcal infections during childhood appear to confer some immunity in adult life.
- f) Rapid diagnosis and treatment of early streptococcal infections in military units markedly reduce the incidence of new infections, and can prevent epidemics.
- g) Fungal infections are more prevalent and severe in the tropics. Most are due to exacerbation of the "American" types of body ringworm and tinea pedis caused by the anthropophilic species; T. rubrum, E. floccosum, and T. mentagrophytes var. interdigitale. Under special circumstances, such as Vietnam, highly inflammatory epidemics of the zoophilic strains of T. mentagrophytes can occur. As in bacterial infections, surveys of adult native populations may give misleading information regarding potential hazards. Vietnamese adults, including military personnel, suffered from a chronic dermatophytosis due to a strain of T. rubrum, rare in our troops, and not endemic in the United States.

- h) Heat, humidity and occlusive clothing are the principle determinants of the prevalence and severity of dermatophytosis.
- i) Candidiasis may share an equal role as a cause of groin dermatitis in hot climates such as Fort Benning in the summer. Repeated water immersion in wet terrain operations actually improves the condition and lowers prevalence rates.
- j) Oral griseofulvin offers some value in prevention of dermatophytosis. Treatment of chronic hyperkeratotic Tinea pedis requires more than one month of therapy. Prolonged ingestion of pills on a daily basis is almost impossible to enforce. The future lies mainly with improved broad spectrum, cosmetically acceptable topical agents.

3) Human models of Dermatophytosis: Human models for dermatophytosis were developed in Miami, including the inflammatory type caused by zoophilic T. mentagrophytes, and the more chronic circinate lesions of T. rubrum. These models led to later extensive work on host susceptibility, immunology, and prophylactic studies, conducted at LAIR and by two other civilian contractors (Reigelman/Epstein and Stoughton).

4) New diagnostic methods: Adequate field methods have been developed or evaluated under this contract for dermatophytosis, streptococcal pyoderma, and staphylococcal infections. Dermatophyte Test Medium (D. T. M.) has proven its value in Vietnam, Venezuela, Uganda, Colombia and in military units at Fort Benning, Eglin AFB, and Lackland AFB. In addition to enabling field teams to identify dermatophyte fungi without previous training, it was used in all clinical trials conducted by the pharmaceutical industry in the development of the new Imidazole antifungal agents. Trypticase Soy Agar Sheep Blood with 1 microgram/ml. Crystal violet has been compared with other selective media and plain blood agar in many studies under this contract, and clearly emerges as the best available culture medium for Streptococcus pyogenes. Calcium alginate swabs desiccated with silica gel in the field was shown in Uganda to be as useful as direct plating for the recovery of Strep pyogenes from infected lesions. Methods of transporting bacterial isolates from the field to base labs in the US have been developed. We can now safely return cultures of S. pyogenes, S. aureus, and Dermatophyte fungi without loss of viability for periods up to six weeks.

5) Gram-negative infections:

- a) Injuries in wet terrains - Continuing studies on the effect of wet terrain exposure on the human cutaneous microflora prepared us for a rapid response to the Lockheed 1011 aircraft crash in the Everglades. Virtually all survivors with severe open injuries developed gram negative infections from aquatic bacteria present in the Everglades, the most important species being Aeromonas hydrophila. A guinea pig model of injury using samples collected at the crash site, accurately reflected the human infections. The few cases of clinical gas gangrene which developed were related to primary closure of wounds most probably contaminated by the spilled contents of the aircraft toilets, since we could not isolate human strains of Clostridia from

the Everglades water or mud immediately upstream of the impact site. Sixteen further cases of Aeromonas infection of wounds have occurred to our knowledge following injuries in Florida wetlands.

b) Hospital infections - Our work in the Burn Unit at Jackson Memorial Hospital indicates that environmental reservoirs and contaminated solutions and equipment play a greater role in infections than endogenous microflora. Several outbreaks of Pseudomonas infection have been traced to a point source. Flower vases in hospitals were discovered by us to contain enormous numbers of potentially pathogenic gram negative bacteria, many of which were resistant to gentamicin. Gentamicin resistant isolates are now frequently encountered in our hospital and include strains of P. aeruginosa, Serratia, E. coli, and most recently, several outbreaks of gentamicin resistant Klebsiella infections. We believe that the increase in gram negative infections in general and the emergence of multiple drug resistant strains are in part due to ecological pressures in high risk areas in which antibiotic usage, available reservoirs and susceptible patients all play a role. Efforts to diminish these pressures by removal of reservoirs, restrictions on antibiotic usage, particularly for prophylaxis, and on appreciation of the environmental hazards have resulted in lower infection rates. The skin plays an important role as an initial site of colonization, and as a transient means of transmission, for example on nurses' hands. In addition, much of what we have learned from the studies of cutaneous flora in the tropics has been directly applicable to the prevention of burn wound infections.

6) Antibacterial soaps for the prevention of bacterial infections:

In contrast to the still standing recommendation that the use of antibacterial soaps should be encouraged in the Armed Forces to prevent skin infections, our controlled study at a boarding school in Arizona showed that an antibacterial soap used daily under supervision for two months offered no advantage over an identical placebo bar soap in the prevention of common skin infections due to strep and staph. In vitro studies revealed that the antibacterial soap had no increased activity against S. pyogenes when compared with plain soap. At the end of the two month study, S. pyogenes was recovered from normal skin sites more frequently from antibacterial soap users than those using plain soap. A detailed account of this study is included in this report.

7) Survey of Colombian Army: A large scale survey of skin diseases in the Colombian Army was conducted by a field team from LAIR and the University of Miami. Preliminary liaison with the Military Hospital, Bogota, the commanding general Colombian Armed Forces (Gen. Herrera Calderon), the U.S. Embassy Bogota, and the University of Antioquia, Medellin was established by David Taplin during two visits to Colombia. In the field, mycological work was handled by the Miami component (Taplin and Mertz). Clinical aspects and bacteriology were conducted by the LAIR team (Allen, King and Ritchie). The study was unique in using for the first time standardized

methods of examination and diagnosis, including laboratory techniques. A large amount of data was generated, which is currently under analysis by computer at LAIR. The first results indicate that we will be able to precisely determine the various signs and symptoms, severity and extent of skin infections in military populations in relation to occupation, climate, time in service and other applicable variables.

In addition to the main epidemiologic objectives of the study, several spin-off projects have evolved from observations made in the field, including what we believe are very important findings by Dr. Robert King at LAIR, relating to the mechanisms of pathogenicity of dermatophyte fungi.

8) Mobile field epidemiology unit: Following the experiences in Vietnam and Uganda, it became clear that if further effective epidemiologic studies were to be conducted in endemic areas in the tropics, we could not expect to have available in the field the kind of laboratory facilities provided for the team in Vietnam. By definition, the areas in which the highest prevalence of disease occurred were those in which laboratory support was least available. Mobility was also essential in order to get to the populations at risk and to make the best use of time in country. We were also concerned over potential risks to the field team exposed to endemic infectious diseases. The concept was developed of a mobile diagnostic facility which would also serve as the living quarters for the field team. Originally proposed for Nigeria, a site visit to Lagos, Ibadan and surrounding areas quickly indicated to us that the chances of getting the unit in to Nigeria intact and on time were slim. In addition, the high costs of operating in country and the unavailability of spares, cast further doubts on attempting to launch the project in Africa, at least within the time and funding available.

We, therefore, switched the project to Costa Rica, where we had strong support from the University of Costa Rica and the Ministry of Health. Following trial runs in Florida and modifications to improve battery capacity and security features, the trailer was shipped from Miami to Costa Rica, together with a Jeep Wagoneer purchased by the University of Miami. Details of the studies conducted with the trailer in Costa Rica are included in this report.

In summary, the concept of a mobile epidemiology team operating from the trailer facility proved to be valid, and adaptable to a wide variety of functions, including microbiology and parasitology, general health surveys and provision of clinical laboratory facilities.

9) Field epidemiology in Costa Rica: Field studies using the mobile facility described above were conducted with a team trained in Costa Rica. Bacterial infections were confined to young children living in a hot, humid lowland area, where biting insects and Hippelates flies were abundant. Forty percent (40%) of children below age 10 had laboratory proven streptococcal pyoderma. Fewer than 10% of children living above 300 M altitude had bacterial skin infections. Dermatophytosis, Tinea pedis and Tinea versicolor occurred only in young post-pubertal individuals over age 13. Discrete populations, suitable for future research projects, were identified.

10) Evaluation of diagnostic media for S. aureus: A field trial in Costa Rica of two diagnostic methods showed that a selective indicator medium developed in Miami was 95% accurate in identifying coagulase positive S. aureus from skin lesions, without microbiological expertise or need for further laboratory confirmation. Another method developed at LAIR gave slightly higher recovery rates, but needed additional laboratory tests to confirm the isolation of S. aureus.

End of Summary of Projects

See body of report for detailed account of projects 6, 8, 9 and 10.

ANNUAL PROGRESS REPORT

Double Blind Trial of an Antibacterial Soap for the
Prevention of Common Skin Infections

It is evident from our studies in Vietnam and with the Colombian Army that the most common cause of bacterial infection of the skin among military personnel exposed to rugged environments is likely to be Streptococcus pyogenes.

In the late sixties, a recommendation was made from the Armed Forces Epidemiological Board that the use of antibacterial soaps should be encouraged as an aid to the prevention of bacterial infections of the skin. This recommendation was based previously on studies conducted at West Point and Annapolis military academies, which showed an apparent statistical reduction in primary skin infections among the students of these institutions who used an antibacterial soap. The number of infected individuals was very small, and it is clear that the investigators were dealing with cases of staphylococcal infections, including boils and paronychia infections.

No controlled studies have been conducted to our knowledge to determine the value, if any, of antibacterial soaps in the prevention of the more common streptococcal infections of the skin.

The value to the military of such a study is obvious. We were, therefore, enthusiastic when offered the opportunity to conduct such a study in a population with a high prevalence of infections.

The results are presented in two parts. The first part, describing two months of closely monitored soap usage, represents the most complete and best controlled study to date on the effects of antibacterial soaps on the prevalence of clinical infection. The second part describes an uncontrolled ad lib use of the soaps and for practical purposes can be disregarded in terms of scientific validity or statistical significance because we simply did not know what actually went on in the dormitories during this phase. It is included for completeness in the event that others may see value in it that we may have missed.

There is perhaps some value in reporting the second uncontrolled phase of this study, because it provided a lesson to us and hopefully to other investigators, that it is essential to monitor the actual use of the medication by on site inspection. We did not do this because the staffing and expense of having our own monitors at the school to supervise showers every day for eight months were beyond the funding resources available to us.

The Effect of Daily Controlled Use of Antibacterial
Soap on Prevention of Common Skin Infection

David Taplin, William Eaglstein, M.D., Richard Feinstein, M.D.,
Patricia M. Mertz, Jane Walker Ellison, R.N.

SUMMARY

Part I

Two Months Supervised Daily Showers

In a two-month period of carefully monitored showering on a daily basis, we could detect no effect of antibacterial soap in the prevention of skin infections among school children aged six to thirteen.

The prevalence of S. aureus on normal skin was consistently lower in the antibacterial soap group than in the placebo group. There was an increase in the prevalence of Group A S. pyogenes on normal skin related to the use of active soap for two months, particularly among the girls. However, recovery of Staph and Strep from normal skin by the RODAC system did not correlate well with clinical disease, and probably represents transient contamination rather than skin colonization.

In vitro studies showed enhanced activity of active soap when compared with the placebo soap against S. aureus but not against Group A S. pyogenes.

The distribution and streptococcal etiology of the skin infections were typical of common skin infections seen in the United States, Africa, and South America.

Part II

Six Months Ad Lib Use

One month of unsupervised use of the soaps, for a total of three months' use, was followed by a decreased prevalence of clinical infections from that seen at two months. This was particularly evident in the girls. At three months, prevalence of infection was lower with active soap than with the placebo. These effects were not maintained, and after six months' ad lib use, no differences could be determined between the soaps in the prevention of infections.

The high prevalence of S. pyogenes on normal skin associated with the use of antibacterial soap at two months was not followed by an increase in clinical

infection. No adverse effects of active soap were seen during the study, and no data were generated to suggest that antibacterial soap increased the risk of clinical infections.

Further studies are needed on the methodology of sampling normal skin for Staph and Strep and to determine the relationship, if any, between streptococcal colonization and use of antibacterial soaps.

BACKGROUND

Following planning meetings with the University of Miami investigators listed below, the Bureau of Indian Affairs (Chinle, Arizona), the Principal of Chinle Boarding School, and the Food and Drug Administration, a study was initiated at the Chinle Boarding School, Chinle, Arizona, in an attempt to answer the question:

Is there value in the daily controlled use of an antibacterial soap bar* compared to plain soap bar in the prevention of bacterial skin infections?

The investigational team from the University of Miami consisted of:

David Taplin	-Associate Professor of Dermatology and Epidemiology & Public Health, Principal Investigator and Project Coordinator
William Eaglstein, M.D.	-Assistant Professor of Dermatology, Chief Clinical Research, Department of Dermatology
Richard Feinstein, M.D.	-Clinical Dermatologist and previous Health Officer, U.S.P.H.S., Chinle, Arizona
Patricia M. Mertz	-Research Assistant, Department of Dermatology
Jane Walker Ellison, R.N.	-Clinical Health Nurse and Research Assistant, Department of Dermatology
Daria Feinstein, R.N.	-Clinical Health Nurse and previous Health Nurse, U.S.P.H.S., Chinle, Arizona

*Active soap contained 1.5% 2:1 TCC:TFC

Munroe Benhaim

-Senior Medical Student, University of Miami, on site monitor for Miami investigators

Edgar B. Smith, M.D.

-Professor of Dermatology, University of New Mexico, Albuquerque

Essentially, we assigned commercially available soap bars to specific dormitories, of the type which would routinely be used in the school.

In the scientific sense, Chinle Boarding School was ideal for the study of this kind because the children were for most of the time in residence, separated into dormitories by age and sex, each with its own shower facilities physically separated from each other. A daily attendance sheet was available, and we had excellent cooperation from the school administration with permission to monitor soap usage. Previous reports suggested that skin infections had been endemic in the school for some years.

The results of this study are submitted in two parts. Part I represents a two-month study during which time the use of soap was closely monitored, other topical medications were eliminated, and children who received systemic antibiotics were recorded and excluded from the results of the following examinations.

Part II represents further six months of ad lib use of soaps, during which there was no monitoring of showering, no control over the use of topical agents (e.g., Neomycin/bacitracin ointment), and no record of sick calls or administration of systemic antibiotics.

We therefore consider the first two months' data as the most important, whereas the next six months represents a "true life" situation in which many other factors not under our control played a role.

MATERIALS AND METHODS

All children enrolled at the school were included in the study. Color coded, but otherwise identical, soap bars were assigned to each dormitory. Half of the population were assigned an active bar and half the placebo. The pattern of distribution of the soap was assigned to achieve as far as possible equal weighting according to age and sex and the results of baseline clinical studies (prevalence of infection), but the code was not known by the investigators.

Prior to the start of the study, all soaps and shampoos were removed

from the school and replaced with plain soap and shampoo for a period of one month.

Throughout the first two-month period, soap usage was monitored by the dormitory supervisors and by our own monitor. Children were checked off a roster each time they showered (once per day), so that we are confident that soap use was adequately monitored.

On each of three occasions, every child available was examined from head to toe by two clinical dermatologists (Drs. Eaglstein and Feinstein). Any trauma of the skin (cuts, scratches, abrasions, bites, rashes) was recorded on a body diagram sheet. In addition, all infected lesions were recorded, according to the following criteria:

A lesion surrounded by erythema and containing pus, regardless of shape, size or extent of lesion, was scored as a clinical infection. Crusted lesions surrounded by erythema were recorded as possibly infected. When the crust was removed from lesion at station 2 (microbiology) it was confirmed as infected if pus was present under the crust. If the lesion was dry, and no pus could be expressed by superficial incision and firm pressure, the clinical observation was changed to not infected.

All lesions scored as infected or possibly infected at station 1 (clinical) were cultured. All children with infected lesion, and all children with odd study numbers, were sampled for the presence of staph and strep on normal skin sites (mid scapular and inner aspect left wrist) using RODAC contact plates. The choice of these sites was decided on the basis of studies by Dr. Mary Marples (The Ecology of the Human Skin) relating to bacterial densities in various skin sites, and to provide a basis of comparison with the work of the University of Minnesota group under Dr. Wannamaker, who used the same sites in Red Lake Indian Children.

Media used were:

1) Trypticase Soy Agar with 6% sheep blood and 0.8 µg/ml crystal violet for Streptococcus pyogenes (hemolytic strep), accepted by Wannamaker, Dillon, Parker, Potter, Allen and ourselves as the best available medium for isolation of strep.

2) PYM II (miami), a well tested selective medium for Staph aureus contains Polymyxin B, Mannitol, Brom Cresol Purple and yeast extract. This medium has consistently given higher recovery rates and is more selective than standard Mannitol Salt agar, sheep blood agar, or glycine tellurite agar.

All media used in this two-month study were made from the same batches of ingredients of equal shelf life since manufacture, autoclaved identically,

incubated at 37° C, and read at the same time interval after culture (24 to 36 hrs.).

We therefore consider the quality control of the media and methods to be as consistent as could be obtained.

Part I Two Months of Controlled Use

Results

In compiling these results, we have removed from the study all children who received systemic antibiotics (Penicillin, Ampicillin, Tetracycline, Erythromycin), all children who were absent from school for more than one week in any one month, and children who were not present for examination at the time of the three surveys.

This left 401 children who were known to have used the soaps under the supervision of our own monitors, remained in school and who received no antibiotics. These are termed "completers" in this report. 301 children were listed as "non-completers" and have been evaluated separately.

Clinical Infections

Figure 1 shows the point prevalence of clinical infection for all "completers." There was no significant difference in the prevalence of clinical infection between the children using antibacterial soap and those using placebo after two months of supervised daily use.

Figures 2 and 3 show the point prevalence of clinical infections by sex. Differences in prevalence between the two groups of soap users were not significant. The boys remained with approximately the same differences in prevalence at the three survey points. The girls, by chance, had all of their infections at the baseline survey in the group that were to become antibacterial soap users, and remained after two months with more infections in the active soap group.

To evaluate whether the "non-completers" were significantly different from "completers," data for the entire population (with the exception of the few who received systemic antibiotics) are shown in Figure 4. It can again be seen that there were no differences in prevalence between those assigned to active soap dormitories and those in placebo dormitories.

Figure 5 shows "non-completers" only. The point prevalence rates are almost identical with the raw data for the entire population and for the "completers."

Recovery of Staph and Strep from Normal Skin

The influence of soap use on the prevalence of recovery of Staphylococcus aureus from normal skin sites is shown in Figure 6.

There was slightly higher recovery of S. aureus from the group assigned to placebo bars at the baseline study, and this differential remained the same throughout the two months if all the "completers" are considered together. Analysis of Figure 7 and 8, however, shows that this effect was almost entirely due to the girls, who we believed practiced more careful hygiene throughout the study.

Note: We were consistently impressed with the fact that the girls were always cleaner, better groomed and sweeter smelling than the boys. We could detect no obvious differences in the dormitory routines, and we came away with the impression that the girls were more concerned with their personal appearance and cleanliness than the boys, or that more attention was paid to the girls in this respect due to the fact that most of the dormitory aides and counselors were women.

Figure 9 illustrates the point prevalence of recovery of Group A Streptococcus pyogenes from normal skin sites. There appears to be a significant change in the recovery rates of S. pyogenes relating to the use of antibacterial soap. In spite of the fact that there was twice the recovery rate at the baseline from the placebo group compared with the active groups, at the end of two months this ratio was reversed, and there were more children with positive cultures for S. pyogenes from normal skin in the active soap group. Figure 10 and 11 show that this apparent increase in Strep recovery was most marked in the girls.

Recovery of Staph and Strep from Normal Skin in Relation to Clinical Infection

Figures 12 and 13 show the point prevalence of S. aureus from normal skin sites with and without clinical infections.

As might be expected, there were more recoveries of S. aureus from normal skin sites in children with active infections than from non-infected children.

The type of soap used had little effect on Staph recovery in children with no infections. Children with infections at two months had fewer recoveries of Staph in the active soap group when compared with the baseline and one-month data.

Figures 14 and 15 show that Group A - Streps were more often recovered from normal skin sites than was S. aureus, and that Streps were more often recovered from children with infections than without infections. The figures also demonstrate that there were more children with positive Strep cultures from normal skin in the active soap group at the end of two months, regardless of whether they had clinical infections.

Recovery of Staph and Strep from Lesions

Figure 16 shows that there were fewer recoveries of S. aureus from infected sores in the active soap group as the study progressed.

Figure 17 illustrates that the two groups (active vs. placebo) started the study with a difference in recovery of Strep from lesions, and that this differential was maintained throughout the study with no effect noted which could be related to the type of soap used.

Environmental Sampling

Because we were beginning to have doubts by the end of two months concerning the RODAC system of sampling as a measure of "colonization" as opposed to transient contamination, we sampled 25 bed sheets in two dormitories using RODAC plates. The children at that time were continuing to shower on a daily basis and sheets had been changed the day before. We sampled the bottom sheet 12" below the pillow. Seven of 25 sheets (28%) yielded Group A Strep. Three of 25 (12%) yielded S. aureus. These figures were almost identical with the levels we obtained from normal skins, indicating, we think, that the skin surfaces were simply another surface which could be contaminated by transient microorganisms.

In vitro Testing of Soaps

At the end of the two month study, we investigated the effect of the soaps on the Staphs and Streps isolated at Chinle. One gram of soap chips was dissolved in 100 ml distilled water and serially diluted in Trypticase Soy Broth to produce a 9 tube assay. Standardized suspensions of S. aureus and S. pyogenes were added to each tube. Duplicate sets were made with the addition of Fetal Calf Serum to give a final concentration of 10% in the broth. Table 1 shows good activity for the active soap to the 7th tube against Staph aureus. Activity was inhibited somewhat by the addition of serum. Little activity could be detected in the placebo soap.

Table 1

Soap Solutions in Broth Inoculated with Chinle S. aureus

<u>Tube #</u>	<u>Soap Conc, %</u>	<u>Antibacterial soap c̄ dis- tilled water</u>	<u>Antibacterial soap c̄ Fetal Calf Serum</u>	<u>Placebo soap c̄ dis- tilled water</u>	<u>Placebo soap c̄ Fetal Calf Serum</u>
1	10 ⁻¹	no growth	no growth	1+ growth	2+ growth
2	10 ⁻²	no growth	no growth	1+ growth	2+ growth
3	10 ⁻³	no growth	no growth	2+ growth	1+ growth
4	10 ⁻⁴	no growth	no growth	2+ growth	4+ growth
5	10 ⁻⁵	no growth	1+ growth	2+ growth	4+ growth
6	10 ⁻⁶	no growth	4+ growth	4+ growth	4+ growth
7	10 ⁻⁷	no growth	4+ growth	4+ growth	4+ growth
8	10 ⁻⁸	1+ growth	4+ growth	4+ growth	4+ growth
9	10 ⁻⁹	4+ growth	4+ growth	4+ growth	4+ growth

Table 2 shows activity for both soaps against S. pyogenes presumably due to the fatty acids present in the soap. This activity was greatly diminished by the addition of serum, but we could detect no increased activity of the antibacterial soap over the placebo.

Table 2

Soap Solutions in Broth Inoculated with Chinle Strep. pyogenes

<u>Tube #</u>	<u>Soap Conc, %</u>	<u>Placebo with dis-tilled water</u>	<u>Placebo with Fetal Calf Serum</u>	<u>Antibacterial soap c̄ dis-tilled water</u>	<u>Antibacterial soap c̄ Fetal Calf Serum</u>
1	10 ⁻¹	no growth	no growth	no growth	no growth
2	10 ⁻²	no growth	no growth	no growth	no growth
3	10 ⁻³	no growth	no growth	no growth	no growth
4	10 ⁻⁴	no growth	no growth	no growth	no growth
5	10 ⁻⁵	no growth	4+ growth	no growth	4+ growth
6	10 ⁻⁶	no growth	4+ growth	no growth	4+ growth
7	10 ⁻⁷	no growth	4+ growth	no growth	4+ growth
8	10 ⁻⁸	no growth	4+ growth	no growth	4+ growth
9	10 ⁻⁹	4+ growth	3+ growth	4+ growth	4+ growth

Evaluation of Clinical Scoring

At the first two examinations, Dr. Feinstein recorded 49 subjects with clinically infected lesions, of whom 45 harbored Staph and/or Strep (92%). Dr. Eaglstein recorded 33 subjects with clinical infections, of whom 30 carried the organisms in their lesions (91%). Thus, the accuracy of diagnosis was very closely associated with positive laboratory findings, and the two observers were very closely matched in their diagnostic criteria.

Analysis by Dormitory

Data were analyzed by dormitory as well as by individual children because of the possibility that infected children were spreading the organism among their dormitory mates.

There was no relation of antibacterial soap usage to changes in the prevalence of infection or recovery of S. aureus from normal skin, between the baseline period and months one and two. However, the increase in prevalence of S. pyogenes from normal skin of antibacterial soap users between months one and two (Table 3) was significantly greater than in the control subjects. This analysis supports the findings based on point prevalence for individual children that S. pyogenes was more frequently recovered from normal skin in the active soap dormitories after two months, and that the prevalence was significantly greater in the girls' dormitories using this soap. The results also suggest there was exchange of bacteria among children living in the same dormitory. This factor should be considered in the design of any future studies.

Table 3

Prevalence of S. pyogenes on Normal Skin
of Completers During First Two Months, by Dormitory

<u>Treatment</u>	<u>Dorm-itory</u>	<u>Sex</u>	<u>Age</u>	<u>Prevalence of S. pyogenes</u> <u>(as percent) on normal skin at</u>		
				<u>Base-line</u>	<u>One Month</u>	<u>Two Months</u>
Placebo Soap	ID	M	>10	11.1	11.1	11.1
	IE	M	<10	20.7	10.3	6.9
	IID	M	<10	5.3	10.5	15.8
	IIE	M	<10	25.9	14.8	7.4
	IIIE	F	<10	8.1	13.5	2.7
	IVD	F	>10	0.0	11.1	0.0
	IVE	F	>10	0.0	11.1	3.7
Anti-bacterial Soap	IIA	M	<10	4.0	16.0	24.0
	IIB	M	<10	9.4	9.4	6.3
	IIIA	M	>10	12.0	4.0	4.0
	IIID	F	>10	3.4	10.3	20.7
	IIIE	F	<10	2.7	8.1	16.2
	IIVA	F	>10	0.0	7.7	15.4
	IVB	F	>10	3.3	6.7	6.7

"t" for difference between placebo and active groups in change of prevalence of S. pyogenes on normal skin between months one and two: 3.11.

Discussion

Despite various methods of analysis, we could detect no effect of the antibacterial soap on the prevalence of clinical infections after two months of vigorously monitored use. This was true whether the population was analyzed by point prevalence in individual children or by dormitory, or by comparing "completers" alone or the entire population at risk.

The correlation of laboratory microbiology with clinical judgment of the two physicians was high, and we have little doubt that we were dealing with an endemic situation of skin infections in which Group A - Streptococcus pyogenes was the principal agent involved, with S. aureus as a secondary colonizer perhaps contributing to the severity of delayed healing of the infections.

The microbiology of the infections was very similar to other populations we have studied (Table 4).

Table 4

<u>Location</u>	<u>Climate</u>	<u>Number Cultured</u>	<u>Recovery of S. aureus (%)</u>	<u>Recovery of S. pyogenes (%)</u>
Miami		252	59	95
Apartado, Colombia	Tropical	91	90	90
Medellin, Colombia	Temperate	50	62	84
Chinle (Baseline)	Arrid	40	60	80

The results are similar to those of our studies among combat soldiers in Vietnam and Colombia, and to those of studies in children by Dillon (Alabama), Esterley (Baltimore), Poon King (Trinidad), and Wannamaker (Minnesota).

All of these authors studied common skin infections in populations at large and recovered S. pyogenes Group A from 68% - 93% of lesions, and S. aureus from 39% - 75%. We therefore believe that the Chinle infections were rather typical of common skin infections in the United States. Throughout the Chinle study we did not find a single case of staphylococcal bullous impetigo.

The lack of benefit of the antibacterial soap over a period of two months' use in the prevention of infections was in accordance with our in vitro data, which showed no advantage of the active over the placebo soap against Streptococcus pyogenes.

There did appear to be a consistent reduction of S. aureus on normal skin of the antibacterial soap users, particularly in the girls, who may have used the soaps more diligently. This is reflected in the lower recovery from lesions. This is also not surprising in view of the in vitro data, which showed activity against S. aureus.

However, the effect on S. aureus was not reflected in the prevalence of clinical infection. It can be seen for example, by comparison of Figures 2 and 7, that the increased recovery of S. aureus from girls in the placebo groups was not related to an increase in infections.

On the other hand, the higher prevalence of infection in the active soap girls after two months (Figure 2) occurred at a time when we obtained a higher recovery of S. pyogenes from normal skin (Figure 9).

The two month data in girls seem to make sense with regard to infection, RODAC recoveries of Streptococci from normal skin and in vitro data, but is difficult to reconcile the many other examples in this study where the RODAC sampling of normal skin sites does not correlate well with prevalence of infection, or subsequent expression of clinical disease. We wish, therefore, to expand the discussion of the normal skin flora because it is likely to give rise to the most controversy and may represent either an important discovery, or a red herring.

We are well aware of the importance of this study in helping to establish the benefit to risk ratio of antibacterial soaps. We believe that the two-month supervised portion of the study represents the best available investigation so far conducted, and we conclude that no effect could be shown for prevention of streptococcal pyoderma over a period of two months.

Obviously, if the use of this or any other antibacterial soap encourages the colonization of normal skin by S. pyogenes, and if this can be correlated with an increase in clinical infection, it would be an important fact to take into consideration on the risk side of the evaluation. This study has shown a statistical increase in the recovery of S. pyogenes on normal skin associated with the use of an antibacterial soap. However, the RODAC system has never, in our knowledge, been adequately evaluated with regard to sensitivity or reproducibility. The fact that bed sheets produced similar recoveries suggests that our information from skin is no more relevant than that from inanimate surfaces, and although it may reflect the general level of contamination of the environment, it adds little to our knowledge of skin 'colonization', and nothing to the evaluation of health risks. In fact, we could find no evidence

that the prevalence of Streptococci recovery from normal skin was followed by an increase in clinical infection, unlike data of Wannamaker and colleagues working with Red Lake Indian children.

Before suggesting that use of antimicrobial soaps results in an increase in colonization of the skin by S. pyogenes, we would like to confirm this by additional studies. We would also like to know much more about the value of the RODAC surface sampling technique in determining the difference between colonization and transient contamination.

Part II

Six Months Ad Lib Use

Following the two-month supervised study reported in Part I, it was decided that the children should continue to use the soaps on an ad lib basis for a further period of six months according to the usual showering schedules normally practiced in the school previous to the initiation of the study. This, we were told, was twice per week with an additional shower on Sunday evening for those children who went home for the weekend.

We understood that this schedule would be practiced by all dormitories. Unfortunately, at least two dormitories continued mandatory daily showering on the initiative of the dormitory supervisors, but we did not discover this fact until five weeks after the ad lib part of this study was initiated. We could obtain no documented evidence to indicate which dormitories followed a twice-per-week schedule and which insisted on daily showers.

After the two-month period, we had no monitor on site, and no control over the use of antibiotics. To further confuse the situation, by the end of the two-month study, the physicians at the local health clinic had become convinced that systemic benzathine penicillin G (Bicillin) was the preferred method of treating skin infections as opposed to topical medications (Neomycin/bacitracin).

It is likely, therefore, that after the two-month controlled study any child with infections requiring medical attention would have received I.M. Bicillin.

Thus, the results of the four-month examination (clinical only) and the results of the eight-month examination (full survey with microbiology) should be viewed with the foregoing limitation in mind.

In Figures 1-3 and 6-15, "completers" at three and eight months are those children who were "completers" at two months, although many had intervening absences before the last two examinations.

We are confident, based on investigation of the supply situation and questioning of the children, that the correct colors of soaps were issued to the dormitories during the two-month to three-month period.

Results

After one month of ad lib use (total 3 months) there was a significantly lower prevalence of clinical infection (Figures 1,2,3) in the active soap group, as compared with that seen at two months. This was especially notable in the girls. At three months, prevalence of infection was lower with active soap than with the placebo. After a further five months (total 8 months), the prevalence of infections was approximately the same as the baseline data obtained at the start of the study.

It can also be seen (Figures 6,7,8) that at the end of eight months, the recovery of S. aureus from normal skin was not statistically different from the baseline data.

Figures 9, 10 and 11 show that the recovery of S. pyogenes from normal skin sites at 8 months was lower than any previous survey point. This may be due to the effect of climate during the winter months.

Discussion

The dramatic decrease in prevalence of clinical infection among the girls using active soap, after a month of ad lib use, is difficult to explain in light of the in vitro data and the lack of efficacy of antibacterial soap during the two-month supervised portion of the study. If it represents a cumulative effect, then this effect was not maintained during the next five months.

The three-month data throw even more doubt on the RODAC surface sampling technique as a method of predicting infections.

Clearly, the high prevalence of Streptococci recovery from normal skin at two months was not followed by an increase in infection.

We, therefore, cannot prove in this study that this soap represents a health risk, even if the increase in Streps on normal skin is real. The most logical explanation at this time is that the RODAC system is an insensitive, or perhaps misleading tool, and we would again suggest the need for a much closer look into the whole area of streptococcal skin "contamination",

"colonization", "carriage", and the relevance of these parameters in relation to clinical disease.

The overall study from baseline to the eight-month point showed no difference between the soaps in prevention of clinical infection. Thus, after two months supervised soap use, followed by six months of ad lib use (which included times when adequate control of soap use was not practical), the children ended up essentially with the same prevalence of infection as they started, with no demonstrable difference between the soaps.

The immediate result of this study suggests that we cannot expect to achieve any benefit from antibacterial soaps in military populations subject to streptococcal infections. These soaps may actually enlarge the cutaneous reservoir of streptococci, but further work is indicated to determine the extent of any ecological effects and subsequent health risks.

POINT PREVALENCE OF CLINICAL INFECTIONS
 COMPLETERS ONLY
 Children with one or more clinical infections
 as a percent of each population at risk.

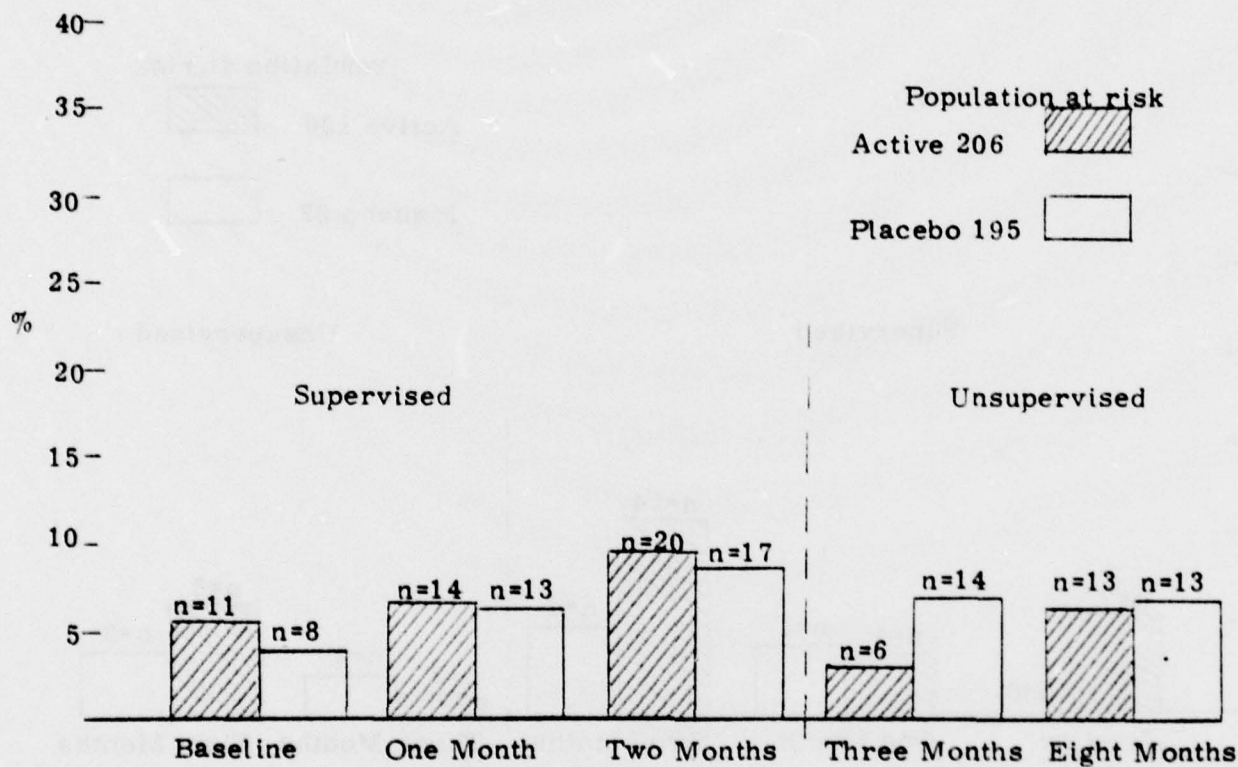


Figure 1

POINT PREVALENCE OF CLINICAL INFECTIONS
FEMALE COMPLETERS ONLY

Female children with one or more clinical
infections as a percent of each population
at risk.

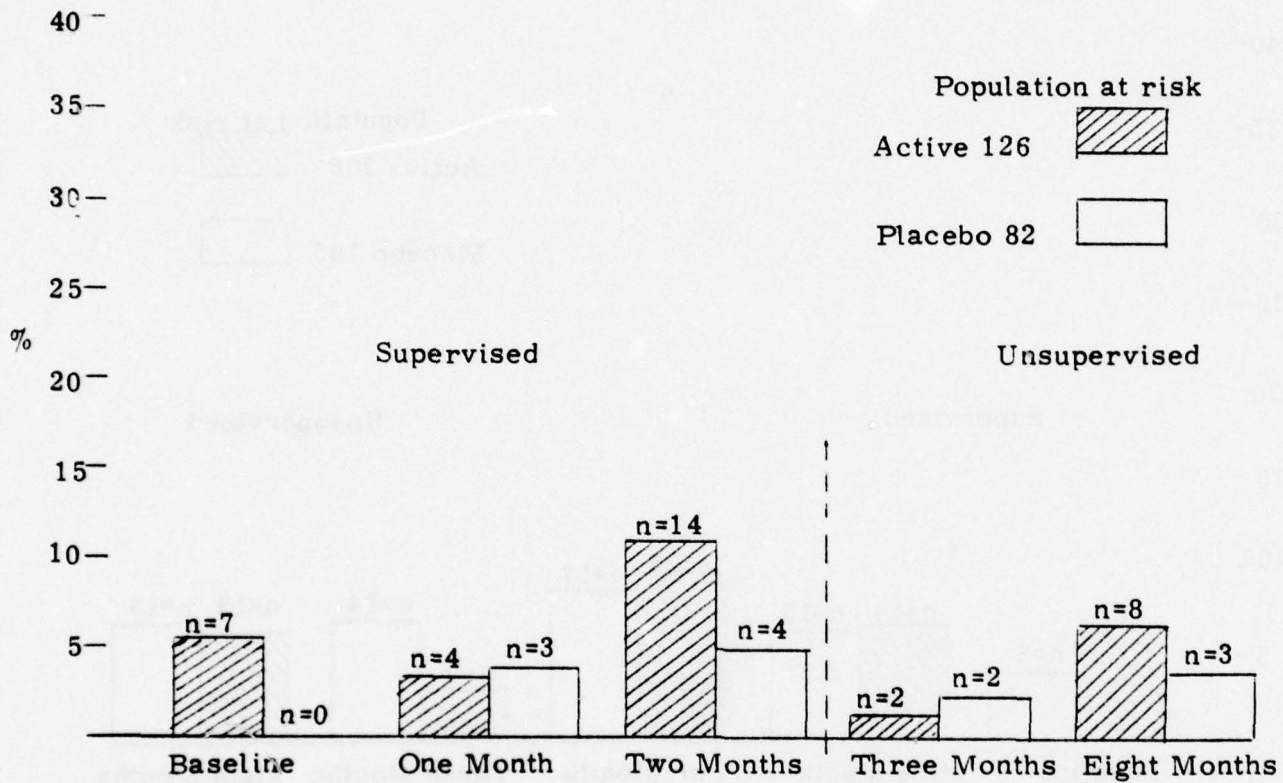


Figure 2

POINT PREVALENCE OF CLINICAL INFECTIONS
MALE COMPLETERS ONLY

Male children with one or more clinical infections as a percent of each population at risk.

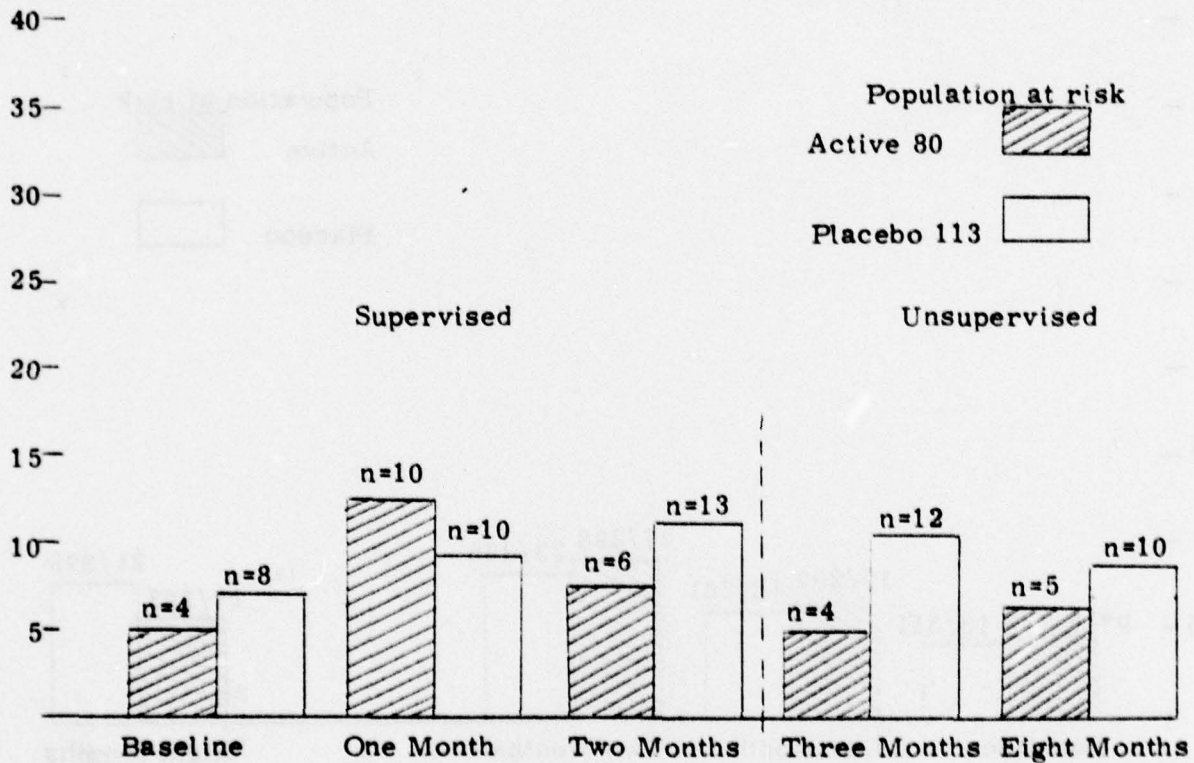


Figure 3

POINT PREVALENCE OF CLINICAL INFECTIONS. RAW DATA
All children except those receiving systemic antibiotics, with one or more clinical infections as a percent of each population at risk.

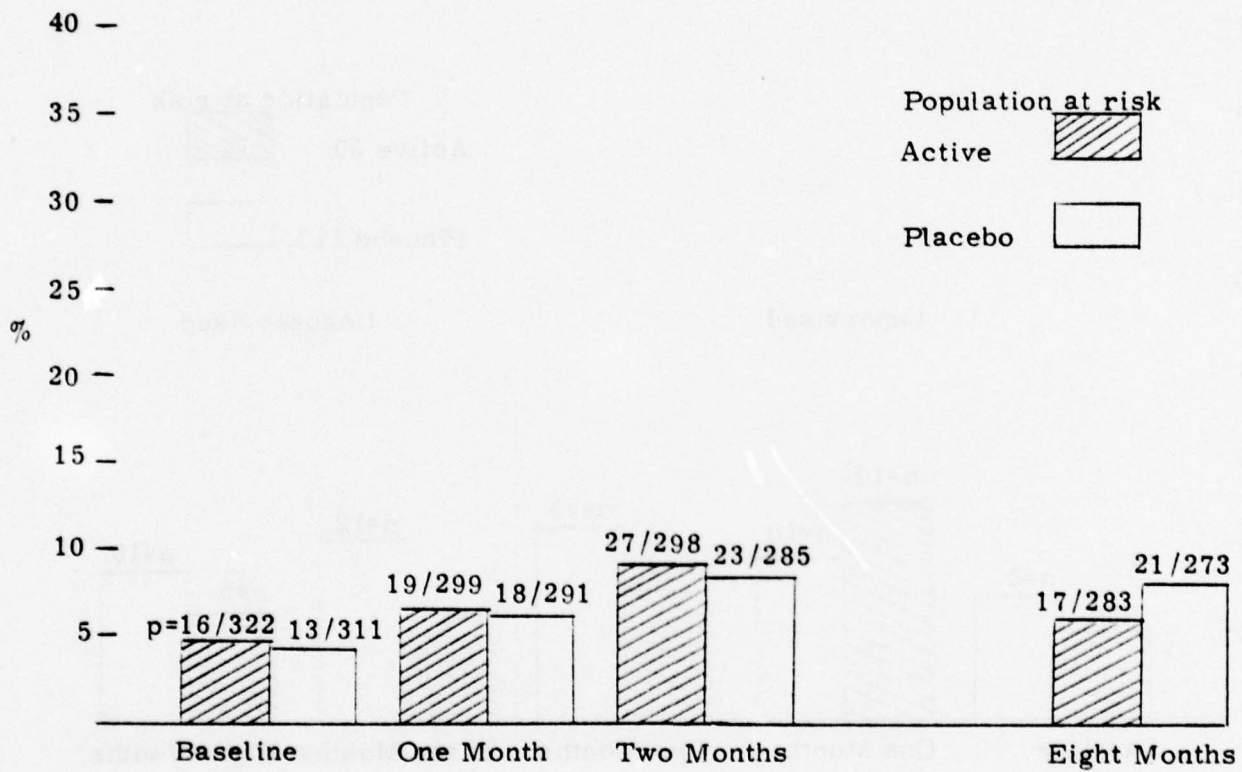


Figure 4

POINT PREVALENCE OF CLINICAL INFECTIONS
NON-COMPLETERS ONLY

Children absent one week or more and/or absent one or more survey visits, with one or more clinical infections as a percent of each population at risk.

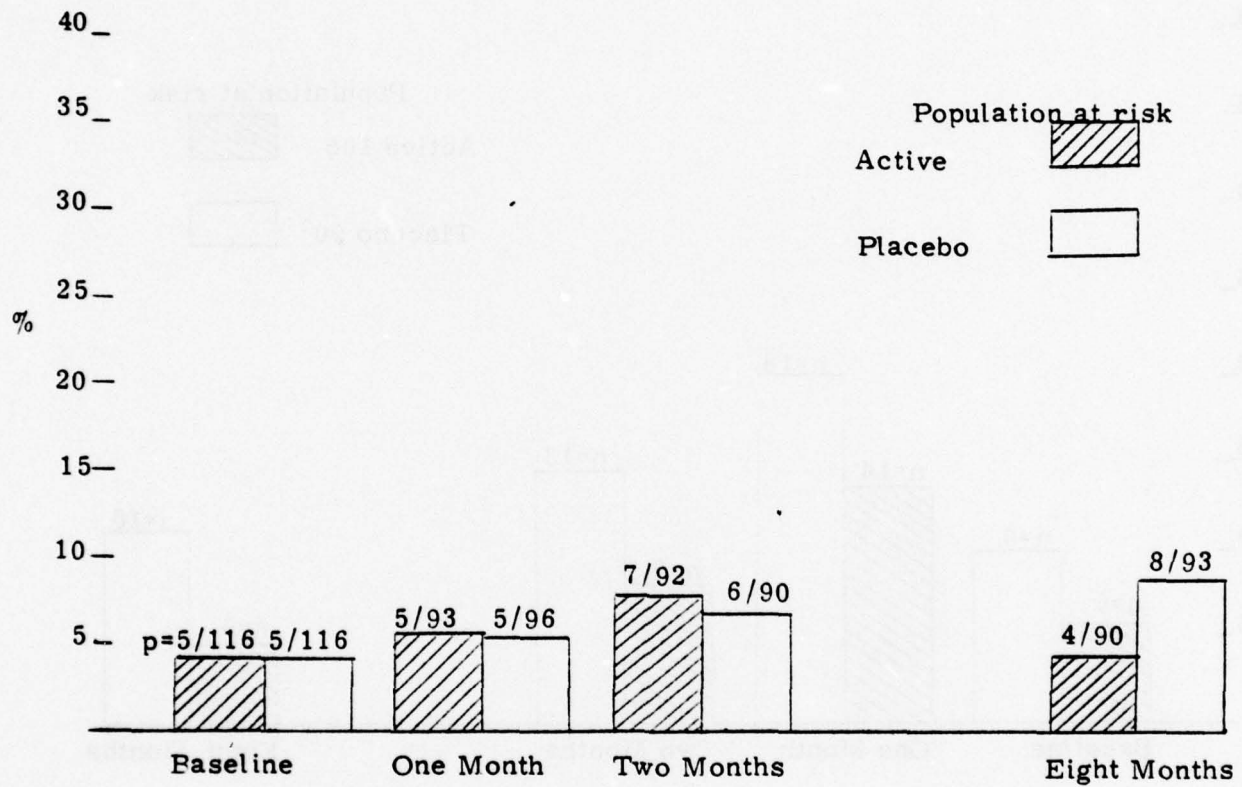


Figure 5

POINT PREVALENCE OF STAPH AUREUS
NORMAL SKIN, COMPLETERS ONLY
Children with one or more positive skin sites by RODAC
method as a percent of the population at risk.

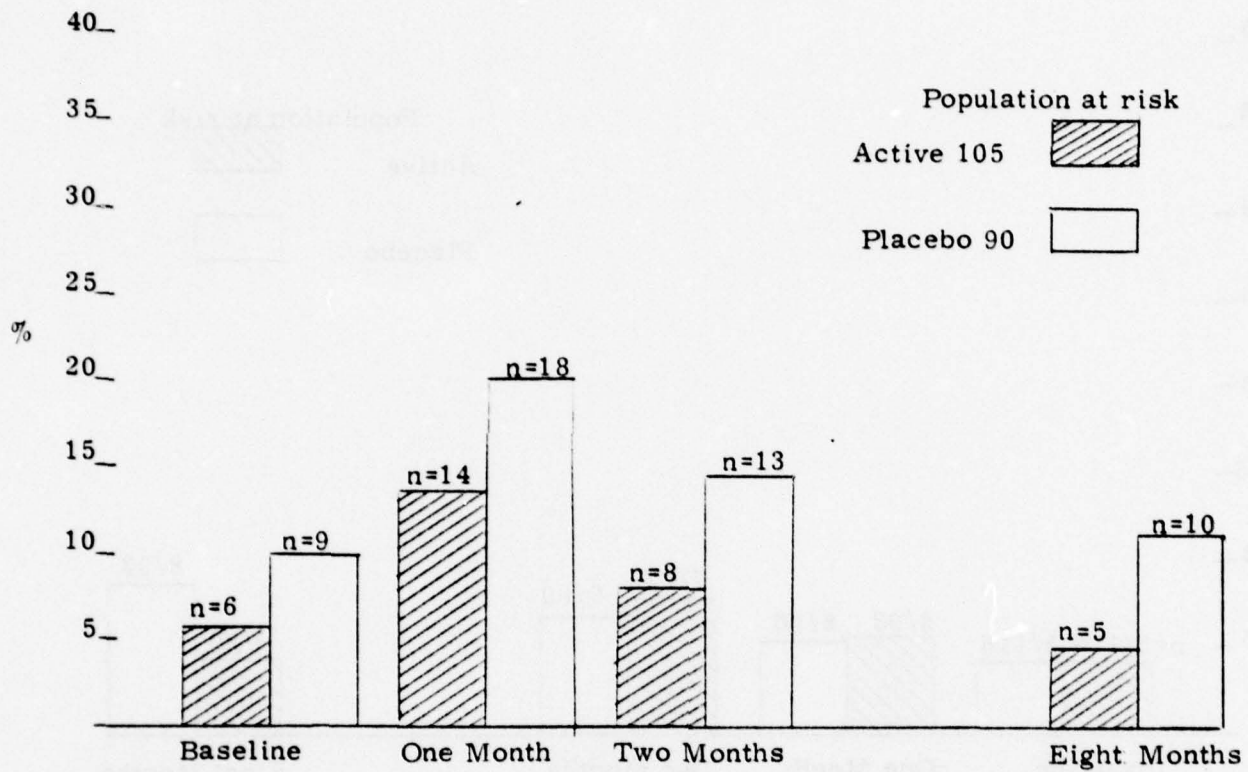


Figure 6

**POINT PREVALENCE OF STAPH AUREUS
NORMAL SKIN, FEMALE COMPLETERS ONLY**
Female children with one or more positive skin sites by RODAC
method as a percent of the population at risk.

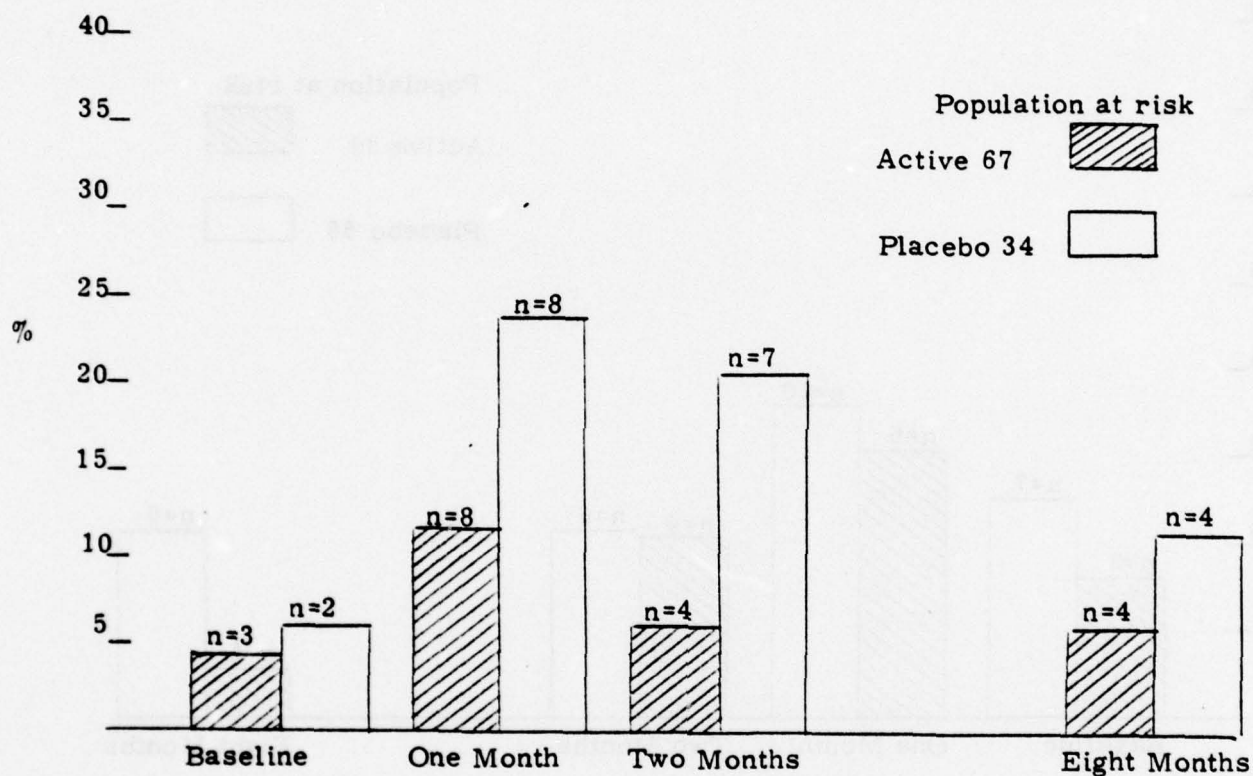


Figure 7

POINT PREVALENCE OF STAPH AUREUS
NORMAL SKIN, MALE COMPLETERS ONLY
Male children with one or more positive skin sites by RODAC
method as a percent of the population at risk.

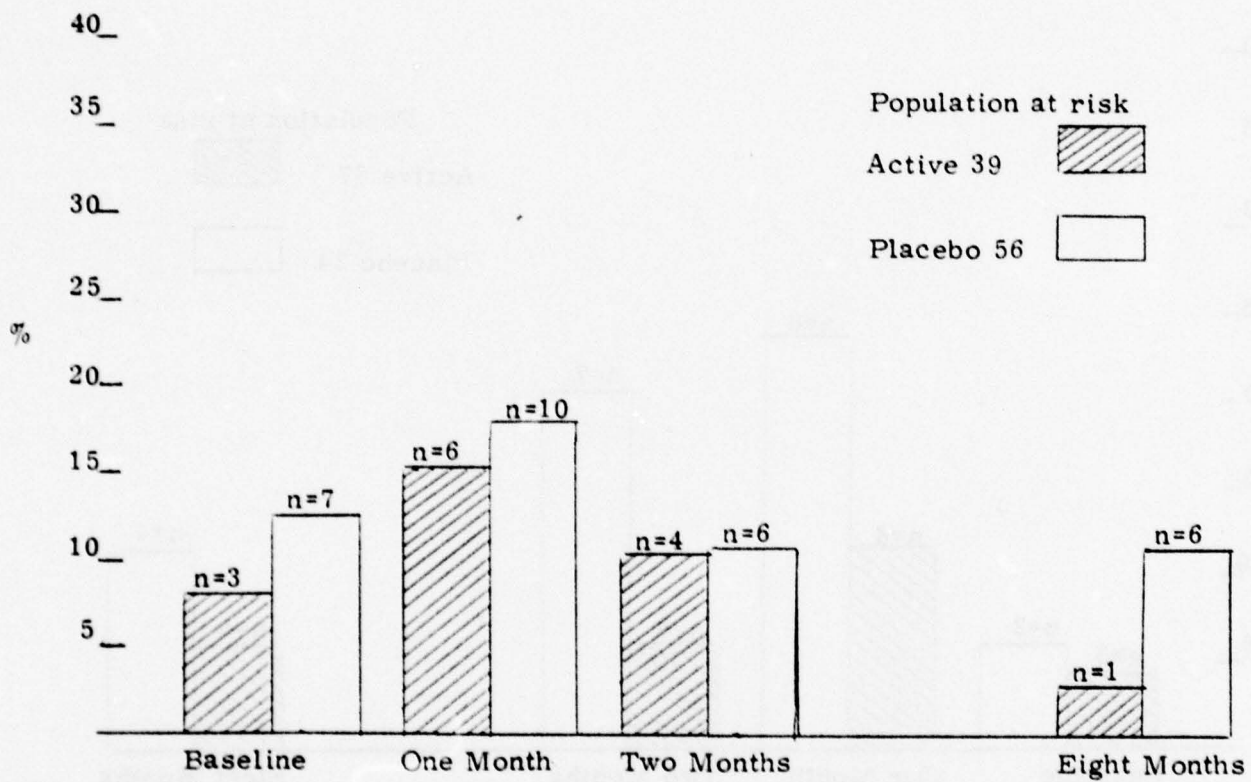
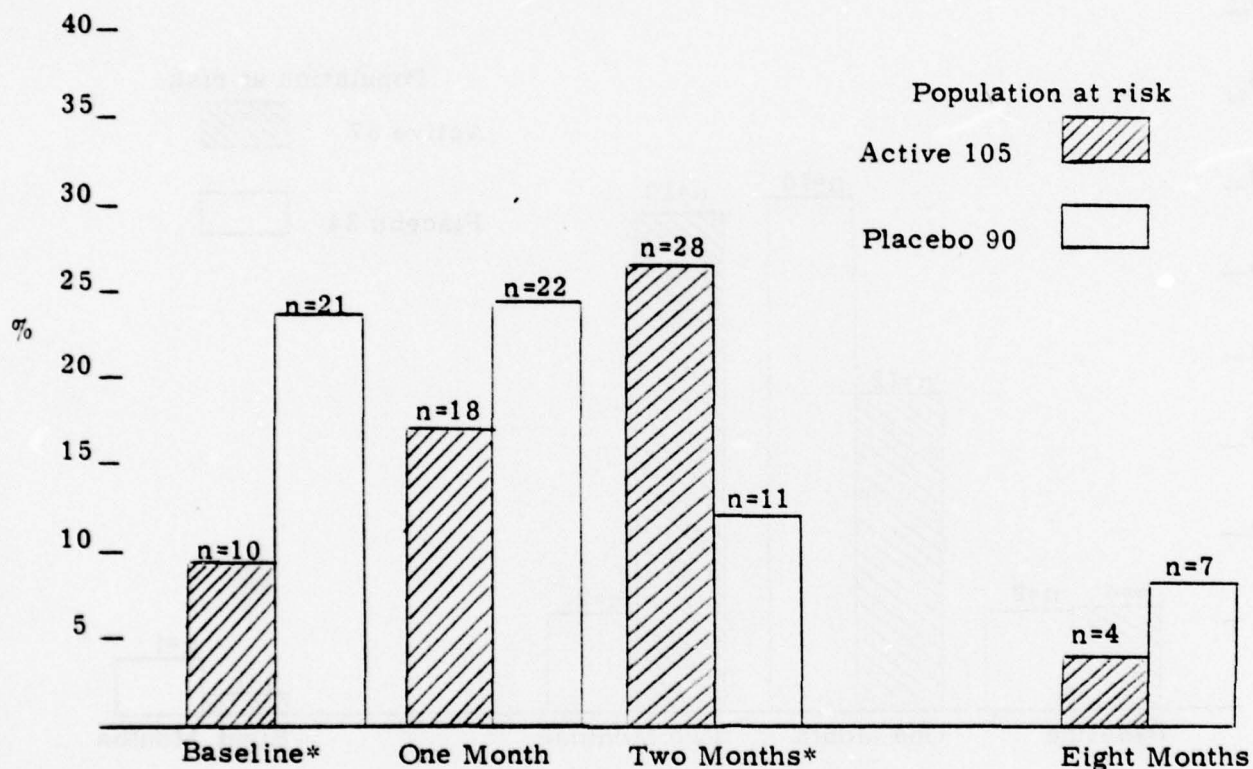


Figure 8

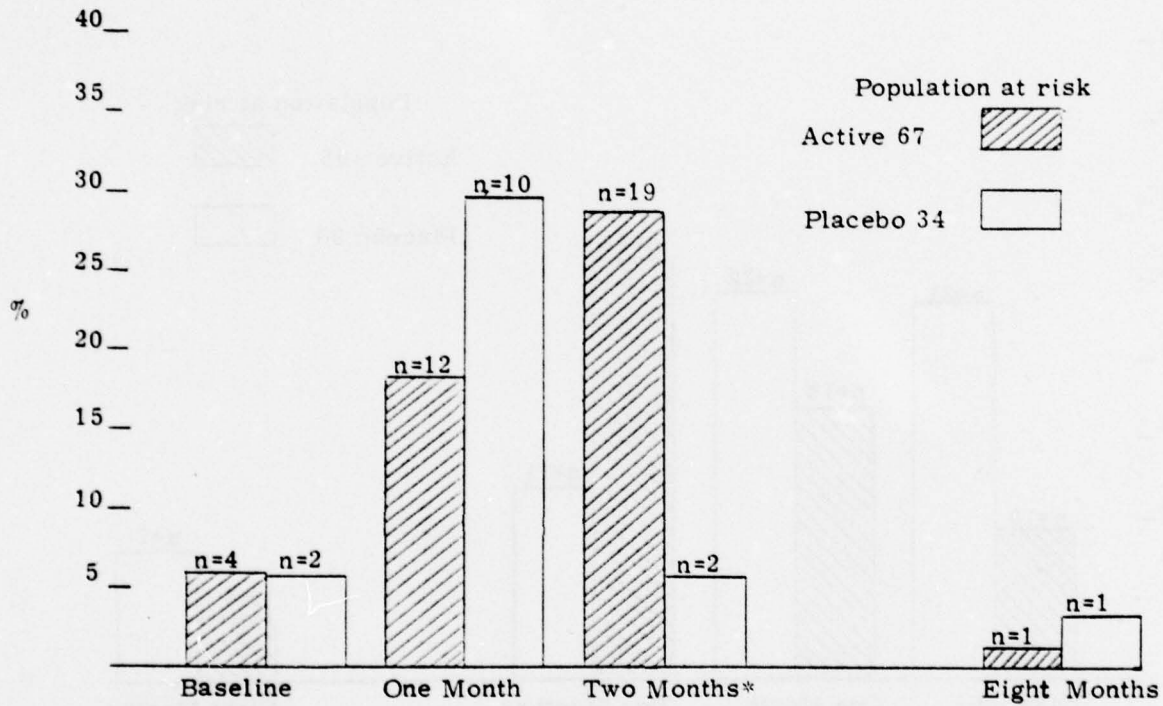
**POINT PREVALENCE OF STREP PYOGENES
NORMAL SKIN, COMPLETERS ONLY**
Children with one or more positive skin sites by RODAC method
as a percent of the population at risk.



*The differences between active and placebo at these points prove to be significant at the 0.05 level according to Chi-Square.

Figure 9

POINT PREVALENCE OF STREP PYOGENES
 NORMAL SKIN, FEMALE COMPLETERS ONLY
 Female children with one or more positive skin sites by RODAC
 method as a percent of the population at risk.



*The differences between active and placebo at this point prove to be significant at the 0.05 level according to Chi-Square.

Figure 10

POINT PREVALENCE OF STREP PYOGENES
 NORMAL SKIN, MALE COMPLETERS ONLY
 Male children with one or more positive skin sites by RODAC
 method as a percent of the population at risk.

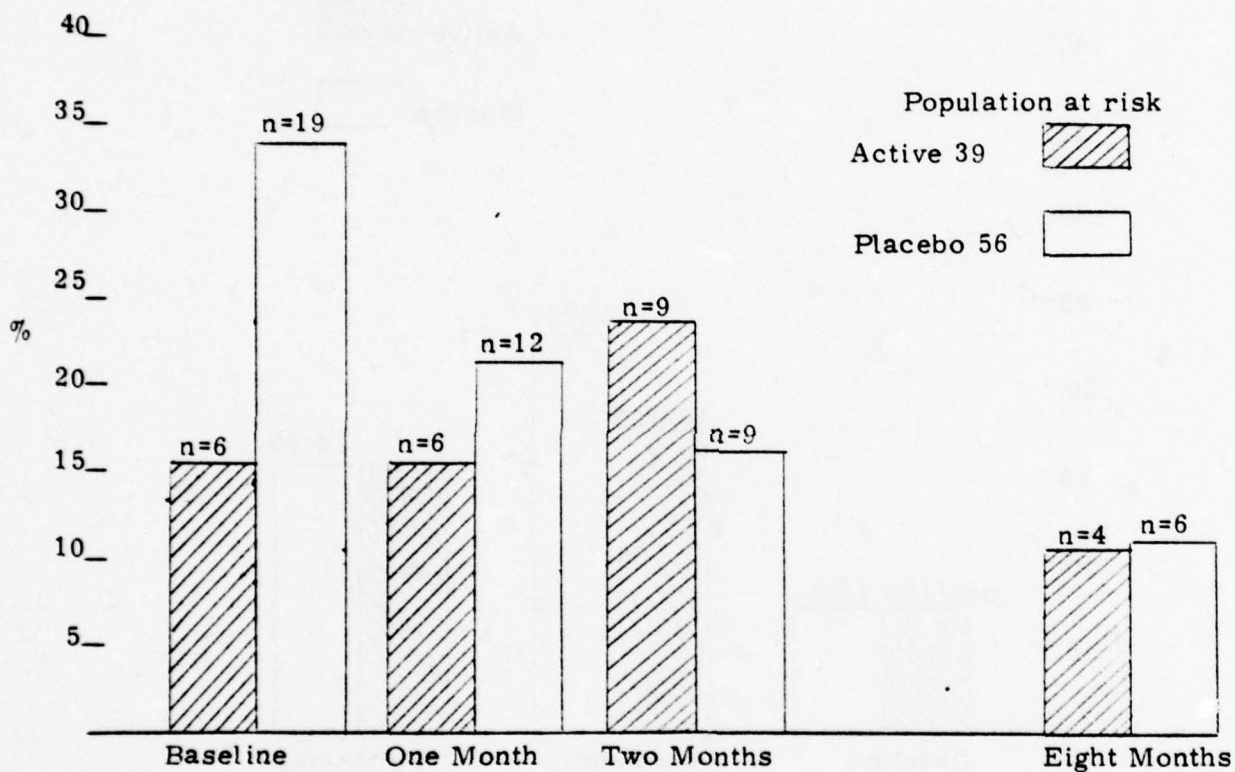


Figure 11

POINT PREVALENCE OF STAPH AUREUS
 ODD-NUMBERED COMPLETERS WITHOUT CLINICAL INFECTIONS
 Odd-numbered children with one or more positive skin sites by RODAC
 method, without clinical infections, as a percent of each population
 at risk.

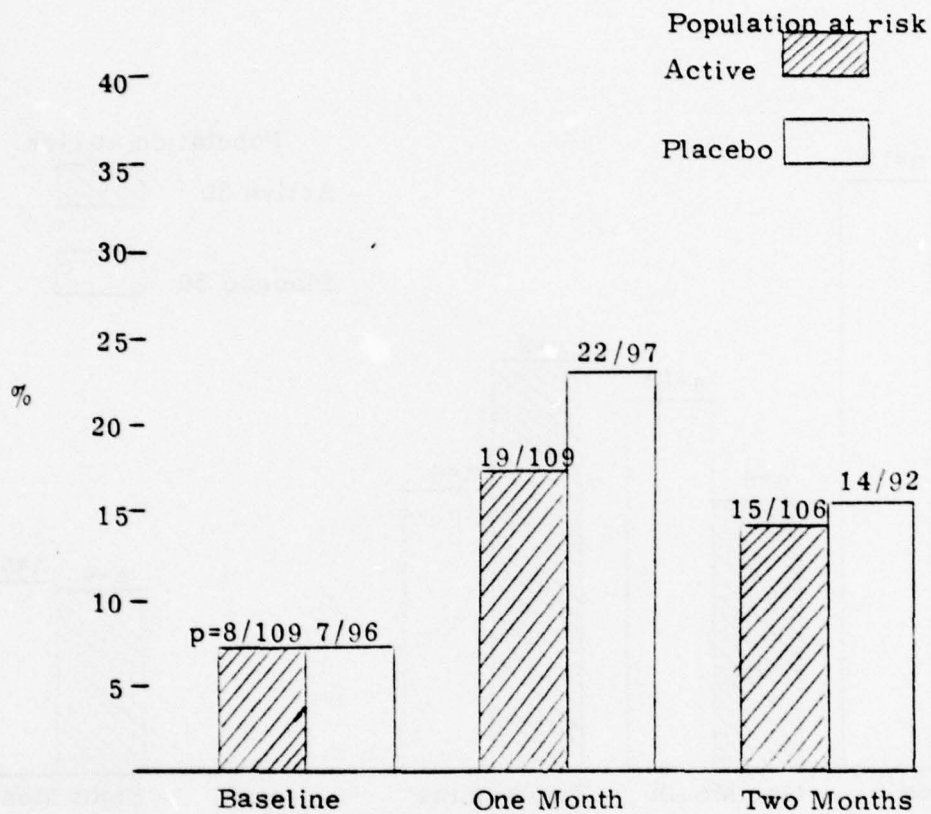


Figure 12

POINT PREVALENCE OF STAPH AUREUS
 NORMAL SKIN, ALL CHILDREN WITH CLINICAL INFECTIONS
 All children with one or more positive skin sites by RODAC
 method, with one or more clinical infections as a percent of
 each population at risk.

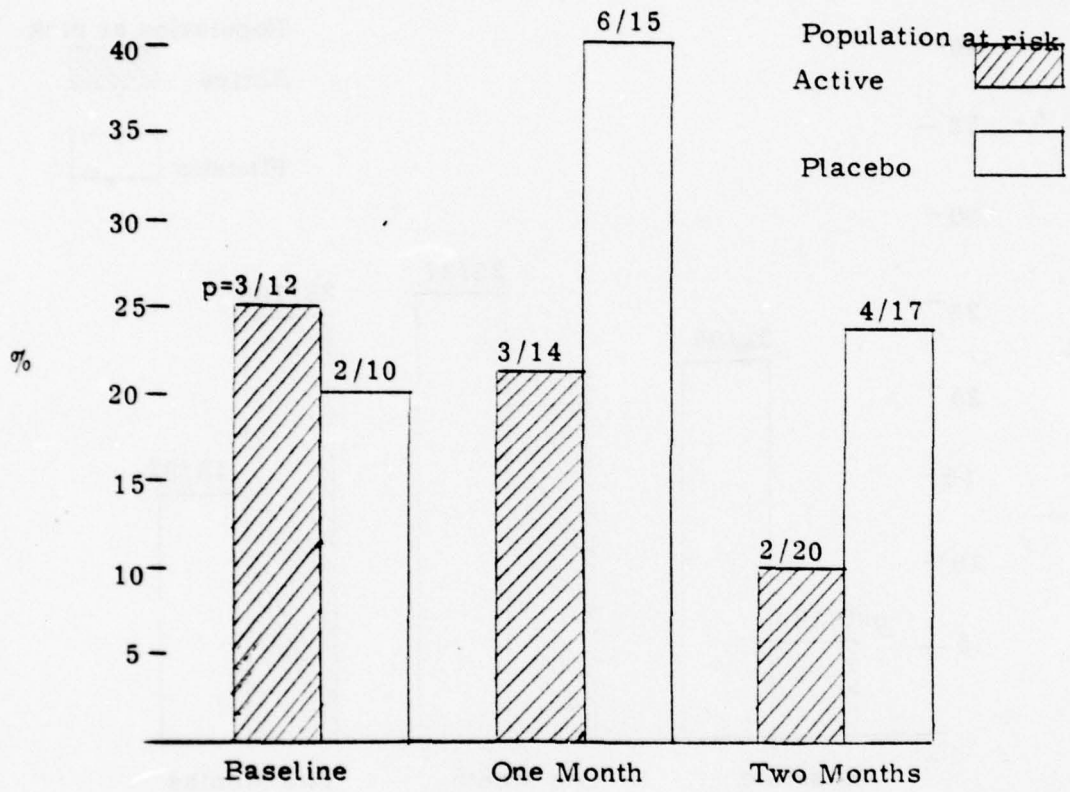


Figure 13

POINT PREVALENCE OF STREP PYOGENES
 NORMAL SKIN. ODD-NUMBERED COMPLETERS WITHOUT
 CLINICAL INFECTIONS

Odd-numbered children with one or more positive skin sites by
 RODAC method, without clinical infections, as a percent of each
 population at risk.

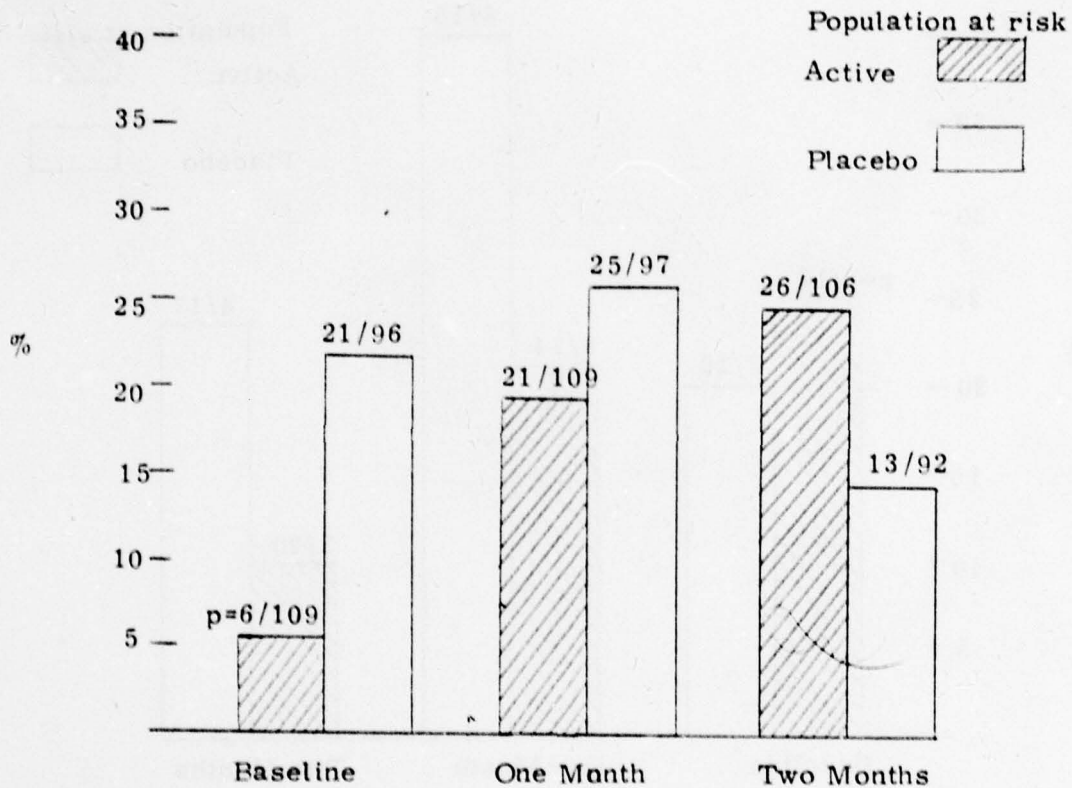


Figure 14

POINT PREVALENCE OF STREP PYOGENES
NORMAL SKIN, ALL CHILDREN WITH CLINICAL
INFECTIONS

All children with one or more positive skin sites by RODAC
method, with one or more clinical infections, as a percent
of each population at risk.

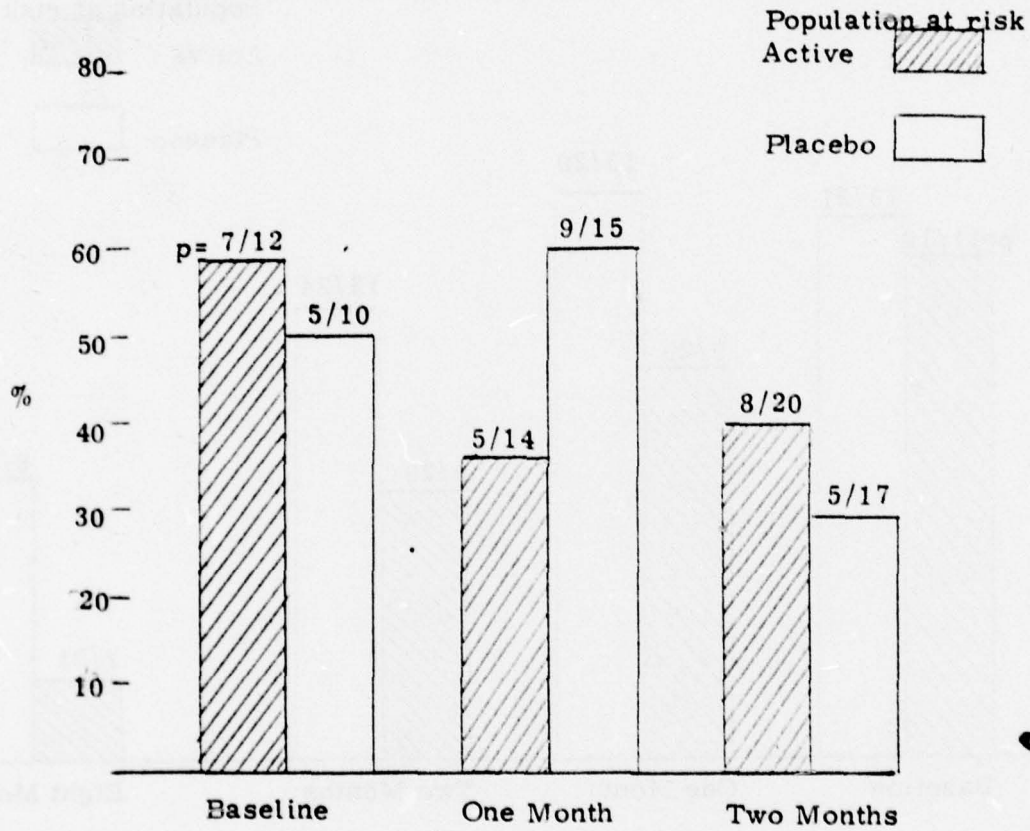


Figure 15

POINT PREVALENCE OF STAPH AUREUS
RECOVERY FROM SORES OF CHILDREN WITH CLINICAL
INFECTIONS, RAW DATA

Children with one or more positive skin sores as a percent of
each population at risk.

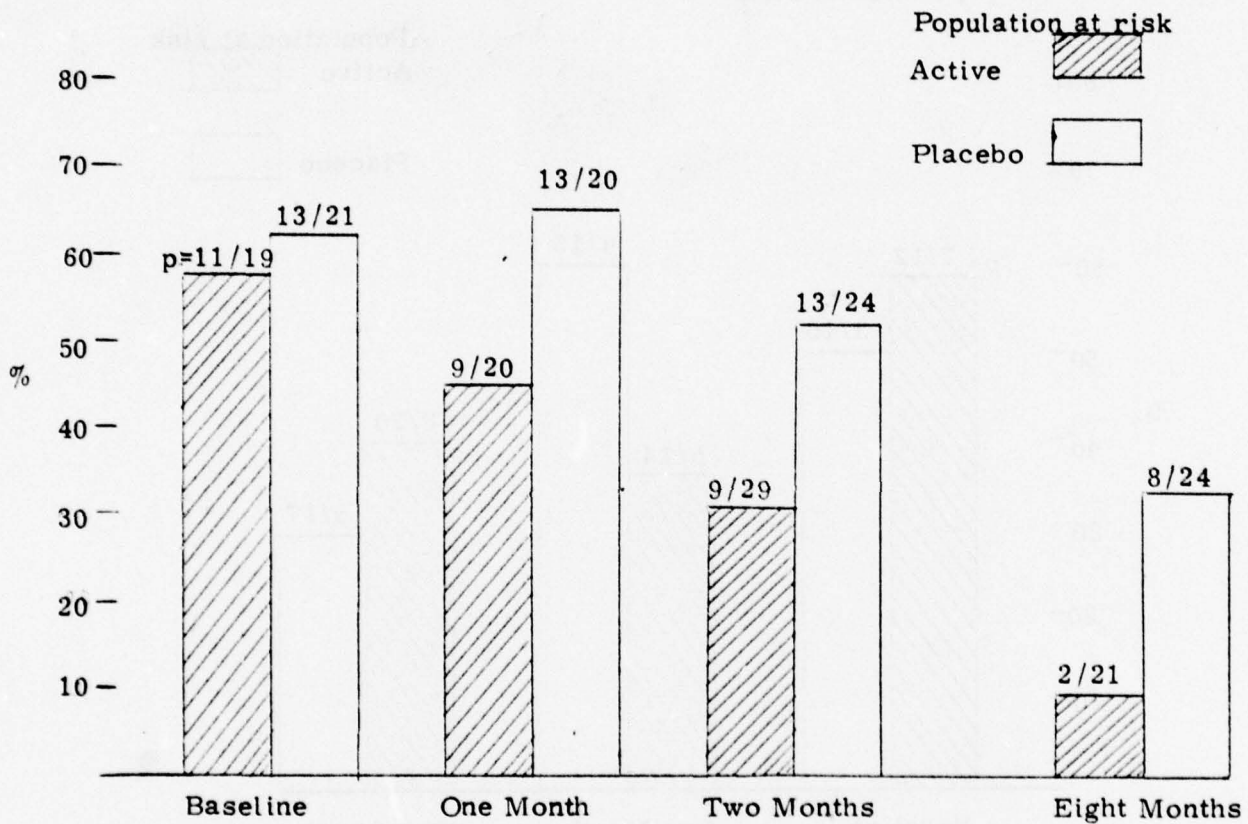


Figure 16

POINT PREVALENCE OF STREP PYOGENES
RECOVERY FROM SORES OF CHILDREN WITH CLINICAL
INFECTIONS, RAW DATA

Children with one or more positive skin sores as a percent
of each population at risk.

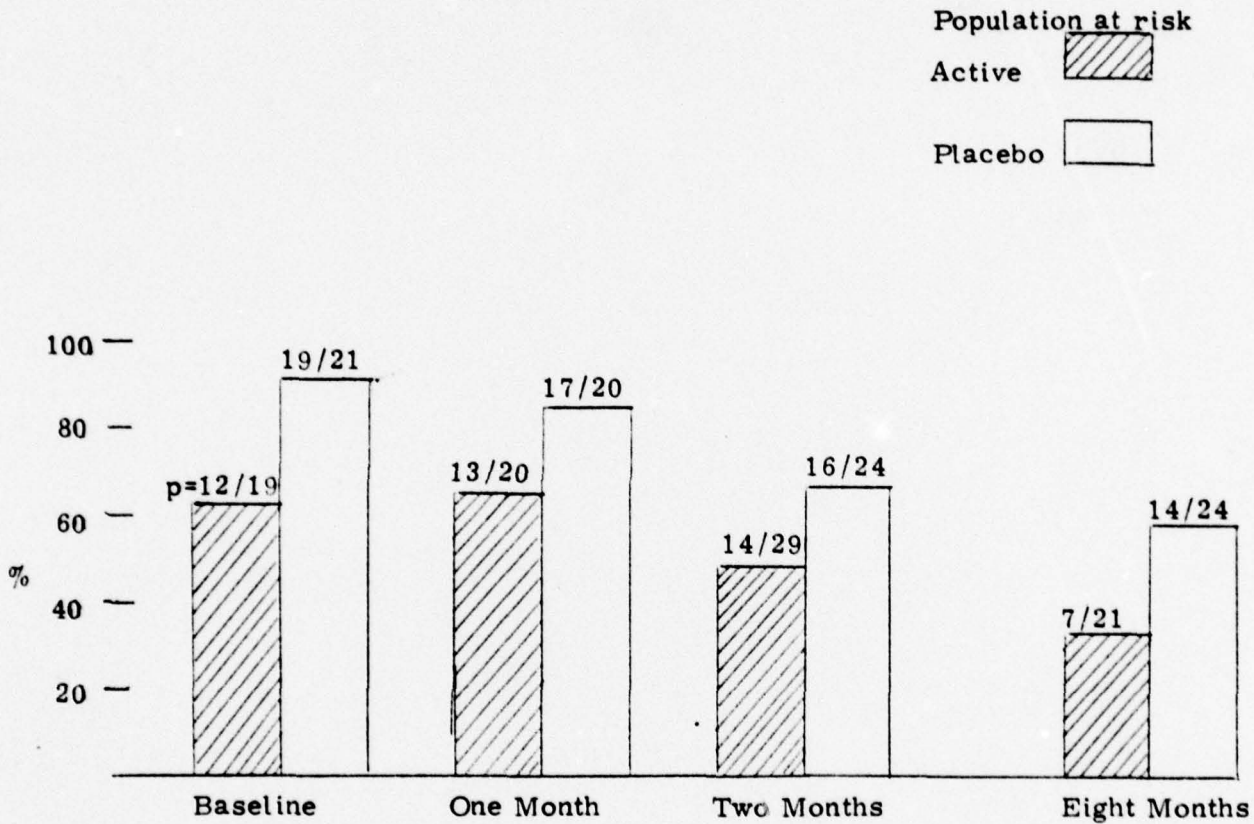


Figure 17

Field Epidemiology

"Through direct observation of health and disease in nature, the aim is to identify varying distributions of a condition, in place and in time, and according to characteristics of the people affected, with the objective to determine factors of man and of environment active in causation. It is the diagnostic instrument of mass disease. It rests on principles derived from Thoreau, that if one would learn about nature, it is necessary to go where it is An epidemiologic analysis leading to identification and assembly of those characteristics of man and environment contributing to the complex of causality is clearly the prime requisite to a programme for prevention and control of a community disease. Control operates on epidemiological facts."

from "Ecologic Interplay of Man, Environment and Health", John E. Gordon, American Journal of Medical Sciences 252, September 1966.

The principles expressed by Dr. Gordon almost ten years ago represent the backbone of our field studies under this contract, and as we see it, our major contribution to the needs of the USA Medical R & D Command; that is to establish the epidemiological facts on which future control measures and research efforts may be based. This portion of the report concerns itself with our progress in translating these well-meaning phrases into actual productive work in the field.

In 1968 and 1969, the field epidemiologic team under Captain (now LTC) Alfred M. Allen was provided with a sophisticated laboratory facility without which this productive period of USAMR & D sponsored research would not have been possible. The many techniques and experience developed under this contract contributed much to the success of this team, but it still required a base laboratory in the field to support the team in microbiologic capabilities. Since 1969, we have attempted to continue our programme in field epidemiology in Uganda, Colombia, Venezuela and Nigeria. In each of these countries we were provided facilities and laboratory space by the University centers in Kampala, Bogota, Caracas and Lagos. Although our hosts were hospitable and generous, the projects were often in jeopardy because of seemingly small problems which arise under the stress and time considerations imposed by handling hundreds or sometimes thousands of culture isolates. In large scale mobile epidemiology studies, efficiency in media preparation, handling of cultures on a daily basis and preparing them for safe shipment back to the United States becomes of paramount importance, and all of this must be accomplished while finding relatively safe food and water for the field team, arranging schedules for the population studies, finding sleeping accommodations and taking care of the many liaison and logistical problems.

Much of what we take for granted in our home laboratories (incubation space at the correct temperature, glassware, disposable petri dishes, refrigerator storage and electricity 24 hours a day) were often not available in the area of operations. We, therefore, conceived the idea of a self-contained mobile epidemiology laboratory and living facility for a field team, able to go anywhere in the world to conduct surveys, maintain the health and comfort of the team, and be able to handle the intake of specimens on a daily basis. We were concerned with the total logistical problems of getting field teams into endemic and often remote areas and out again with the least encroachment on the limited resources of the host country and with maximum efficiency and welfare of the team. The following is a report of progress and our experience in Costa Rica.

Planning: In the early months of 1975, arrangements were made with the University of Costa Rica in San Jose to train a field team in Costa Rica and to begin epidemiologic surveys in areas which had not been previously evaluated. Appropriate permissions were obtained from the Ministry of Health, the University of Costa Rica, the College of Physicians and Surgeons and the Costa Rican Dermatologic Society. The team was to consist of senior microbiology students and physicians from the University of Costa Rica and the project was to be coordinated by David Taplin. The mobile facility consisted of a modified HI-LO trailer which collapsed for towing and shipping, but could be extended to full height when on station. The interior was equipped with 110 V and 12 V wiring and lighting, two work benches, double sink, toilet-shower, stowaway beds to sleep six, 110/12 V incubator, centrifuge, microscope and consumable supplies to conduct microbiology. Our hosts informed us that the project should get underway not later than June 1st, due to the encroachment of the rainy season and the likelihood that some of the roads in remote areas might not be usable.

The trailer and Jeep Wagoneer were shipped from Miami to Puerto Limon in mid May, 1975, where we met the ship and drove the unit directly to the University of Costa Rica. The first week was spent in conferences and liaison meetings with faculty members of the University, selecting students for the team, orientation lectures and planning. It was decided to make a preliminary run to two isolated villages, Naranjal and Cangrejal in the center of Costa Rica, which were highly endemic for cutaneous leishmaniasis. The students selected were about to graduate from a four year programme and were skilled in clinical microbiology, parasitology, immunology and mycology, but had little exposure to organized field epidemiology. Their faculty advisors were highly enthusiastic at the prospect of sending their best students into the field. With less than a week to prepare, five students were assigned a variety of projects to tackle, in addition to our primary objective of estimating the prevalence and etiology of skin infections. The projects selected for them included:

a) Collection of blood samples from cases of healed and active cutaneous leishmaniasis for later serological testing with an equal number of matched controls.

- b) Evaluation of immediate and delayed skin test reactions to two antigen preparations from Leishmania braziliensis.
- c) Trapping and collecting slugs (Vaginulus (Sarasinula) plebeius) and small rodents, the intermediate and definitive hosts of Angiostrongylus costaricensis.
- d) Evaluating the prevalence and etiology by cultural methods of common skin infections, both bacterial and fungal.

Any one of these projects was worthy of a field study in itself, and considering the difficult roads, an untested mobile unit staffed by a newly formed team, and only a vague idea of the population distribution, it was unlikely that any of the projects would be completed in the seven to ten days we estimated we could remain on station. However, rather than introduce a negative approach, we tackled all of the tasks, believing that this would be the quickest and most effective way of gaining experience as a team. It was indeed a valuable lesson. The trailer was stationed in San Ignacio, and we proceeded to the villages of Cangrejal and Naranjal using the Jeep Wagoneer. The roads to these two villages consisted of 25 kilometers of narrow uneven cart tracks of red clay winding through mountainous terrain, which although navigable in the morning, became treacherous mud slides after the afternoon rains. We eventually resorted to packing supplies on a horse and walking the team in, but even this presented problems for man and horse in attempting to maintain a foothold on the steep inclines. After four days a bruised and exhausted team succeeded in culturing 12 cases of pyoderma, identified 11 cases of leishmaniasis, trapped one cotton rat and collected ten slugs. Landslides cut off our water supply in San Ignacio, and the water supply in the trailer became contaminated with transmission fluid when local boys who had climbed a mountain to bring us water in cans used the funnel from our tool kit to fill the on-board holding tank. In four days we wore out the disc brakes on the jeep, had problems with the lights and transmission and split the fuel tank, losing an entire day and night for repairs. In short, we spent more time solving mechanical and logistical problems than conducting field epidemiology, which was not likely to be meaningful in any case because the population at risk lived in isolated dwellings among the mountains rather than in two discrete "villages" as we had expected. Therefore, no estimate of prevalence of disease could be obtained because we had no denominators.

Thus, in the first week this fledgling team learned some valuable lessons:

- a) A prior scouting expedition to the proposed target population is essential to evaluate road conditions, population distribution, and to arrange for a census or appropriate sampling technique if prevalence data is sought.
- b) Tackling a variety of clinical problems dilutes the team effort and is likely to produce no worthwhile results from any one project.
- c) Testing of hardware (e. g. the Jeep) must be conducted under conditions to be expected in the target area. While suitable for the flatlands of Florida, the rough mountain tracks in Costa Rica resulted in major mechanical

failures after only one week.

d) No essential functions (supply of drinking water for example) should be delegated to non-team members.

e) The basic laboratory field methods worked well.

f) The team could work in harmony under conditions of severe stress.

On returning to San Jose, meetings were held with the Dean of the School of Microbiology, other Faculty and the field team students. It was decided that the lessons learned in the first week were valuable, and that the team was now prepared to tackle a definitive single study related to skin infections. David Taplin was appointed team chief, and the field team was to consist of four students and a physician, with another physician to follow up cases in need of urgent medical care. In this way the field team could concentrate on epidemiology without ignoring the need of health care delivery, at least for the most deserving patients. Three objectives were selected:

1) To estimate the prevalence of skin diseases among school children in rural areas in relation to environment (altitude, climate, vegetation, insect vectors, agricultural practices, housing), age and sex.

2) To identify populations with significant problems to guide future allocation of medical personnel and funds, or areas where future clinical studies would be rewarding.

3) To test two methods (LAIR medium and MLAMI medium) for recovery and presumptive identification of Staphylococcus aureus from skin lesions under field conditions.

Background: Between Santa Ana, 20 kilometers west of San Jose and the town of Parrita on the Pacific coast of Costa Rica, there are 23 schools on or near the highway over a distance of approximately 140 km. None is more than 8 km from the next school and each has an enrollment of between 60 and 230 students, ranging in age from 7 to 16. These schools are situated at altitudes ranging from 1,100 M at Cerbetana to sea level at Parrita. All children are of similar ethnic derivation, and all populations in the study were rural. These schools, therefore, represented populations at risk living at altitudes which could be much more closely defined than in previous studies. Moreover, nothing was known concerning the relative frequency of skin diseases, nor their etiology.

Mindful of the lesson learned in the first field experience, Dr. Roger Bolanos and David Taplin made a preliminary visit to all 23 schools along the highway, interviewed the school principals and evaluated the logistical problems. From this trip it was immediately evident that there was no clear understanding of the prevalence of skin infections, except that the reports from higher elevations suggested that there were "very few" whereas in Parrita the teachers reported "a lot". We were also impressed with the myriads of Hippelates flies in Parrita which we had not encountered at the higher elevations. It was also apparent that the temperature at 100 M and only ten Km distant was sensibly lower than the sea level area of Parrita.

The situation appeared ideal for our studies, and nine representative rural schools between Puriscal and Parrita were selected for surveys. Be-

cause the highest prevalence and consequently, the greatest work load was likely to be found in Parrita, it was decided to drive the mobile unit directly there and to work back, thus taking advantage of a fresh rested team where working conditions would be most adverse. The team was reassembled, the trailer stocked with food and media, the Jeep repaired and we drove uneventfully to Parrita to begin the studies.

Methods: At each school, attendance was checked against enrollment and all children not in school at the time of the survey were accounted for and their reason for non-attendance recorded. All children were examined from head to toe by one observer (D. T.) and all skin lesions recorded on a body diagram sheet. A notation of clinical diagnosis and stage of disease were recorded at this time with instructions for the two microbiology stations, according to the following criteria:

Active pyoderma - culture (well circumscribed single lesion with pus, erythema and crust, obviously infected),

Healing pyoderma active - culture (obviously has been worse and now healing, but still clinically infected),

Healing pyoderma dry - no culture (still evident lesion, but not an old scar, no evidence of infection, dry intact epidermis, no crust).

Old scar - no culture (obviously was once a pyoderma, but now only residual superficial scar or hyperpigmentation).

Pyodermas active - take best one for culture (used for clusters of lesions or multiple lesions, most purulent or active selected).

Very early small - culture (any very small lesion not yet echthymatous, but looks infected, e. g. pustule, infected insect bite, etc.).

Infected abrasion - culture (any abrasion showing serous or purulent exudate with erythema).

Fresh abrasion not infected - no culture.

Old leishmaniasis scar - record origin (no active cases of leishmaniasis seen in this survey, all old scars were in children who once lived in endemic area).

Two microbiology stations were set up, each manned by two students using identical methods. Crusts were lifted to expose pus and healing lesions were gently knicked with a scalpel and squeezed to express underlying pus. Material was collected on two calcium alginate swabs; one was used to inoculate Trypticase Soy Agar with Sheep Blood (TSAB) and the same media containing 0.8 $\mu\text{g/ml}$ Crystal Violet; the other was used to inoculate a plate of LAIR Staphylococcal medium and a plate of MIAMI Staphylococcal medium. Clinical photos were taken of typical lesions. All plates were streaked identically within 2 hours of collection, and incubated at 35 to 37°C. All cultures were read at 24 hours, scored and picked by the same observer (D. T.). Cultures were preserved for return to Miami, where S. aureus was confirmed by tube coagulase test. S. pyogenes was identified by hemolysis on Sheep Blood Agar and bacitracin sensitivity. Antibiotic sensitivities were evaluated by the Kirby-Bauer technique.

Results: Figure 1 shows the relationship of altitude to prevalence of

PREVALENCE OF CULTURE CONFIRMED CASES OF STREPTOCOCCAL PYODERMA
IN RURAL SCHOOLS COSTA RICA JUNE 1975
RELATED TO ALTITUDE

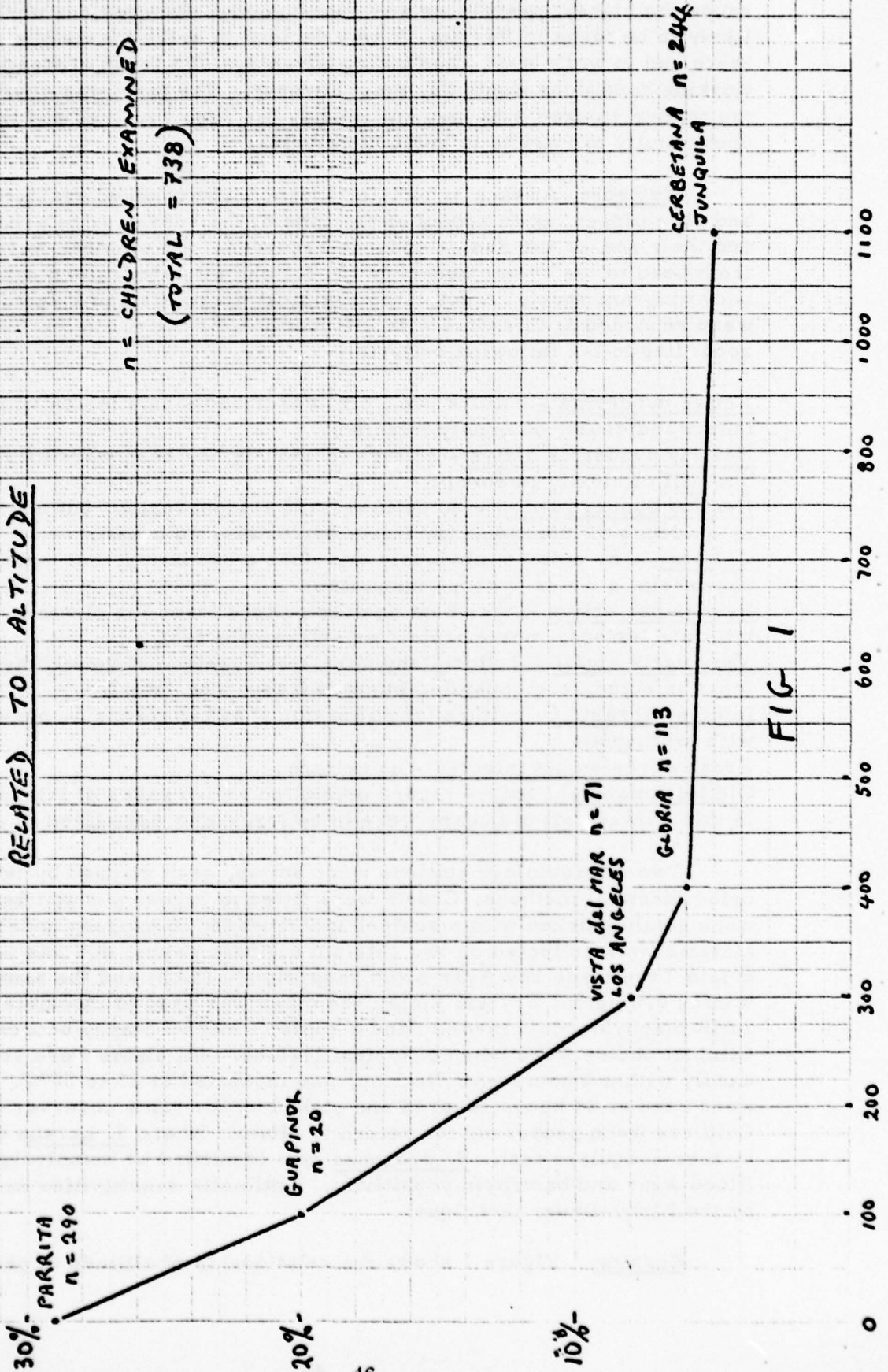


FIG-1

TABLE 1

Prevalence of Pyoderma
PARRITA School - Costa Rica

Age	BOYS	Age	GIRLS
	# infected/ # examined		# infected/ # examined
6	10/10	6	8/15
7	3/15	7	4/16
8	6/14	8	8/13
9	5/12	9	6/20
TOTAL	24/51=47%		26/64=41%
10	4/13	10	7/19
11	3/13	11	1/9
12	1/10	12	2/12
13	1/6	13	0/5
TOTAL	9/42=21%		10/45=22%

Overall TOTAL 69/202 children with active clinical lesions
= 34% prevalence of pyoderma

64/202 children with positive strep culture
= 32% laboratory proven strep pyoderma

TABLE 2

PARRITA School - Costa Rica

<u>Type of Lesion</u>	<u>Total Children Sampled</u>	<u>Recovery of S. aureus</u>	<u>Recovery of Strep pyogenes</u>
early	18	10 (55%)	12 (67%)
active	55	46 (84%)	54 (98%)
healing	10	7 (70%)	10 (100%)
TOTAL	83	63 (76%)	76 (92%)

TABLE 3

Antibiotic resistance in S. aureus. Clinical isolates
Costa Rica. Kirby-Bauer method. 322 strains tested.

<u>Antibiotic</u>	<u>% resistant</u>
Penicillin G	68
Tetracycline	15
Erythromycin	5
Chloramphenicol	3
Cloxacillin	1.2
Cephalothin	0.6

TABLE 4

63 Post-Pubertal High School Students
Gloria High School

Diagnosis	Males (28)	Females (35)	Combined Prevalence
T. pedis	7	11	26%
T. corporis	3	4	11%
T. versicolor	7	5	19%
Pyoderma	3	4	11%*
Old leishmaniasis	6	6	19%

*only 4 pyodermas yielded streps = 6% proven strep pyoderma

confirmed streptococcal pyoderma. Many were severe or multiple infections. Only 12 Km distant, at an altitude of 300 M, the prevalence was only 8%. This was little different from schools at altitudes up to 1,100 M. Table 1 shows the prevalence of clinically active pyoderma in one Parrita school by age and sex. There was no difference in prevalence relating to sex, but there was a difference relating to age. The number of children examined represents the age distribution of the school enrollment (only 6 of 208 children were absent from school). The number of children at each year of age from 6 to 13 is too small for prevalence determination by year, but it can be seen that children of either sex under 10 years of age were twice as likely to be infected than their older school friends. The data further suggest that the six year olds were most at risk. Unfortunately, we could not examine preschool children without designing a separate study. However, the 70% prevalence (18 of 25) among the six year olds certainly suggests that younger children might also suffer a high prevalence of infection.

Table 2 is an attempt to determine the initial etiology of the common skin infections. Lower recoveries of S. aureus and S. pyogenes were obtained from early lesions when compared with active or healing lesions. Overall, however, the figures are close to those we obtained in previous tropical areas, i. e. over 90% of all purulent skin infections, regardless of the initiating factors or clinical appearance, yield a Group A Streptococcus pyogenes.

Antibiotic resistance: Antibiotic sensitivities were performed by the Kirby-Bauer technique on the S. aureus and S. pyogenes recovered during these studies.

Table 3 shows the percent of strains of S. aureus resistant to six antibiotics. There was no geographic clustering of resistant strains. This data is interesting because of the high percentage of strains resistant to Penicillin G (68%), from skin lesions of children in areas where antibiotics were not available and few if any children had ever received penicillin. This is similar to Dr. Allen's findings in a remote village in Panama, and indicates that penicillin resistance is not necessarily related to the therapeutic use of penicillin. We even found strains resistant to Cloxacillin, not available at all to the populations at risk, again indicating the presence of small reservoirs of resistant strains in remote rural areas. One strain from a patient was resistant to Penicillin G, Ampicillin, Tetracycline, Cloxacillin and Chloramphenicol. The same patterns of resistance were found in strains isolated from Hippelates flies; 9 of 18 strains were resistant to Penicillin G. One was also resistant to Cloxacillin. All S. aureus isolated from any source was sensitive to Neomycin.

Ninety-five percent (95%) of all S. pyogenes tested (129 strains) were sensitive to Bacitracin (i. e. presumptive Group A). Only 5 cases in the entire survey yielded a non-group A strep as the only hemolytic streptococcus recovered. Eighty percent (80%) of isolates were resistant to Neomycin, 5%

were resistant to Tetracycline, and all were sensitive to Penicillin G, Chloramphenicol, Erythromycin and Cephalothin.

We were impressed by the abundance of Hippelates flies seen feeding on the purulent skin lesions and fresh abrasions of the Parrita children. Two students captured 14 flies on or near skin lesions in individual sterile containers. Three days later they were squashed in sterile saline and one drop was streaked on culture media. Six yielded S. aureus and four yielded β Hemolytic Streps.

Table 4 depicts the results of a survey in a high school at Gloria (altitude 400 M), where the students were aged 13 to 19. This was the only population in which we found dermatophytosis and Tinea versicolor. One in every four students had Tinea pedis, one in ten had Tinea corporis, one in five suffered from Tinea versicolor, and every fifth student carried one or more scars of cutaneous leishmaniasis (all of these had active leishmaniasis while living as children in Turalba or Puriscal). We found no cases of the "post-pubertal" skin diseases listed above among 113 children, 7 to 13 years old, in a school only 2 Km distant and only 60 M higher, but we did find exactly the same prevalence (6%) of streptococcal pyoderma.

Evaluation of Miami and LAIR media for isolation and identification of Staphylococcus aureus under field conditions:

For several years under this contract, we have been developing a method for the isolation and identification of Coagulase positive S. aureus which would be suitable for field epidemiology and not require microbiological expertise.

Our current formation (PYM) is a nutrient agar base containing polymyxin B to selectively inhibit gram negative bacteria and coagulase negative gram positive bacteria, actidione to inhibit molds, yeast extract as a growth supplement, mannitol and Brom Cresyl Purple to indicate acid production from mannitol.

Independently, the LAIR team had been working with a mannitol, salt, phenol red, egg yolk formulation to indicate mannitol utilization and lecithinase production. The Costa Rican survey of pyoderma provided an opportunity to evaluate both methods in the field.

Methods: Duplicate swabs were taken at the same site on each lesion. One swab was used to inoculate a plain blood agar plate (TSAB) and a selective medium for S. pyogenes (TSAB-CV). The other swab was used to inoculate a plate of LAIR medium and a plate of polymyxin, yeast mannitol (PYM). The order of inoculation alternated.

All plates were streaked in a standard manner, incubated within 2 hours of culture at 35-38°C and examined by one observer (D. T.) at exactly 24 hours. The amount of growth (+ to +++) was recorded together with color change in the media (red to yellow LAIR), (purple to yellow PYM). Lecithinase activity on LAIR medium was also recorded. A typical colony was picked for later testing. The criteria for picking were as follows:

LAIR - Any circular colony surrounded or overlying a halo of lecithinase activity or exhibiting acid production, or both. If no such colonies could be seen, a circular colony representing the predominant growth was picked.

PYM - Any circular colony showing surrounding zones of acid production from mannitol. If no such colony was present, a circular colony representing the predominant growth was taken.

This data is based on 125 pairs of cultures. Table 5 shows the distribution of all combinations of pairs obtained in the study.

TABLE 5

Recovery of coagulase positive S. aureus

	LAIR	PYM	No. of occurrences
	Pos	Pos	92
	Pos	Neg*	1
	Neg*	Pos	8
	Pos	no growth	14
	Neg*	no growth	10
<u>Total S. aureus</u>	107	100	

* grew an organism resembling S. aureus, but coagulase negative

There were 14 recoveries of S. aureus on LAIR which were missed on PYM. Eight (8) recoveries were obtained on PYM and missed on LAIR.

There were 18 false positives on LAIR (growth of an organism resembling S. aureus, but coagulase negative) and one false positive on PYM.

The recovery rate of S. aureus in 125 attempts was 86% for LAIR and 80% for PYM.

Table 6 expresses results in terms of the chances of any growth being S. aureus on each medium. For this table five contaminants on PYM were excluded because they were spreading growths of aerobic spore formers easily differentiated from S. aureus.

TABLE 6

125 Pairs

	<u>Growth</u>	<u>Confirmed as S. aureus</u>	<u>% Confirmed</u>
LAIR	125	107	86
PYM	101	100	99

One in every seven cultures on LAIR was a false positive.

One in every hundred cultures on PYM was a false positive.

Table 7 investigates the value of lecithinase activity alone as an indicator of S. aureus on LAIR medium. For this parameter, all cultures taken on LAIR were considered, since PYM does not contain egg yolk and paired comparison was not applicable.

TABLE 7

All cultures taken on LAIR medium. 127 attempts.

<u>Coagulase</u>	<u>Lecithinase</u>	<u>No. of Occurrences</u>
POS	POS	87
POS	NEG	17
POS	Doubtful	3
NEG	POS	15
NEG	NEG	16

Using lecithinase alone as the method of identifying S. aureus, the method

would have missed 20 of 107 isolates of S. aureus (19%) and incorrectly scored 15 of 20 other organisms as S. aureus.

The value of including mannitol fermentation in both media is outlined in Tables 8, 9 and 10. Since there were no culture plates which changed color with no growth (remained sterile), the results are expressed as growth and color change.

TABLE 8

125 Pairs

	<u>Coagulase POS</u> <u>S. aureus recovered</u>	<u>Color change</u> <u>in medium</u>
LAIR	107	90
PYM	100	94

TABLE 9

	<u>Other bacterial growth</u> <u>(Coag. neg.)</u>	<u>Color change</u> <u>in medium</u>
LAIR	12	12
PYM	6	1

TABLE 10

False results. 125 Pairs. Color change only.

	<u>Growth</u>	<u>False POS</u>	<u>False NEG</u>	<u>Total</u>	<u>%</u>
LAIR	125	12	17	29	23
PYM	106	1	6	7	7

Color change alone, as a method of identifying S. Aureus in 125 attempts, would have missed 17 of 107 isolates (16%) and incorrectly scored 12 of 18 other organisms as S. aureus using LAIR.

Using PYM, 6 of 106 isolates would have been missed on color change alone (6%), and incorrectly scored one other organism as a S. aureus.

Mannitol utilization and lecithinase together as indicators of S. aureus are shown in Table 11.

TABLE 11

125 Cultures on LAIR

	Coagulase POS staph	Other growth
Mannitol and Lecithinase Positive	77/107	5/18

In 125 culture attempts, an identification based on color change and lecithinase reactions combined would have missed 30 of 107 S. aureus (28%) and incorrectly identified 5 of 18 other organisms as S. aureus.

Discussion: The objective of developing a field method for recovery of S. aureus from skin infections without laboratory facilities (except for an incubator) and no knowledge of microbiology appears to have been largely achieved in this study using the Miami PYM medium.

Without any prior training, a person would have been 94% accurate in identifying any growth on PYM as S. aureus. Instructions to ignore any flat, spreading growth (Bacillus spp) would increase the accuracy to 99%.

The penalty for this accuracy may be a loss of 6 potentially positive cultures in every 100 attempts, since there was a difference in overall recovery of S. aureus of 6% in favor of the LAIR medium. Surprisingly, the addition of mannitol and a pH indicator contributed nothing to the medium in terms of accuracy.

The LAIR medium, although producing luxurious growth and possibly 6% greater recovery rates, needs further laboratory confirmation of cultures to be useful. Growth alone was of no help to the untrained person, since all

125 plates grew bacteria, and 95% of the cultures were scored as ++ growth.

Color change in the LAIR medium was not reliable. There was a 23% error based on color change alone; too high to be a useful epidemiologic tool. Lecithinase reaction in the LAIR medium was also of doubtful value, producing a 25% error.

If lecithinase reaction and mannitol fermentation were required to identify S. aureus, this would result in a 28% error.

In summary, the LAIR medium resulted in a 6% higher recovery rate than PYM, was less likely to become contaminated during air transport and produces rapid luxurious growth, but required considerable additional lab testing to separate S. aureus from other organisms.

PYM is more expensive because of the antibiotics incorporated, is more susceptible to airborne contamination by Bacillus spp., and is more inhibitory to S. aureus than LAIR medium, but required no experience or additional laboratory confirmation.

We plan to experiment with additional growth supplements to improve the yield and shorten incubation time required for PYM.

DISCUSSION

These studies clearly illustrate what can be achieved in a short period of time by well-motivated and carefully selected field teams, and further illustrates the potential value of the mobile laboratory field unit. Following the "shake down" trip in the first week, the entire series of studies outlined here were completed during one additional week in the field. Thus, a team which had never worked together before, was able to conduct studies within a month of the arrival of the trailer unit in Costa Rica, and arrived at the following conclusions:

- 1) Throughout the entire school system between San Jose and Parrita, the only populations at serious risk to skin infections were children in the delta area of the Rio Parrita. Skin infections in all schools over 300 M altitude are unlikely to reach prevalences over 10% of the school enrollment.
- 2) In Parrita, the youngest children had the highest prevalence, suggesting further investigation of pre-school children. Boys and girls are equally at risk.
- 3) The high prevalence of infection was associated with an abundance of biting insects (mosquitoes and culicoides), and the presence of wound feeding eye gnats (Hippelates). Musca domestica were found at all altitudes, but Hippelates were only found below 300 M.
- 4) Group A Streptococcus pyogenes were recovered from over 90% of purulent lesions. Endemic or epidemic Acute Glomerulonephritis could pose serious problems among the younger children of Parrita, and should be investigated.
- 5) Penicillin resistant S. aureus are abundant even in rural areas where little or no penicillin is used.
- 6) Further studies on bacterial skin infections including clinical therapeutic trials should be conducted in Parrita. Other locations would not be worth the effort.
- 7) A comparison of two diagnostic methods for S. aureus indicated that accurate epidemiology may be conducted with little or no microbiological expertise even under adverse field conditions.
- 8) Dermatophytosis and Tinea versicolor are post-pubertal diseases. Children under 14 would be inappropriate subjects for further studies.

We considered these experiences an excellent start to the field programme in Costa Rica. The concept of a mobile field epidemiology unit was so impressive to our Costa Rican colleagues that plans were made to establish a division of Epidemiology at the University, build around the trailer project. In addition, our experiences in Costa Rica opened up rich new potential for future studies, particularly in programmes of prevention and therapeutic trials. Following the use of the trailer system as a mobile epidemiology unit, another team evaluated its potential as a travelling clinical laboratory. Their report appears in Appendix A.

By July 1, 1975, all preparations were completed to begin a new contract year. The mobile unit was established in Costa Rica, the field team had proven its capabilities, and populations had been identified for further research, prevention and clinical evaluation of currently available therapy. Most important, we had formed a close working collaboration with our Costa Rican hosts and had cleared all formalities with the appropriate University and Government agencies. Unfortunately, we learned on July 10, 1975, that continued funding of this contract would not be forthcoming, and all activities in Costa Rica were put in a holding situation. With no guarantee of a future programme, which depended on our logistical support and involvement, the original team was disbanded in September 1975. We have in the interim kept our options open and hope that the funding situation will eventually allow us to regenerate the programme.

A revised contract proposal which emphasised prevention and therapeutic trials of topical versus systemic treatment of the skin infections in Parrita, passed the University of Miami Committee for the Protection of Human Subjects in Research, but was turned down by the USAMR & D contract review board, primarily, we believe, because of the sensitive nature of the study, which involved children. It is our opinion that few proposals have been more carefully prepared with regard to the protection and welfare of the patients and attention to the laws and customs of the host country, nor received such whole-hearted support from the appropriate in-country agencies.

An on-going and highly productive programme geared to prevention and therapy of significant medical problems is still feasible, and can satisfy all current USA MR & D requirements to be pertinent, unique, cost-effective, and conform to the guidelines for the protection of human subjects. To this we would add our proven ability to work in close collaboration with inservice laboratories, and establish excellent liason with our overseas colleagues.

ACKNOWLEDGEMENTS

We owe a great deal to the Faculty and students of the University of Costa Rica, the Ministry of Health, the Costa Rican Dermatological Society and the Costa Rican College of Medicine and Surgery, and the many other agencies and individuals who shared in this project. To Doctora Eugenie Rudín de Monge, Dean of the Faculty of Microbiology, and to Dr. Roger Bolaños Herrera, Director, Instituto Clodomiro Picado we owe a very special thanks for their untiring efforts on our behalf.

APPENDIX

The following report was submitted on August 8, 1975 to Dr. Eugenie Rudín de Monge, Dean of the Faculty of Microbiology, University of Costa Rica from Dr. Roger Bolaños Herrera, Coordinator, Mobile Laboratory project. It describes the use of the trailer as a clinical laboratory in the field.

"I wish to report on further work conducted under the cooperative programme between the University of Miami and the University of Costa Rica, utilizing the mobile laboratory, which was accomplished between July 20th and July 25th, in the area of Nicoya.

For this evaluation, the mobile unit was equipped as a general diagnostic laboratory to handle the following types of tests: Sputum for T. B., serology for syphilis and autoimmune diseases, blood grouping, blood chemistry, urinalysis, hematology, intestinal parasitology and studies of cutaneous lesions.

In four days of work, we were able to see a total of 103 patients, on whom 239 laboratory tests were conducted as follows:

Hemoglobin	30	WBC's	17
Hematocrits	30	ABO grouping	5
E. S. R. 's	13	Rh factor	5
Blood glucose	6	Microscopy for TB	7
Urinalysis	31	Culture for TB	7
DRL	10	Fungal cultures	2
Rheumatoid factor	1	Serology for toxoplasmosis	5
Faeces (parasitology)	67		

In addition, we preserved serum for later studies of free amino acids in relation to nutrition.

The laboratory was stationed at San Antonio de Nicoya during the four days of work. Doctors of the hospital "La Anexión" referred patients from the hospital and surrounding areas. Every opportunity was afforded the laboratory personnel to see the patients with the physicians.

As a result of this experience, we offer the following conclusions.

- 1) The mobile unit is extremely valuable as an addition to the programme of rural medicine.
- 2) It can best be utilized to support the central medical facility serving population of a particular zone (county or jurisdictional area).
- 3) With a five person team we could handle 500 to 1,000 tests per week.

- 4) We can conduct laboratory studies of high quality and we have the technology to tackle blood chemistry, bacteriology of sputum, hematology, etc. under field conditions.
- 5) The physicians at the Hospital de Nicoya recognized the tremendous effectiveness of this type of laboratory in the practice of rural medicine. Many patients are denied sophisticated diagnostic services because of work obligations, domestic responsibilities or economic factors. The mobile laboratory, to a large extent, provides a solution to these problems.
- 6) We strongly support the addition of a permanent physician on the field team, who could offer in addition to laboratory services, medical consultation, and provide a measure of continuity of patient care.
- 7) The facilities in the laboratory such as shower, beds, air-conditioning, extractors, etc., are totally adequate and indispensable. Future mobile units should include similar items.

(The report also included technical and mechanical recommendations relating to improved type of vehicle for towing, increasing ground clearance of trailer and increasing water storage capacity).

One of the factors not mentioned in this report, but conveyed in strong terms to us by the students, is the significant contributions to morale and comfort provided by the trailer. Previously, they slept wherever they could; on mud floors, in school rooms, or even in the open. The opportunity to sleep comfortably, protected from biting insects, to drink clean water and to take a hot shower, were in their opinion, important to the ability and desire of the team to remain in the field.

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ABSTRACT

(Current reporting period only)

A bar soap containing Trichlorocarbanilide and Trifluorocarbanilide demonstrated no value in the prevention of common skin infections in a double blind study against a plain soap bar, when used daily under supervision for two months. Lack of efficacy against Streptococcus pyogenes was confirmed by in vitro testing.

Field studies in Costa Rica showed that most skin infections were of streptococcal etiology. Children below 10 years of age were most at risk, and children of all ages were at significantly higher risk below 300 M altitude. Sixty-eight percent (68%) of Staphylococcus aureus recovered from lesions were resistant to penicillin even in remote rural areas where penicillin was not available. Dermatophytosis and Tinea versicolor were confined to post-pubertal age groups.

The hazards of bacterial and parasitic skin infections of military importance can be more accurately predicted by surveys of native children than native adults. Anthrophilic dermatophyte infections should be studied in post-pubertal population, but inflammatory zoophilic infections are best studied in children.

A mobile unit was shown to be the most effective way of conducting field epidemiology and research in rural populations. The unit also served well as a mobile clinical diagnostic laboratory, and in addition provided comfortable sleeping quarters, washing facilities and safe food and water for the field teams.

Populations suitable for further work in prevention and evaluation of current therapeutic agents have been identified, but the programme is suspended, pending approval and funding from USA Med R & D.

A diagnostic method to enable untrained personnel to recover and identify Staphylococcus aureus from skin lesions has been developed. The method is 94% accurate without microbiological expertise. Additional simple instruction increases accuracy to 99%.