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US ARMY MEDICAL RESEARCH LABORATORY

FORT KNOX, KENTUCKY

REPORT NO. 511

EXTERNAL-ENVIRONMENTAL FACTORS AND HOST-PARASITE RELATIONSHIPS

EFFECT OF ARTIFICIAL ACCLIMATIZATION TO HEAT ON THE NASAL CARRIAGE OF STAPHYLOCOCCI

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Studies of Physiological Effects of Cold on Man
Task 01
Environmental Medicine
USAMRL Project No. 6X24-12-001

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Studies of Physiological Effects of Cold on Man
Task 01
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USAMRL Project No. 6X64-12-001

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ABSTRACT

EXTERNAL ENVIRONMENTAL FACTORS AND HOST-PARASITE RELATIONSHIPS

EFFECT OF ARTIFICIAL ACCLIMATIZATION TO HEAT ON THE NASAL CARRIAGE OF STAPHYLOCOCCI

OBJECT

To determine the role of external environmental factors in the establishment and maintenance of the staphylococcal nasal carrier state in personnel not associated with hospitals.

RESULTS

Artificial acclimatization to heat of a group of soldiers produced significant changes in the composition of the nasal flora characterized by an increased recovery of pathogenic and potential pathogenic strains of staphylococci. The number of carriers of these types of staphylococci increased significantly in the group of soldiers undergoing artificial acclimatization to heat. A similar group of soldiers serving as controls experienced no comparable changes in the carrier state.

CONCLUSIONS

The results of this study suggested that external environmental factors, specifically ambient temperature and relative humidity, influenced the establishment and maintenance of the staphylococcal nasal carrier state in humans.

RECOMMENDATIONS

A similar study should be made on a group of soldiers undergoing artificial acclimatization to cold. Attempts should be made to relate the changes observed in the nasal carrier state with changes in the types of staphylococci carried on the skin.

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I. INTRODUCTION

The role of nasal carriers in the dissemination of staphylococcal disease among patients and staff personnel has been studied extensively within the semi-closed environment of hospitals (1-3). Although much valuable epidemiological information has been derived from such investigations of the carrier problem, little precise information is available concerning the factors which influence the establishment and maintenance of the staphylococcal nasal carrier state in non-hospital populations.

Basically, the nasal carriage of staphylococci may be considered as a special and often extended phase of the classical relationships existing between host and microbe preceding overt disease. It has been repeatedly shown that nasal colonization precedes staphylococcal infection among hospital patients and that such carriers represent a threat to themselves as well as to other patients (4-6). In the case of staphylococcal nasal carriage, however, the classical concept of the host-parasite relationship suffers from our lack of adequate methods for the estimation of the virulence of different strains of staphylococci and the degree of resistance of the host to infection by these organisms. Anima. experiments designed to measure these variables in the host-parasite equation have often proven unreliable and, in some cases, misleading (7).

Reports in the literature have tended to minimize or discount completely the role of external environmental factors in the establishment and maintenance of the staphylococcal nasal carrier state (8-10) although correlations have been established between external environmental factors and the incidence of some viral diseases of humans (11). Changes in temperature and relative humidity have also been shown to produce local alterations in the normal nasal microflora of mice (12).

The role of external environmental factors in host-parasite relationships is difficult to assess and the correlation of changes of such factors with alterations in the characteristics of the carrier state does not necessarily establish a cause and effect relationship. However, because of the delicate nature of balance between opposing forces of host

and microbe immediately prior to the appearance of overt disease, it is difficult to understand how the outcome of these interactions could be completely independent of the influence of external environmental factors. This is especially true when such factors are known to produce physiological changes in the host associated with the stress phenomenon.

This study was undertaken to determine the influence of two external environmental factors, ambient temperature and relative humidity, on the type of staphylococci carried by soldiers and on the duration of the carrier state. The changes occurring in the carrier state of non-hospital personnel undergoing chronic exposure to heat are presented and discussed.

II. MATERIALS AND METHODS

Experimental subjects. The experimental subjects were regular US Army paratroopers from the 2d Platoon, Company E, 503d Airborne Battle Group, 82d Airborne Division, stationed at Fort Bragg, North Carolina. Forty-one volunteers from this unit were selected and flown to Fort Knox to take part in a study of artificial acclimatization to heat prior to their scheduled participation in OPERATION SOLIDARITY in the Canal Zone during February and March of 1961. The subjects ranged in age from 18 to 34 years, with the average age being 22 years. Soldiers with records of hospitalization within the six month period prior to the study were not accepted as experimental subjects. Upon their arrival at Fort Knox the soldiers were divided into two groups. Nineteen enlisted men were placed under the supervision of a non-commissioned officer and designated as a control group. The remaining 20 men and the officer in charge were placed in groups to be artifically acclimatized to heat.

The initial laboratory phase of the study began on 23 January and continued until the departure of the soldiers for the Canal Zone on 17 February 1961. Participation in OPERATION SOLIDARITY by the soldiers covered the period from 20 February through 10 March 1961. The second phase of the laboratory study was conducted during the period from 13 March through 13 March 1961.

Collection of control data. Control data concerning the distribution and types of staphylococci being carried by the experimental subjects were collected during a three day preliminary control period (table 1). Both groups of soldiers were exposed to a strenuous exercise regime in the climatic chambers at an ambient temperature of 65°F with

46 per cent relative humidity. Only cotton shorts and combat boots were worn during the preliminary control period. Essentially, the exercise regime consisted of walking 14 miles daily at a rate of 2.2 miles per hour. Appropriate physiological measurements designed to determine the status of the groups with regard to previous thermal experience were taken at 30 minute intervals. Pulse rate and rectal temperature were recorded for each member of the control and experimental groups.

Acclimatization procedure. Following the completion of the collection of preliminary control data, the experimental group was moved into a climatic chamber with an ambient temperature of 105°F with 56 per cent relative humidity (table 1) for three days. The exercise program in the heat was identical to that followed during the collection of preliminary control data. Exposure to 105°F was followed by successive three day exposures to ambient temperatures of 110°F, 115°F, and 120°F with relative humidity values of 46, 37, and 31 per cent, respectively (table 1).

While the experimental group was undergoing acclimatization, members of the control group followed the standard exercise regime in a climatic chamber with a constant ambient temperature of 65°F and 46 per cent relative humidity.

Standard stress tests. The progress of the experimental group was assessed periodically by a standard stress test conducted in a climatic chamber with an ambient temperature of 110°F and 46 per cent relative humidity (table 1). Members of the control group were also subjected to the stress tests to evaluate their performance under elevated environmental temperature. The stress test involved walking on a treadmill traveling at 3.5 miles per hour. During the tests physiological measurements were recorded every 15 minutes. Approximately 2.8 miles were walked by each soldier during the 48 minutes spent on the treadmill.

The brief exposures (table 1) of members of the control group to heat during the stress tests represented the only important thermal experiences encountered during the initial laboratory phase of the study. These experiences were not of sufficient duration to produce a significant degree of acclimatization based on physiological measurements and performance tests.

Collection of nasal cultures. During the preliminary control period nasal cultures were collected from each subject in the control

and experimental groups immediately prior to entering and on leaving the climatic chamber. Cultures were taken from members of each group at the end of each succeeding exposure period (table 1). Determinations of qualitative changes in the types of staphylococci composing the nasal flora were based on the examination of the pooled cultures from each group after each exposure period. Changes in the carrier state of individuals within each group were determined by examination of all cultures collected from each subject during the exposure period. Results for each exposure were plotted at the midpoint of the period. Cultures collected during the standardized stress test were not included in the results.

Nasal cultures. Approximately 1,100 nasal cultures were collected from the members of the control and experimental group during the study. Sterile cotton swabs moistened in sterile water were used to collect the primary cultures. The swabs were inserted 1 to 2 cm into the vestibule and rotated against the septum and the alae nasi. The swabs were withdrawn and streaked immediately onto the surfaces of petri dishes containing mannitol-salt agar (Difco) and Staphylococcus Medium No. 110 (Difco) fortified with 1 per cent non-fat milk solids. The milk solids were added to enhance pigment production during initial isolation. The plates were incubated at 37°C for 18 hours and then permitted to stand at room temperature for 24 hours to further enhance pigment production. Colonies producing orange or yellow pigment and fermenting mannitol were picked from the plates and transferred to culture tubes containing trypticase soy broth. These broth cultures were incubated at 37°C for 24 hours and morphology was checked by gram staining.

Coagulase production by pigmented mannitol-positive strains of staphylococci was determined by the slide technique. When the results of the slide test were equivocal a microcapillary tube test was performed (13). All strains that coagulated plasma within 15 seconds by the slide technique or within 18 hours by the microcapillary tube method were classified as coagulase-positive reactors. Strains producing coagulase were transferred to trypticase soy agar slants for storage at -10°C.

Types of nasal carriers. For the purpose of this study an occasional nasal carrier was defined as any individual who gave only one coagulase-positive culture per experimental exposure. Individuals who had a series of coagulase-positive cultures followed by a coagulase-negative culture or vice versa were classified as intermittent carriers. A persistent carrier was defined as any individual who had coagulase-positive strains in every culture taken throughout the entire study.

Airborne contamination. Airborne contamination in the climatic chambers was estimated by the particle fall-out method. The method is based on the fall-out rate of staphylococcal-carrying particles onto the surfaces of petri plates containing mannitol-salt agar. The number of staphylococcal-carrying particles settling out of the air in one minute represents approximately 1/12 of the number of such particles per cubic foot of air above the plate (9). Counts obtained from fall-out plates are not absolute values. They are, however, satisfactory for comparing changes in the airborne contamination of rooms under similar flow conditions (table 2).

Statistical analyses. The chi-square test was used for comparing experimental results with those obtained during the preliminary control period. Differences were considered significant only when the chi-square test yielded $P \leq 0.02$. In one instance (fig. 5) the means of recovery rates for coagulase-positive strains of staphylococci from pigmented, mannitol-positive isolates were compared for significance by the Student "t" test.

III. RESULTS

Recovery of pigmented, mannitol-positive strains. The recovery of pigmented, mannitol-positive strains of staphylococci, expressed as a recovery factor (RF), from the primary nasal cultures is presented in figure 1. There was no significant difference between the RF's of the control and experimental groups during the study. Within both the control and experimental groups there were significant increases in the recovery of pigmented, mannitol-positive strains. Following the return of the soldiers from the Canal Zone there was a slight, but insignificant, decrease in the recovery of pigmented, mannitol-positive strains from the nasal cultures of members of both the control and experimental groups.

In the control group the distribution of pigmented, mannitol-positive strains in the primary nasal cultures increased significantly during the initial laboratory phase of the study (fig. 2). A similar increase in the distribution of these strains was also observed in the experimental group (fig. 3). In both groups the increased distribution of pigmented, mannitol-positive strains isolated from primary nasal cultures was accompanied by a simultaneous decreased distribution of non-pigmented, mannitol-negative strains.

Recovery of coagulase-positive strains. The recovery of coagulase-positive strains of staphylococci from pigmented, mannitol-positive

primary isolates is shown in figure 4. In the control group the distribution of coagulase-positive strains among the pigmented, mannitol-positive isolates did not become significantly different from the distribution observed during the preliminary control period. Throughout the entire study, however, the distribution of coagulase-positive strains tended to increase.

In the experimental group the distribution of coagulase-positive strains among the pigmented, mannitol-positive primary isolates became significantly different ($x^2 = 5.76$, $df = 1$, $P > 0.02$) from the distribution observed during the preliminary control period following exposure of the group to an ambient temperature of 115°F with 37 per cent relative humidity (fig. 4). Following exposure of the experimental group to an ambient temperature of 120°F with 31 per cent relative humidity the increased distribution of coagulase positive strains became highly significant ($x^2 = 11.57$, $df = 1$, $P > 0.01$).

After the return of the soldiers from the Canal Zone and subsequent exposure to an ambient temperature of 110°F with 46 per cent relative humidity cultures from the control showed a slightly increased number of coagulase-positive strains (fig. 4). Cultures from the acclimatized group yielded significantly fewer strains of this type.

Effects of temperature and humidity. The recovery of coagulase-positive strains of staphylococci from pigmented, mannitol-positive isolates in relation to changes in ambient temperature and relative humidity is shown in figure 5. In the control group the recovery of coagulase-positive strains did not change significantly during the study. In the experimental group, however, the recovery of coagulase-positive strains increased significantly ($t = 2.69$, $df = 12$, $P > 0.02$) during the initial laboratory phase of acclimatization. There was a substantial degree of positive correlation ($r = 0.70$) between the increased recovery of coagulase-positive strains and the increased ambient temperatures to which the experimental group was exposed. A substantial degree of negative correlation ($r = 0.61$) was also observed between the recovery of coagulase-positive strains and relative humidity.

Nasal carriage of staphylococci. Changes in the number of nasal carriers within the control and experimental groups are shown in figure 6. In the control group the number of all types of staphylococcal carriers increased to 35 per cent following the first and second exposure periods. Following the third and fourth exposures of the control group there was a decrease in the number of all types of carriers to 25 per cent at the end of the initial laboratory phase of the study.

In the experimental group the number of all types of staphylococcal carriers decreased from 29 to 25 per cent following the exposure to an ambient temperature of 105°F with 56 per cent relative humidity. Following exposure to ambient temperatures of 110°F and 115°F, the number of all types of staphylococcal carriers increased to 67 per cent and remained at that figure during the rest of the initial laboratory phase of the study.

Following the return of the soldiers from the Canal Zone and subsequent exposure to an ambient temperature of 110°F with 46 per cent relative humidity there was a 17 per cent increase in the number of all types of staphylococcal carriers to 42 per cent in the control group. The number of all types of staphylococcal carriers decreased from 67 to 35 per cent during the corresponding period.

Persistent carriers. The number of persistent carriers of coagulase-positive strains of staphylococci in the control group increased from 10 to 20 per cent during the initial laboratory phase of the study. In the experimental group during the same period, the number of persistent carriers increased from 9.5 to 48 per cent.

Upon return of the soldiers from the Canal Zone and exposure to an ambient temperature of 110°F with 46 per cent relative humidity the number of persistent carriers in the control group increased 17 per cent to 37 per cent of the group. In the acclimatized group during the second phase of the laboratory study the number of persistent carriers decreased from 48 to 25 per cent.

Air contamination. Estimations of airborne staphylococcal contamination in the control and heat chambers, based on calculations from fall-out counts, indicated that the rooms were comparable in this regard. Both chambers received filtered outside air at an exchange rate of 1000 cubic feet per minute. This exchange rate produced a rapid removal of particles and resulted in a stabilized fall-out rate shortly after the exposure periods were started (table 2).

IV. DISCUSSION

The effect of stressful climatic environments on resistance to infection is of particular importance to the Army as modern soldiers are rapidly deployed from temperate climates into potentially hostile environments ranging from tropical jungles to Arctic wastelands (15). Thus, the primary interest of the Army in the staphylococcal carrier

state lies in its potential relation to wound infection, especially by microorganisms carried on the soldier himself.

The most common pathogen recovered from war wounds in all theaters of operations during World War II was Staphylococcus aureus (16-18) and it is now apparent that self-infection of wounds by strains of staphylococci present on the skin of victims, as well as cross-infection, played a major role in wound sepsis. Several reports in the recent literature support the concept that nasal and skin carriers of staphylococci are usually the sources of their own infections (6, 10, 19-22).

Despite the recognized importance of carriers in the dissemination of staphylococcal disease, very little precise information is available concerning the factors involved in the establishment of the staphylococcal nasal carrier state. It is difficult to understand how some persons can resist colonization indefinitely, even in the presence of heavy contamination, while others rapidly become colonized. Factors inherent in the biology of the host and microbe, as influenced by the environment, undoubtedly determine whether staphylococci can live and multiply on the nasal membranes.

With regard to the microbe, there appears to be differences in the ability of various strains of staphylococci to colonize the nasal membranes (23-26) as well as in the cohesiveness exhibited by various types of staphylococci present on these membranes (1).

In the case of the host, various factors have been suggested as determinants of staphylococcal nasal colonization. Among the factors suggested have been anatomic abnormalities of the nasal passages, the presence of inhibitory substances in the nasal secretions, and the presence of inhibitory agents produced by bacterial commensals of the nose.

In general, however, the influence of external environmental factors has been ignored in studies of the host-parasite relationships as exemplified in the staphylococcal nasal carrier. This negligence is difficult to comprehend in view of the sensitivity of the nasal circulatory system to changes in temperature and relative humidity (27), the variations occurring in secretions of mucous membranes during changes in temperature and humidity (14, 28), and effects of changes in temperature and humidity on the virulence of staphylococci (29).

This study, although limited in range, offers a promising approach to the study of the influence of external environmental factors on the

establishment and maintenance of the staphylococcal nasal carrier state in an non-hospital population. All of the recognized variables that have complicated previous studies of the carrier state conducted within the semi-closed hospital environment were controlled within acceptable limits. Under these experimental conditions, it has been shown that two external environmental factors, namely ambient temperature and relative humidity, influence the staphylococcal nasal carrier state.

From the standpoint of the parasite the influence of increased ambient temperatures accompanied by reduced relative humidity was expressed by changes in the distribution of staphylococcal biotypes within the nasal flora. There were significant increases in the distribution of strains with biochemical properties associated with virulence in the nasal cultures of soldiers chronically exposed to heat.

In the case of the host the influence of increased ambient temperatures with decreased relative humidity was reflected by increases in the number of all types of nasal carriers of pathogenic staphylococci biotypes.

The changes in the carrier state of members of the acclimatized group could be correlated with changes in temperature and relative humidity. The interpretation of the correlated changes, however, requires caution and the establishment of such a relationship under the conditions of this experiment does not necessarily justify its extension to all combinations of ambient temperature and relative humidity. Likewise, the epidemiological behavior of the staphylococci is known to be variable and the results of studies made on one group of individuals in one environment do not have universal application.

Although the results of this investigation do not permit the precise determination of the mode of action of external environmental factors in the establishment and maintenance of the staphylococcal nasal carrier state, we feel that the confirmation of the existence of external environmental influences on the carrier state under controlled experimental conditions is important in the ultimate solution of the staphylococcal problem.

Further studies designed to elucidate more precisely the roles of temperature and humidity on the staphylococcal carrier state are presently under way in this laboratory.

V. SUMMARY

Studies of the nasal cultures of soldiers undergoing artificial acacclimatization to heat indicated that external environmental factors,

including ambient temperature and relative humidity, influence the establishment and maintenance of the staphylococcal nasal carrier state. Significant changes in the composition of the nasal flora, characterized by an increased recovery of pathogenic and potential pathogenic staphylococci, were observed in cultures from the experimentally acclimatized group. The number of all types of staphylococcal carriers also increased in the experimentally acclimatized group. No comparable changes were observed among cultures or carrier rates obtained from the group of soldiers that served as experimental controls. The implications of the findings are discussed in relation to the epidemiology of staphylococcal wound infections among members of modern, highly mobile military organizations subject to rapid deployment into potentially hostile climatic environments.

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TABLE 1. REGIME FOLLOWED BY TWO GROUPS OF SOLDIERS DURING THE STUDY OF THE EFFECT OF ACCLIMATIZATION ON THE STAPHYLOCOCCAL NASAL CARRIER STATE.

Period (Fort Knox)	Control Group			Acclimatized Group		
	Amb. Temp-F°	Rel. Humidity-%	Time	Amb. Temp-F°	Rel. Humidity-%	Time
Preliminary Control	65	46	3 days	65	46	3 days
Stress Test	110	46	1 hr	110	46	1 hr
1st Exposure	65	46	3 days	105	56	3 days
2nd Exposure	65	46	3 days	110	46	3 days
Stress Test	110	46	1 hr	110	46	1 hr
3rd Exposure	65	46	3 days	115	37	3 days
Stress Test	110	46	1 hr	110	46	1 hr
4th Exposure	65	46	3 days	120	31	3 days
Stress Test	110	46	1 hr	110	46	1 hr
CANAL ZONE	Ave. 1030 hrs 87	Ave. 1030 hrs 65	19 days	Ave. 1030 hrs 87	Ave. 1030 hrs 65	19 days
(Fort Knox)						
Stress Test	110	46	1 hr	110	46	1 hr
Post-Panama Exposure	110	46	2 days	110	46	2 days
Stress Test	110	46	1 hr	110	46	1 hr

TABLE 2. AIRBORNE STAPHYLOCOCCAL-CARRYING PARTICLES.

	Amb. Temp-F°	Rel. Humidity-%	1000 hrs PC*	1400 hrs PC*	Ave. PC	Particles Sq. Ft. †
Control Chamber						
Preliminary Control	65	46	30(6)**	19(4)	24.5(5)	294(60
1st Exposure	65	46	40(12)	25(8)	32.5(10)	390(120)
2nd Exposure	65	46	45(16)	36(13)	40.5(14.5)	486(174)
3rd Exposure	65	46	26(8)	33(10)	29.5(9)	354(108)
4th Exposure	65	46	27(7)	48(12)	37.5(9.5)	450(114)
Post-Panama	110	46	29(12)	21(9)	25.0(10.5)	300(126)
Ave.			32.8(10.2)	30.3(9.3)		379(117)
Heat Chamber						
Preliminary Control	65	46	31(9)	25(8)	28.0(8.5)	336(102)
1st Exposure	105	56	33(8)	17(5)	25.0(6.5)	300(78)
2nd Exposure	110	46	40(15)	29(11)	34.5(13)	414(156)
3rd Exposure	115	37	39(20)	39(25)	34.0(22.5)	408(270)
4th Exposure	120	31	23(16)	40(27)	31.5(21.5)	378(258)
Post-Panama	110	46	39(14)	14(5)	26.5(9.5)	318(114)
Ave.			32.5(13.7)	27.3(13.5)	27.3(13.5)	359(163)

*Average colony counts from duplicate settling plates.
**Coagulase-positive strains in parentheses.
† Calculated from settling plates.

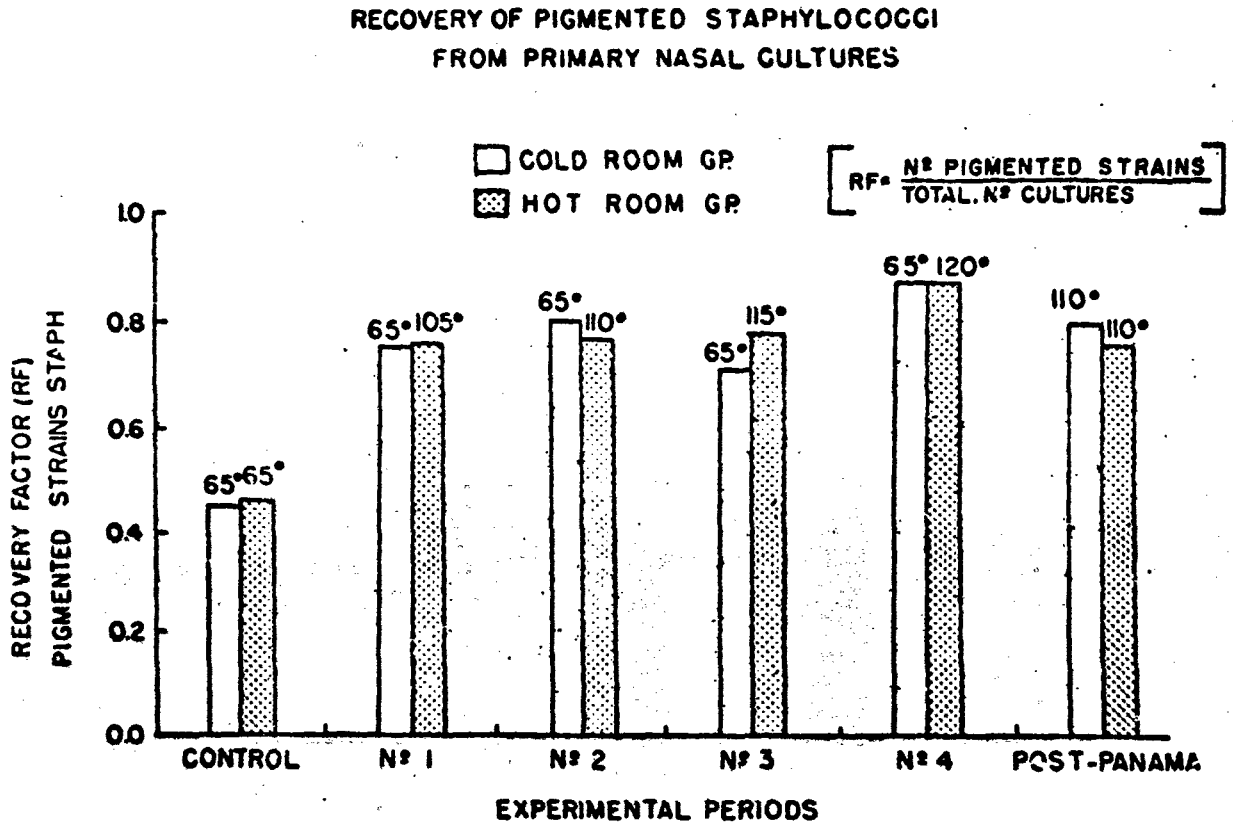

Fig. 1. Recovery of pigmented, mannitol-positive strains of staphylococci from the primary nasal cultures of soldiers undergoing artificial acclimatization to heat.

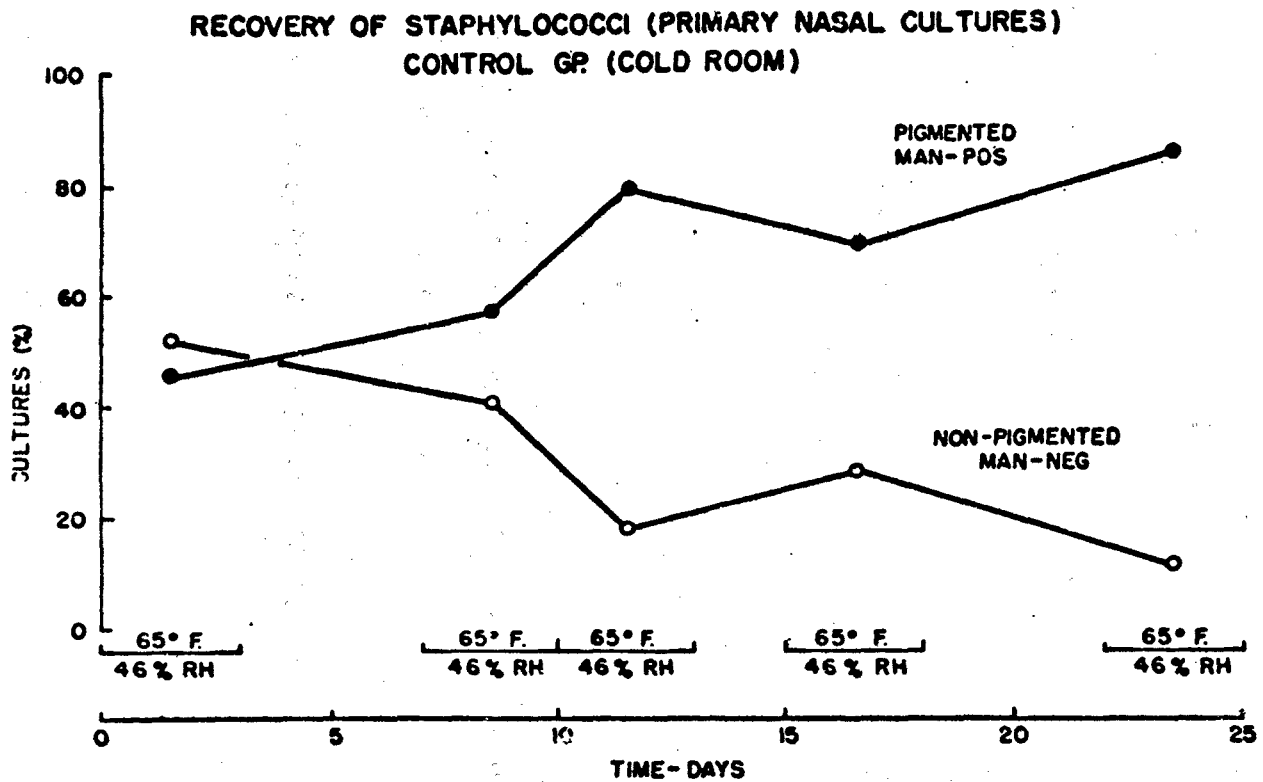

Fig. 2. Recovery of two types of staphylococci from the primary nasal cultures of soldiers exposed to an ambient temperature of 65°F with 46 per cent relative humidity. Values are plotted at the midpoints of the exposure periods. Bottom scale shows elapsed time.

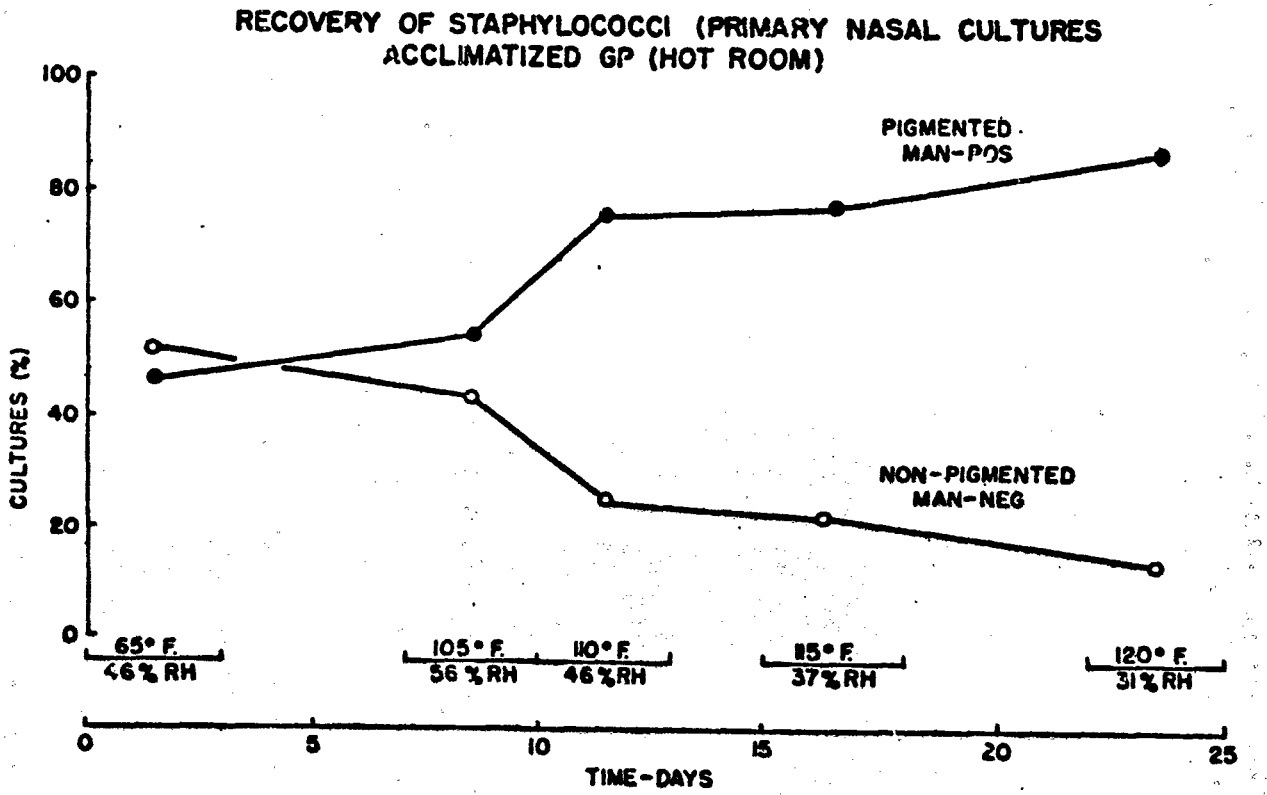

Fig. 3. Recovery of two types of staphylococci from the primary nasal cultures of soldiers undergoing artificial acclimatization to heat. Values are plotted at the midpoints of the exposure periods. Bottom scale shows elapsed time.

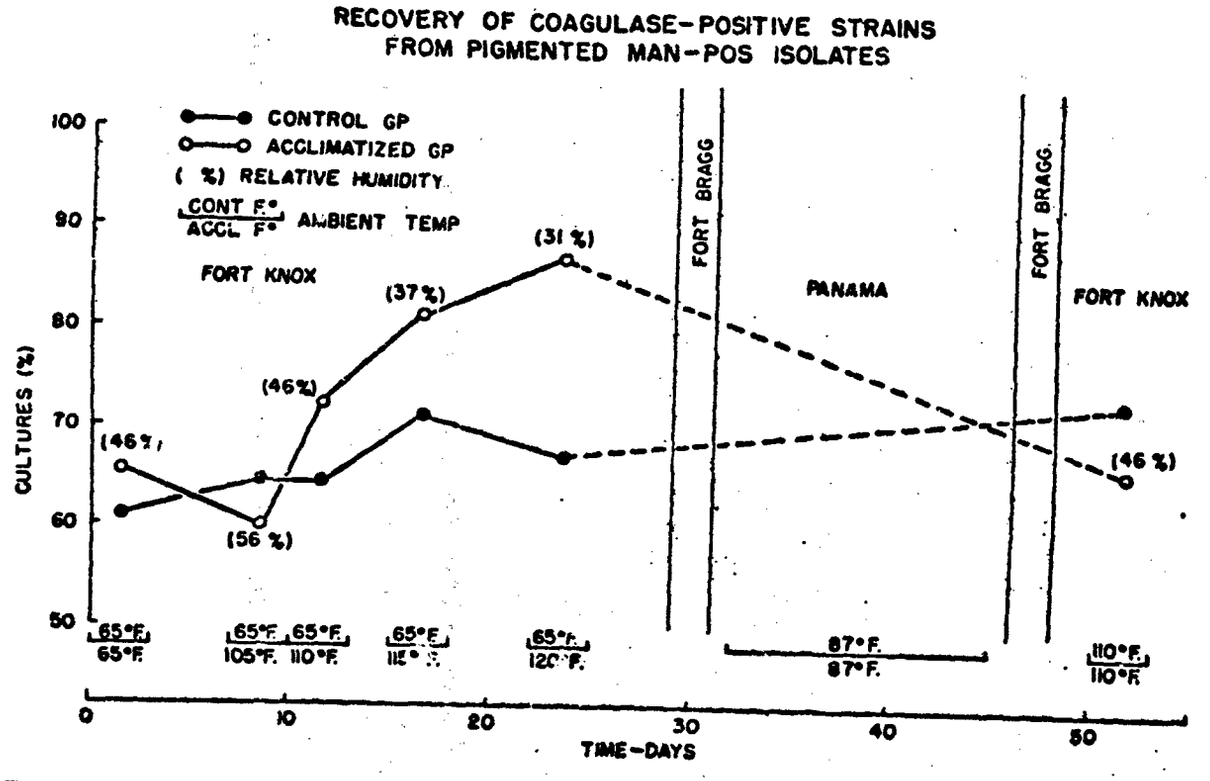

Fig. 4. Recovery of coagulase-positive strains of staphylococci from pigmented, mannitol-positive primary isolates. Values for each exposure period are plotted at the midpoint of the period. The distribution of coagulase-positive strains became significantly different ($x^2 = 5.76$, df = 1, $P > 0.02$) in the acclimatized group following exposure to an ambient temperature of 110°F with 46 per cent relative humidity. Bottom scale shows elapsed time.

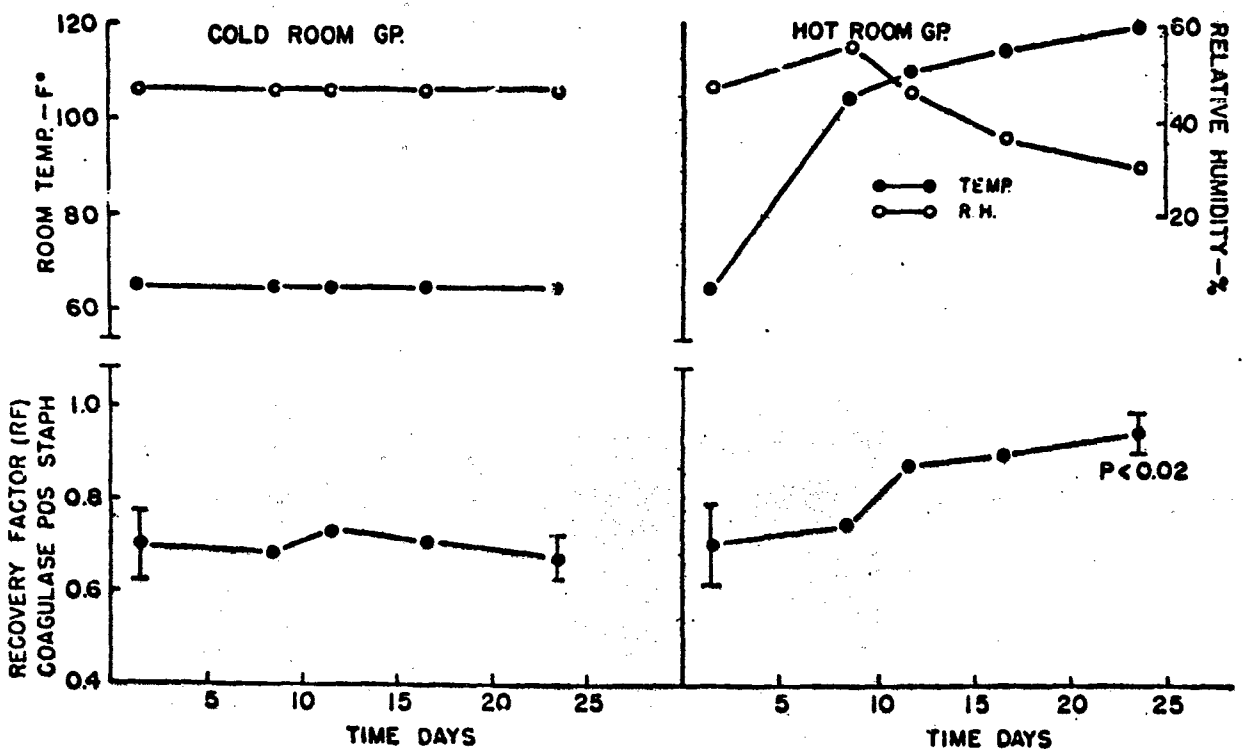

Fig. 5. Relationships between the recovery of coagulase-positive strains of staphylococci from pigmented, mannitol-positive isolates, ambient temperature, and relative humidity. Values are plotted at the midpoints of the exposure period during the initial phase of the laboratory study. Bottom scale shows elapsed time.

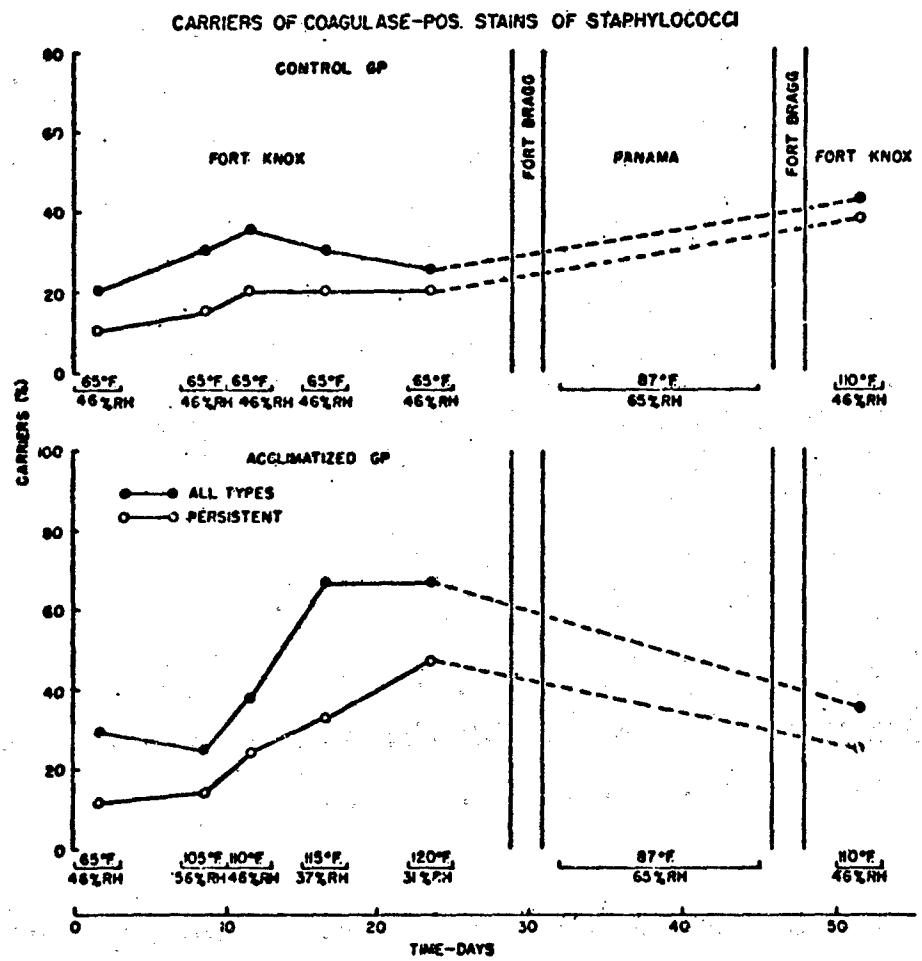

Fig. 6. Changes in the carrier state of subjects during artificial acclimatization to heat. Values are plotted at the midpoints of the exposure periods. Bottom scale shows elapsed time.

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2. Acclimatization to heat
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