INFECTION WITH "PATHOGENIC" MICROORGANISMS BUT WITHOUT CLINICALLY APPARENT DISEASE HAS BEEN VARIOUSLY DEFINED AS LATENT, SUBCLINICAL, DORMANT, OCCULT, AND INAPPARENT. THESE TERMS ARE EMPLOYED WITH VARYING MEANINGS BY CLINICIANS, VETERINARIANS, PHYTOPATHOLOGISTS, AND OTHER BIOLOGISTS. FOR THE SAKE OF GREATER CONSISTENCY, HOWEVER, THREE GENERAL CATEGORIES OF INFECTIONS WITHOUT APPARENT DISEASE HAVE BEEN PROPOSED, DEFINED, AND BROADLY GROUPED UNDER THE TERM "ATTENUATED INFECTION." THESE THREE CATEGORIES ARE THE CARRIER STATE, MICROBIAL PERSISTENCE, AND LATENT INFECTION.

INFECTION IS HEREIN DEFINED AS THE PRESENCE OF MICROORGANISMS WITHIN OR UPON TISSUES REGARDLESS OF WHETHER OR NOT THIS RESULTS IN DETECTABLE PATHOLOGIC EFFECTS. THE CARRIER STATE IS THAT VARIANT OF ATTENUATED INFECTION CHARACTERIZED BY A CONTINUING INFECTION WITH AGENTS WHICH ARE EASILY RECOVERABLE AND RECOGNIZABLE. THE INFECTING AGENTS CAN BE RECOVERED FROM THE HOST BY MEANS OF THE USUAL STANDARD TECHNIQUES. THEY CAN NOT BE DISTINGUISHED MORPHOLOGICALLY, PHYSIOLOGICALLY, OR BIOCHEMICALLY FROM OTHER MEMBERS OF THE SPECIES ISOLATED FROM INSTANCES OF INFECTION DISEASE OR NATURALLY OCCURRING CONTAMINATION.

MICROBIAL PERSISTENCE IS CHARACTERIZED BY A CONTINUING ATTENUATED INFECTION FOLLOWING AN EPISODE OF OVERT CLINICAL DISEASE. THIS MAY OCCUR DESPITE THE USE OF EFFECTIVE ANTIMICROBIAL THERAPY. CLINICAL RELAPSE, WHEN IT FOLLOWS MICROBIAL PERSISTENCE, WILL BE ASSOCIATED WITH THE ORIGINAL INFECTING STRAIN, AND WILL USUALLY NOT BE ASSOCIATED WITH RESISTANCE TO THE ANTIMICROBIAL USED INITIALLY. IN ORDER TO DEMONSTRATE PERSISTERS, HOWEVER, THE STANDARD TECHNIQUES FOR THEIR ISOLATION GENERALLY HAVE TO BE REFINED. IT IS PROBABLE, BUT NOT CLEARLY ESTABLISHED, THAT THE BIOCHEMICAL AND PHYSIOLOGICAL PROPERTIES

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ATTENUATED STAPHYLOCOCCAL INFECTION

of persisters differ from those of the species as usually encountered. The term, "drug indifference," (3) describes one of the more important aspects of persisters. This characteristic is demonstrable in vitro (4).

Latent infection denotes an infection wherein the infecting agents differ quantitatively and qualitatively from those encountered in the other varieties of attenuated infection. In this sense, qualitative differences refer to alterations in morphology and physiology of the microorganisms from those characteristic of the usually recognized form. Latency is most readily recognized by means of suitable indicator hosts, or through retrospective analysis.

Staphylococcal infections commonly occur as carrier states in the normal population. Microbial persistence among staphylococcal infections has been recognized after abscesses have formed, and has been shown in vitro (3, 4). It has long been suspected that latent staphylococcal infections exist, but proof has been lacking.

The present report is concerned with the establishment of an experimentally induced attenuated staphylococcal infection in guinea pigs. At least two forms of attenuated infection were encountered.

Materials and Methods

Terminology.—Unless otherwise specified, the terms "staphylococcus" and "staphylococci" refer to coagulase-positive, mannitol-positive, hemolytic staphylococci (Staphylococcus pyogenes, var. aureus).

Guinea Pigs.—Normal, adult, male guinea pigs weighing 200 to 300 gm were used after initial studies showed them to be free of detectable coagulase-positive staphylococci on the skin, fur, and in the respiratory tract and the feces. They were housed in separate open, wire-meshed bottomed cages, 16 X 14 X 21 inches (2.7 cubic feet), fed a standard diet, and allowed water ad lib. Daily swab cultures were obtained from the anterior nares and screened for the presence of staphylococci. As the studies progressed, animals were crowded 4 to a cage and were given tetracycline (50 mg perorally twice daily for 1 week) in order to detect staphylococci that might have been missed on routine cultural examination. Only animals from which no staphylococci could be recovered during the control period were used for the studies reported in this communication.

Cultural Techniques.—Sterile, cotton-tipped applicators were prepared, moistened in sterile saline, passed into the anterior nares for a distance of 0.5 cm, twirled, withdrawn, streaked on 5 per cent sheep blood agar plates, and on similar plates into which polymyxin (5 μg per ml of medium) had been incorporated. (Essentially all staphylococci are resistant to this concentration of polymyxin which was therefore used for screening purposes.) Staphylococcal colonies were identified morphologically and subjected to tube coagulase testing. Coagulase-positive staphylococci were then categorized by means of phage typing (5). Antimicrobial susceptibility testing employed the screening method of Rantz and Rantz (6). A minimum of 5 dispersed colonies from each specimen was chosen for phage typing.

Bacterial Strains.—All strains used for aerosol infection were initially recovered from clinical instances of staphylococcal infection.

1 From Horton's Rabbitry, Los Gatos, California.

2 The set of staphylococcal phages and their propagating strains were obtained through the generosity of Dr. John E. Blair, Hospital for Joint Diseases, New York.
Strain 4974 consists of coagulase-positive, mannitol-positive, hemolytic staphylococci of phage type 80/81. It is resistant to more than 100 μg per ml of penicillin G, more than 20 μg per ml of streptomycin, and more than 50 μg per ml of tetracycline. It destroys penicillin G. Strain 4974 was recovered from a patient with multiple infected decubitus ulcers.

Strain 5723 consists of coagulase-positive, mannitol-positive, hemolytic staphylococci of phage type 52A/79. It is resistant to the same concentrations of penicillin G, streptomycin, and tetracycline as strain 4974. Strain 5723 was isolated from the bloodstream of a patient with staphylococcal bacteremia.

Strain 4580 consists of coagulase-positive, mannitol-positive, hemolytic staphylococci of phage type 6/47/53. It is susceptible to 1 μg per ml of penicillin G, 5 μg per ml of streptomycin, and 5 μg per ml of tetracycline. It was isolated from the bloodstream of a patient with osteomyelitis and bacteremia.

Preparation of the Bacterial Inoculum.—Nutrient broth tubes were inoculated with the strain to be used and incubated for 18 hours at 37°C. The broth tubes were then decanted into larger, round-bottomed tubes and centrifuged for 20 minutes at 3000 rpm. The supernatants were removed by decanting and pipetting, and the remaining sediments were resuspended in 1.0 ml of sterile distilled water.

Five ml lots of the aqueous suspensions were combined and recentrifuged at 3000 rpm for 20 minutes, and the supernatants decanted. The sediments were again suspended in 1 ml of sterile distilled water. Two such 1 ml lots were combined, brought to 5 ml volume, and recentrifuged as above. The supernatant was removed by decanting and pipetting, and the sediment brought to 10 ml volume with sterile distilled water. This became the inoculum.

Aliquots of 0.1 ml were removed, serial tenfold dilutions were made, and 0.1 ml aliquots of these dilutions were plated on trypticase soy agar plates and incubated overnight. Colony counts were performed and the appropriate calculations made. The final suspensions contained approximately 10⁸ bacteria per ml, or 10⁹ bacteria per inoculum.

Technique of Aerosol Infection.—A sealed, portable bacteriological hood (isolater/lab, Fisher Scientific Company, New York) of 10 cubic feet capacity was employed and equipped with blower, air filter, and ultraviolet light source. The hand openings were sealed with cardboard and tape. One of the seals was perforated to provide a 2 cm opening which could be sealed completely with a rubber stopper. The aerosol inoculum was introduced through this hole by means of a fine nozzle perfume atomizer activated by squeezing the bulb until the reservoir chamber was dry. The rubber stopper was inserted into its seat after the 10 ml inoculum had been introduced. The air in the chamber was then agitated for 15 minutes by means of the blower motor. Thereafter the ultraviolet lamp was turned on for 15 minutes before the chamber was opened and the animals removed.

Quantification of Aerosol Inoculum.—The blower motor was turned off, and two sterile, 5 ml needle-tipped syringes introduced into the chamber. Five ml of air was rapidly withdrawn, and the blower was turned on again. The needle was removed from the syringe and the contents of the syringe slowly expelled under the meniscus of a 10 ml volume of broth in a tube. Broth was twice drawn into the syringe and expelled again. Serial tenfold broth dilutions were made and 0.1 ml aliquots plated for colony counts.

Culture after Aerosol Exposure.—Following a 15 minutes' exposure to the aerosol, the guinea pigs were dipped and scrubbed in 80 per cent ethanol, only the anterior head portion remaining free. The head and nostrils were lightly washed with a hexachlorophene containing compound (pHisHex®). Cultures for staphylococci were then obtained from the anterior nares, feet, fur, tail, and anus. Cultures were repeated twice daily for the first 48 hours and daily thereafter.

Indicators of Cross-Infection.—During the time following aerosol infection, but not during the procedure itself, guinea pigs from which staphylococci were not recoverable and which
were not exposed to the aerosol were housed in the same animal room with the infected animals. Nasal cultures were obtained from these indicator animals at the same times as those obtained from the artificially infected guinea pigs.

**Techniques Employed for Tissue and Organ Cultures for Staphylococci.**—The animals were sacrificed by a blow on the head. The carcass was immersed in 80 per cent ethanol and then placed on a wooden board covered with a sterile towel. The organs were removed aseptically, minced, and prepared for homogenization employing the methods described by Smith and Dubos (7), and McCune and associates (8). The organs were transferred to Pyrex tubes and nutrient broth added to the calibrated 3 ml mark.

The organs were then homogenized with a teflon grinding head attached to a rotary motor (Precision Scientific Company, Chicago). The resulting homogenates were placed into plates containing 100 ml of nutrient broth. Aliquots were removed and diluted serially 10-fold and 100-fold in nutrient broth. The original flasks were incubated at 37°C for at least 72 hours. The plating technique used for the dilutions was the same as that described by Sellers and LeMaistre (9).

Where indicated under Results, the technique was varied by allowing the ethanol to evaporate from the body surface in a 37°C incubator for 1/2 hour, removing the feet, tail, and mouth (including the teeth), mincing the entire animal under aseptic precautions, and then homogenizing it.

**TABLE I**

Aerosol-Induced Infection (Strain 4974). Establishment, Persistence, and Disappearance of the Carrier State in Guinea Pigs

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>2 wks.</th>
<th>8 wks.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6/6*</td>
<td>6/6</td>
<td>6/6</td>
<td>2/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>2</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
<td>2/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>3</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
<td>2/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>4</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
<td>2/6</td>
<td>1/6</td>
<td>1/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>5</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
<td>2/6</td>
<td>1/6</td>
<td>1/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>6</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
<td>2/6</td>
<td>1/6</td>
<td>1/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>Total . . . .</td>
<td>36/36</td>
<td>36/36</td>
<td>36/36</td>
<td>14/34</td>
<td>4/34</td>
<td>4/34</td>
<td>3/33</td>
<td>2/32</td>
</tr>
</tbody>
</table>

* No. of animals with positive nasal cultures/total No. of animals.
† Sacrificed for organ cultures while nasal cultures were positive.
§ Sacrificed for organ cultures after nasal cultures had become negative.

**RESULTS**

**The Aerosol Inoculum.**—Repeated measurements indicated that the aerosol inoculum consisted of approximately $2.5 \times 10^6$ viable units per animal (compared with a predicted charge of 1 to $2 \times 10^6$).

**Establishment of the Infection by Means of Aerosol.**—All animals became colonized in the upper respiratory tract following exposure to the bacterial aerosol inoculum (Table I). Following immersion in 80 per cent ethanol and
washing of the snout, the aerosol strain was recovered only from the anterior nares.

Duration of Recoverability of Staphylococci from the Anterior Nares.—The results of six aerosol experiments utilizing 6 guinea pigs each are shown in Table I. The staphylococci were uniformly recoverable for 3 days (see Table I). This corresponds to the carrier state. Thereafter, there was a rapid and progressive decline in the number of carrier animals. Only 4, or 11 per cent, of the 36 animals exposed to aerosol persisted as carriers for more than 4 days, and only 2, or 5.5 per cent, seemed to have become permanent carriers.

Attempts to Recover Staphylococci from Tissues of Animals.—Four animals were sacrificed in this phase of the experiment, 2 while still carriers. Staphylococci were found in the respiratory tract of all 4 animals. They were recovered from the upper section which included nasal passages, larynx, trachea, and major bronchi, and from the lower section which included the smaller bronchi and lungs. No staphylococci were recovered from any other organ or tissue in this experiment.

Control of Cross-Infection.—Neither the aerosol strain nor any other coagulase-positive staphylococci were recovered from the anterior nares of the indicator animals housed in cages immediately adjacent to the infected animals.

The Effect of Tetracycline Administration.—
(a) Tetracycline administration to normal, non-carrier guinea pigs: The administration of 50 mg of tetracycline perorally twice daily to normal, non-carrier guinea pigs in all instances reduced the numbers of microorganisms recoverable from the anterior nares of these animals within 12 to 24 hours. No precise quantification or identification was attempted. Apparent pretreatment concentrations were generally restored within 4 to 6 days, although the microflora had changed from predominantly Gram-positive (including large numbers of non-hemolytic streptococci) to predominantly Gram-negative bacteria and yeasts. Diarrhea and some anorrhea were also noted. Six such experiments involving a total of 24 guinea pigs were carried out. None of these animals demonstrated recoverable coagulase-positive staphylococci before, during, or after tetracycline administration.

(b) Tetracycline administration prior to aerosol challenge with strains 4974 and 5723: The administration of tetracycline for 4 to 7 days prior to aerosol challenge with strains 5723 or 4974 slightly altered the nature of the carrier states following infection. When tetracycline was stopped on the day of aerosolization, the carrier state persisted in all animals for 4 days. The fraction of carriers then decreased progressively (Table II). Only 3, or 18 per cent, of the 17 experimental animals seemed to have become permanent carriers.

The tetracycline preparation used was velacycline®, kindly supplied through the courtesy of E. R. Squibb and Sons, Division of Olin-Mathieson Chemical Corp., New York.
(c) Tetracycline administration to carrier animals following aerosol infection with strains 4974 and 5723 (tetracycline-resistant): Tetracycline administered perorally to animals immediately after aerosol infection with strain 4974 and daily thereafter considerably altered the results previously described.

### TABLE II
Carrier State after Tetracycline Administration Prior to Aerosol Challenge with Strain 4974 (Tetracycline Discontinued Day of Aerosol Challenge)

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>No. animals</th>
<th>Aerosol Injection</th>
<th>Day 1</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 8</th>
<th>Day 14</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td></td>
<td>6/6*</td>
<td>6/6</td>
<td>3/6</td>
<td>1/6</td>
<td>1/6</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td></td>
<td>5/5</td>
<td>5/5</td>
<td>3/5</td>
<td>1/5</td>
<td>1/5</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td></td>
<td>6/6</td>
<td>6/6</td>
<td>3/6</td>
<td>1/6</td>
<td>1/6</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>17/17</td>
<td>17/17</td>
<td>9/17</td>
<td>3/17</td>
<td>3/17†</td>
<td>3/17†</td>
</tr>
</tbody>
</table>

* No. with positive nasal cultures/total No. of animals.
† Permanent carriers.

### TABLE III
Effect of Continued Tetracycline Administration on Staphylococcal Carriage Following Aerosol Infection (Strain 4974)

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>No. animals</th>
<th>End wk. 1</th>
<th>End wk. 2</th>
<th>End wk. 3</th>
<th>End wk. 6</th>
<th>End wk. 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>6/6*</td>
<td>6/6</td>
<td>4/6</td>
<td>2/6</td>
<td>2/6</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
<td>2/6</td>
<td>1/6</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
<td>2/6</td>
<td>2/6</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>6/6</td>
<td>6/6</td>
<td>3/6</td>
<td>2/6</td>
<td>1/6</td>
</tr>
</tbody>
</table>

* No. of animals with positive nasal cultures/total No. of animals.
1 Tetracycline stopped after 1 week in Experiments 1 and 4.
2 “ “ “ 2 “ “ “ 2

The carrier state persisted for as long as tetracycline administration was continued (Table III). As can be seen from the table, the carrier state not only persisted for the duration of therapy, but far beyond the anticipated 3 to 4 days after tetracycline was discontinued in the majority of the animals. Moreover, the fraction of animals (6 of 16, or 37.5 per cent) which apparently became permanent carriers was significantly increased after tetracycline administration. However, there seemed to be no correlation between the fraction of animals remaining carriers and the duration of tetracycline administration. There were no significant differences when strain 5723 was used in this experiment.
(d) *Tetracycline administration to animals after the carrier state with strain 4974 or 5723 had disappeared.* The preceding experiments produced a group of animals which had undergone aerosol infection; in addition there remained the uninfected animals previously used as indicators for the detection of cross-infection in the animal room. The aerosolized animals fell into two categories. A small group constituted the "permanent carriers." The largest group was comprised of those animals which had been carriers, but from which no coagulase-positive staphylococci were recoverable. Two weeks to 6 months had elapsed between the last positive cultures and the experiment about to be described.

**TABLE IV**

*Effect of Tetracycline Administration on Recoverability of Staphylococci Following Apparent Clearing of the Carrier State*

<table>
<thead>
<tr>
<th>Time since last positive culture</th>
<th>No. of animals</th>
<th>No. positive after tetracycline</th>
<th>Aerosoled strain</th>
<th>Recovered strain</th>
<th>Duration of secondary carriage after end of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0-7 days</td>
</tr>
<tr>
<td>2 wks.</td>
<td>4</td>
<td>3/4</td>
<td>4974-3</td>
<td>4974-2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5723-1</td>
<td>5723-1</td>
<td></td>
</tr>
<tr>
<td>4 wks.</td>
<td>2</td>
<td>1/2</td>
<td>4974-1</td>
<td>4974-1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5723-1</td>
<td>5723-1</td>
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<tr>
<td>2 months</td>
<td>4</td>
<td>4/4</td>
<td>4974-2</td>
<td>4974-2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5723-2</td>
<td>5723-2</td>
<td></td>
</tr>
<tr>
<td>4 months</td>
<td>2</td>
<td>2/2</td>
<td>4974-2</td>
<td>4974-2</td>
<td>2</td>
</tr>
<tr>
<td>6 months</td>
<td>4</td>
<td>4/4</td>
<td>4974-2</td>
<td>4974-2</td>
<td>4</td>
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<td></td>
<td></td>
<td></td>
<td>5723-2</td>
<td>5723-2</td>
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<tr>
<td>Total</td>
<td>16</td>
<td>14/16</td>
<td>4974-10</td>
<td>4974-9</td>
<td>14</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>5723-6</td>
<td>5723-5</td>
<td></td>
</tr>
</tbody>
</table>

Peroral tetracycline was administered for 1 week to a total of 16 non-carrier animals. Two weeks had elapsed from the last positive cultures in 4 animals, 1 month had elapsed for 2, 2 months had elapsed for 4, 4 months had elapsed for 4, and 6 months had elapsed for 4 other animals (total of 16 animals).

The results were identical in all but 2 of the animals (1 from the 2 week, and 1 from the 1 month group). Within 12 to 24 hours, and coincident with a diminution of the normal respiratory tract flora recoverable from the nares of these animals, *the nasal cultures again became positive for coagulase-positive staphylococci in 14 of the 16 animals.*

In all cases, the phage-typing patterns of the staphylococci recovered were identical with those of the aerosol strain, whether this was 4974 or 5723 (Table IV). No clinically detectable effect of the recalled infection was noticeable.
Whereas most of the animals (10 out of 14, or 72 per cent) lost the carrier status within the 1st week after cessation of tetracycline administration, 4 of 14, or 28 per cent, remained "permanent carriers."

(e) Tetracycline administration to animals infected with strain 4580 (tetracycline-susceptible): Tetracycline was administered perorally to 8 animals prior to infection with a tetracycline-susceptible strain of staphylococci. Discontinued at the time of aerosol challenge, tetracycline administration did not seem to alter the results from those experienced with the tetracycline-resistant strains. The carrier state persisted for 4 days in all animals. The frac-

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No. of animals</th>
<th>Persistence of carrier state</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 day</td>
</tr>
<tr>
<td>Tetracycline before aerosol only</td>
<td>8</td>
<td>8/8</td>
</tr>
<tr>
<td>Tetracycline before and for 1 wk. after aerosol</td>
<td>8</td>
<td>5/8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No. of Animals</th>
<th>Time from last positive culture (wk.)</th>
<th>No. of animals</th>
<th>Recall of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline after carrier state had cleared</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>0/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>3</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>3</td>
<td>0/3</td>
</tr>
</tbody>
</table>

tion of overt carriers then declined progressively. One animal had become a permanent carrier.

Tetracycline was administered to 8 animals prior to aerosol challenge and continued for 1 week thereafter. This program resulted in 5 of the 8 animals demonstrating positive anterior nares’ cultures immediately after aerosol exposure, but not thereafter. Three of the animals apparently did not become colonized. Sacrifice and organ cultures of 2 of the transient carriers failed to reveal staphylococci.

Tetracycline administered perorally to 8 of the animals infected with strain 4580 after their anterior nares cultures had been negative for 2 to 8 weeks failed to recall the attenuated infection (Table V). The existence of an attenuated infection was established only after the animals had been crowded together or had experienced an intercurrent respiratory infection (10).

None of the animals exposed to tetracycline and aerosol challenge (Experi-
ments a, b, c, and e above) appeared to suffer any ill effects from the tetracycline or from the infection. Moderate diarrhea occurred for several days in the 1st week of tetracycline administration.

Investigations into the Effect of Tetracycline on the Latent Infections.—
(a) Ecological observations: As previously recorded, the quantity and nature of microorganisms recovered from animals undergoing tetracycline medication changed rapidly under the influence of this antimicrobial. From predominantly Gram-positive (including non-hemolytic streptococci), the microflora became predominantly Gram-negative. No further ecological studies were done at this time.

(b) Studies on growth curves: The growth rates of and final concentrations attained by strains 4974 and 5723 were investigated utilizing standard, enriched and basal media. All experiments were conducted with and without the addition of graded concentrations of tetracycline.

Tetracycline did not enhance growth at any time. Both strains 4974 and 5723 required more than 20 μg of tetracycline for inhibition on plate-screening testing and on tube-dilution studies. However, significant inhibition of growth occurred at 10 μg of tetracycline per ml regardless of the medium employed in these experiments.

(c) Studies on a standardized intracutaneous staphylococcal infection: These studies are reported separately (11).

DISCUSSION

These investigations were initiated in order to find an experimental model in which to study attenuated staphylococcal infection. If such a model were found, it would be used for studies on the techniques required for the eradication of such infections. The impetus for these studies was provided by a clinical experience.

A paraplegic patient was admitted to the Palo Alto–Stanford Medical Center for rehabilitation. On admission, this patient had multiple decubitus ulcers from which coagulase-positive staphylococci (strain 4974) were recovered in profusion. In addition, he harbored these microorganisms in the anterior nares and on multiple skin sites. Consequently, it was deemed unwise to expose other patients to him, and he was placed in isolation, thereby significantly interfering with the rehabilitation program. Every effort was therefore made to rid him of his infection. Systemically and topically administered antimicrobial agents, germicidal soaps, local therapy for the decubiti, and other supportive measures resulted in the elimination of culturable staphylococci after 4 weeks of intensive treatment.

After 2 additional weeks of close observation and 2 days prior to the planned discontinuation of isolation and the institution of rehabilitation techniques,
this patient experienced a recurrence of a chronic urinary tract infection. He was treated with tetracycline. On the next morning, cultures of various body surfaces and cavities were obtained. These cultures were part of a program designed to aid in the decision for discontinuation of isolation procedures. They revealed heavy growth of staphylococci of the same phage type and antibiogram as were isolated on admission. The patient had not been out of his room, had previously been free of culturable staphylococci, and was thus deemed to have experienced a relapse, rather than a reinfection. Since the hospital was still very new, and no other staphylococci with these characteristics had yet been isolated, the case for relapse was strengthened. This case suggested that investigations aimed at eradicating staphylococcal infections without concomitant disease might be useful.

An aerosol-induced, staphylococcal infection without concomitant disease or dissemination was established in guinea pigs. This infection may be likened to the naturally occurring carrier state. The carrier state has, for the most part, been transient although some instances of permanent carriage were induced.

Staphylococci were no longer recoverable from the majority of guinea pigs after the 5th day following exposure to the aerosol. This somewhat surprising finding was not predicted. Bearing in mind the situation presented by the above patient, however, it was decided to administer tetracycline to the animals which had seemingly lost their infection. When the carrier state promptly recurred, and after contamination had been ruled out, it was decided to change the direction of the investigations with the results reported in this and succeeding publications.

The fact that staphylococci could be recalled from a state of latency or persistence weeks and months after the carrier state had cleared suggested several potential investigations. The observation that this recurrence was not associated with dissemination of the infection led to studies concerned with the dynamics and mechanisms that might be operative.

It was found that major shifts occurred in the indigenous microflora of the guinea pigs. This occurrence was anticipated and may be the most important determinant of these occurrences. However, other possibilities also exist.

A series of experiments was carried out in order to detect any enhancement of growth rate or final population size achieved by tetracycline-resistant staphylococci under the direct influence of tetracycline. Neither effect could be demonstrated.

Another series of experiments was then carried out in order to determine the effect of peroral or parenterally administered tetracycline on an experimentally induced, standardized, intracutaneous staphylococcal infection of guinea pigs and rabbits. These results are reported separately (11). It was shown that the infectious charge required to induce a standardized intracutaneous lesion (erythema, induration, and local necrosis) fell by one to two orders of magnitude
when tetracycline was administered to the test animals at the time of intradermal challenge. It seems, therefore, that the effect of tetracycline on an attenuated infection is complex and may be mediated through more than one mechanism.

The experimentally induced infection described in this and succeeding communications also lends itself well to the study of the epidemiology and pathogenesis of staphylococcal infection, at least in guinea pigs. Many of the findings described bear close similarities to previously described clinical and experimental findings.

Infection with virulent, pathogenic staphylococci occurs commonly without dissemination or resultant disease. The staphylococcal carrier state in man and in animals is very common, yet overt disease due to this organism is comparatively uncommon (summarized by Nahmias and Eickhoff, reference 12, and by Elek, reference 13). Some of the factors predisposing hosts to disease following staphylococcal infection were summarized by Keene et al. (14) and by Elek (13). The effect of antimicrobial administration on staphylococcal infection was discussed by Nahmias and Eickhoff (12), and by Berntsen and McDermott (15). Of the many factors implicated, only the effects of tetracycline administration were studied in the present investigations. The effects of crowding, corticosteroid administration, and respiratory infections on the induced staphylococcal infection are reported separately (10).

Tetracycline markedly alters the indigenous microflora of guinea pigs. Such alterations, whether drug induced (16) or induced through environmental manipulation and control (17), have resulted in increased susceptibility to experimental staphylococcal infections in other laboratory animals. The precise importance of these microfloral changes in the present context is not clear, nor were these changes dissected to establish the factor or factors of most immediate importance. It can be said, however, that the nature and composition of the indigenous microflora do influence resistance and/or susceptibility to infections, although the precise operative mechanisms are not apparent (summarized by Elek, reference 13). It is of note that Bohnhoff and Miller (18) also found that antimicrobials greatly enhanced the susceptibility of mice to an experimentally induced infection and suggested induced microfloral changes as the underlying mechanism.

The recorded studies on the in vitro growth characteristics were carried out to detect tetracycline dependence or enhancement (Arndt-Schulz principle) on the part of the test strains. For example, low concentrations of penicillin G have been shown to enhance the growth of staphylococci (19). It was therefore postulated that small concentrations of tetracycline might possibly enhance the rapidity of cell division or increase the final population size (suggested by Elek, reference 13). Neither of the anticipated results was actually found. Had either of these possibilities been documented, it would then have become
possible to argue that tetracycline acted directly, at least in part, on the parasite, thereby altering a balanced relationship in the direction of favoring the parasite. When this did not prove to be the case, other methods were investigated in order to find out more about the influence of this antimicrobial on the host-parasite balance.

The standardized intracutaneous infection (11) seemed most promising. Efforts were made to minimize and control changes in host physiology which might influence phagocytosis (13). Peroral and intramuscular administration were employed and revealed no significant differences. The results of these experiments show that tetracycline administration can significantly alter the charge required to establish a particular lesion, although the operative mechanism(s) is (are) far from clear.

The effect of tetracycline might be mediated through an interference with host metabolism directly (20), or by means of interference in host metabolism reflected in changes in the efficiency of some aspect of the inflammatory reaction. It is also possible, as Dineen (16) and Dubos and Schaedler (17) have shown, that the ecological changes observed may, in turn, induce changes in the ability of the host to control infection at sites distant from the normal residence of the respiratory and/or intestinal microflora through as yet unknown mechanisms. Additionally, tetracycline may alter the nature and balance of the microorganisms commonly present in the deeper layers of the skin. Such alterations might then result in increased susceptibility to dermal infection. All of these questions require further work for resolution.

There is precedent, at least by analogy, for many of the findings reported in the present communication. Sellers et al. (21) utilized a staphylococcal aerosol infection of mice in their studies on combined bacterial-viral infections. Utilizing very careful, quantitative tissue and organ culture techniques, they found that the inoculum had essentially disappeared from the respiratory tracts of exposed animals within 3 days of infection. No apparent lesions resulted from the infection. Sellers also showed that an antecedent viral infection of the respiratory tract resulted in abnormal persistence of staphylococci in the lungs. An intercurrent respiratory infection in an animal colony also altered the behavior of the staphylococcal inoculum (10).

Tackling (22) reported that the administration of penicillin to rabbits infected with penicillin-resistant staphylococci increased the mortality to 37.5 per cent as compared with no mortality in a group of rabbits similarly infected but not given penicillin. The mechanics underlying this observation are obscure. However, Weaver and Middlebrook (23) demonstrated that prior, in vitro exposure to penicillin increased the capacity of penicillin-resistant staphylococci to initiate an infection despite the use of prophylactic penicillin. The mechanisms underlying resistance to penicillin and to tetracycline are fundamentally different. Nevertheless, an analogous situation exists wherein increase in virulence is mediated through an antimicrobial when the infecting
strain is resistant to that antimicrobial. Much more work is required before the precise mechanisms in either case are elucidated.

The apparent disappearance and later reappearance of staphylococcal infections of the upper respiratory passages is a common clinical occurrence. Thus, Jarvis and Wigley (24) report that “recolonization with the same phage type of staphylococci” occurred in the great majority of patients treated with several topically applied antimicrobial agents.

Varga and White (25) applied topical methicillin to the nares of 22 patients who were carriers and disseminators of staphylococci. No culturable staphylococci were found in 8 patients so long as methicillin was continued. All 22 patients again became carriers after therapy had been discontinued. Approximately one-third of these patients yielded the same phage type as that isolated prior to therapy. However the time required for “recolonization” with the same phage type did not differ significantly from the time required to acquire a new phage type.

There are well known limitations to a clearly defined decision as to whether an individual is colonized with one or more than one phage type. The “reacquisition” interval did not differ significantly between the same phage type “reacquired” and different phage types newly acquired. An hypothesis is nevertheless warranted to the effect that an infection was converted from an overt carrier state to one or another form of attenuated infection. Reversion to the carrier state might then have occurred after the inimical stimulus had been removed.

The effects of tetracycline noted in the present communication also seem to have clinical counterparts. Berntsen and McDermott (15) noted that the administration of tetracycline to hospitalized patients considerably increases the risk of acquisition of drug-resistant strains when compared with an untreated group of patients maintained under identical hospital conditions.

The predominance of drug-resistant strains acquired by these patients has been reported on many occasions. Additionally, an increased risk of postsurgical and postpneumonic staphylococcal sepsis was present in patients given broad spectrum antimicrobial prophylactic therapy (reviewed in references 13 and 14). Furthermore, Berntsen and McDermott showed that the acquired microflora was usually drug-resistant. They also demonstrated a significant increase in the over-all carrier rate of staphylococci when tetracycline was used (15). Their explanation suggested increased transmissibility of staphylococci when tetracycline was employed. These investigators questioned whether this was due to some change in the biological characteristics of the staphylococci or to some other factor. They finally concluded that increased transmissibility was most likely due to drug-induced interference with the usual interspecific relationships existing among the nasopharyngeal microfloral populations. Such microfloral changes also occurred in the present studies, but their exclusive significance seems to be somewhat in doubt (11).
ATTENUATED STAPHYLOCOCCAL INFECTION

The purpose of citing these related and analogous studies is to call attention to the fact that the experiments described in the present communication were conducted on a model that seems suitable for the investigations of a great variety of facets of staphylococcal epidemiology and pathogenesis. Further studies are in progress.

SUMMARY

An aerosol-induced staphylococcal infection of previously non-infected guinea pigs is described. Investigations concerning the dynamics of this infection indicate that:

1. An infection ("carrier state") could be established predictably in every animal exposed to the aerosol inoculum.

2. Infection was limited to the upper respiratory tract and occurred without apparent systemic dissemination.

3. Cross-infection between infected and non-infected animals did not occur.

4. The initially established infection persisted in detectable form for 6 days or less in the majority of exposed animals.

5. Tetracycline administration prior to and following aerosol infection with tetracycline-resistant strains significantly prolonged the duration of the carrier state.

6. When tetracycline-resistant strains were employed, the infection could be recalled predictably by means of tetracycline administration.

7. Infection initiated with a tetracycline-susceptible strain could not be recalled by tetracycline administration.

8. The mechanism(s) of action of tetracycline in recalling the attenuated infection is (are) unknown. It (they) may not be wholly attributable to ecological changes alone, at least as these are usually considered. The indigenous microflora diminished and changed as a result of tetracycline administration, and no growth-enhancing effect of the antimicrobial of the infection strains was detectable in vitro.

9. The experimental model described lends itself well to the study of attenuated staphylococcal infection in guinea pigs, and to more general studies of staphylococcal epidemiology and pathogenesis.

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BIBLIOGRAPHY


10. Simon, H. J., Epidemiology and pathogenesis of staphylococcal infections: II. The effect of crowding. III. The effects of corticosteroid administration and naturally occurring respiratory infection on an experimentally induced attenuated staphylococcal infection of guinea pigs, data to be published.


