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Received February 19, 1963. P.S.E.B.M., 1963, v113.

## Effect of Tetracycline on a Standardized Intracutaneous Staphylococcal Infection in Guinea Pigs.* (28414)

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During investigations into induced staphylococcal infections without concomitant disease it was found that tetracycline administration could recall a latent infection among guinea pigs if the initial infection had been induced with tetracycline resistant staphylococci. Tetracycline administration was followed by the anticipated changes in the indigenous microflora of the animals from predominantly gram-positive to predominantly gram-negative. It seemed possible, however, that tetracycline might influence the guinea pig-staphylococcal host parasite relationship in other ways.

*Materials and methods.* Mature, male guinea pigs were used. Nasal cultures obtained before and after crowding and tetracycline administration failed to reveal coagulase-positive staphylococci. One group of animals was then exposed to a tetracycline resistant staphylococcal aerosol. At least one month was allowed to pass before these animals were used for the present experiments. During this period the induced staphylococcal carrier state disappeared. The other group of animals was not exposed to the staphylococcal aerosol.

Two staphylococcal strains were used. Both were coagulase and mannitol positive, hemolytic, and resistant to penicillin G, streptomycin, and tetracycline. Strain 4974 is of phage type 80/81. Strain 5723 is of type 52A/79. The strains were grown in broth overnight, centrifuged and washed twice, and suspended in saline. The inocula consisted of $10^3$, $10^4$, $10^5$, $10^6$, and $10^7$ viable units/0.1 ml, respectively.

*Procedure.* Forty-eight hours prior to infection, the hair over the dorso-lateral aspects of the animals was shaved and chemically depilated. Every effort was made to prevent breaking the skin.

The skin was prepared with 80% ethanol. The hairless areas were marked off in 3 cm squares, 3 to a side, on both sides. An additional site was prepared adjacent to the test sites on each side. A total of 8 test squares was therefore planned on each animal.

Six 0.1 ml intradermal injections of the bacterial inoculum were introduced into the test sites after preliminary preparation with 80% ethanol. One of the adjacent sites was injected with 0.1 ml of sterile saline, and one was left uninjected. Serial 10-fold dilutions of another 0.1 ml of each bacterial inoculum were made and plated in order to determine the infective charge.

*Results. Untreated animals.* No detectable lesion occurred when $10^3$ or $10^4$ viable units were injected (Table I). At $10^5$ viable units there was usually a transient erythema with or without slight induration beginning at 24 hours after injection and lasting for an addi-

* Supported by a grant from Nat. Inst. of Allergy and Infect. Diseases, Nat. Inst. Health, U.S.P.H.S.

TABLE I. Effects of Intradermal Injections of Tetracycline-Resistant Staphylococci on Normal, Untreated Guinea Pigs.

| GUINEA PIG NO. | INFECTIOUS CHARGE (VIABLE UNITS/0.1ml) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $10^3$ | | $10^4$ | | $10^5$ | | $10^6$ | | $10^7$ | | |
| | REACTION (PEAK)** | | | | | | | | | | |
| | E | I | E | I | E | I | E | I | E | I | N |
| 1.A* | O | O | O | O | + | O | +++ | +++ | ++++ | +++ | O |
| 2.A | O | O | O | O | + | O | +++ | ++ | ++++ | +++ | + |
| 3.A | O | O | O | O | + | O | +++ | +++ | ++++ | +++ | + |
| 4.A | O | O | O | O | ++ | O | +++ | +++ | ++++ | ++++ | ++ |
| 5. | O | O | O | O | + | O | +++ | +++ | ++++ | ++++ | O |
| 6. | O | O | O | O | ++ | O | +++ | ++ | ++++ | +++ | + |
| 7. | O | O | O | O | + | O | +++ | +++ | ++++ | ++++ | + |
| 8. | O | O | O | O | + | O | +++ | ++ | ++++ | +++ | O |

A* = PREVIOUSLY EXPOSED TO AEROSOL
** = IN mm
E = ERYTHEMA
I = INDURATION
N = NECROSIS

KEY TO SYMBOLS

| mm | |
|---|---|
| 1 - 5 | + |
| 6 - 10 | ++ |
| 11 - 20 | +++ |
| > 20 | ++++ |

tional 48 to 72 hours. At $10^6$ viable units, the erythema and induration appeared by 24 hours, averaged more than 1 cm in diameter, and did not begin to resolve until 96 to 120 hours. With $10^7$ viable units, the erythema and induration were greater than with $10^6$, and 5 out of 8 animals experienced some sloughing of the skin.

The dose related responses described above were not appreciably altered by the presence or absence of other injection sites tested concurrently in the same animal. They were approximately the same whether the animals were non-carriers or were aerosol infected, but in the non-carrier stage of the infection. They were not affected by restricting food overnight.

*Effect of parenterally administered tetracycline.* A typical experiment is described in which 7 guinea pigs were used. Three had not been exposed to aerosol infection. Four had been exposed to aerosol infection but had lost their carrier status between 2 and 5 weeks before commencement of tetracycline administration. These animals were not the same as those used in the standardization experiment described above.

Tetracycline was administered in a dose of 50 mg intramuscularly in the morning. Tetracycline pretreatment was begun 48 hours before the intradermal injections and continued for a total of 7 days.

There was no significant difference in the cutaneous reactions of the animals whether or not they had been infected with the test strain via the aerosol route (Table II). However, the nasal cultures of the 4 animals which had been exposed to the aerosol became positive for the aerosol strain within 24 hours following administration of the antimicrobial.

There was no response to the $10^3$ viable unit inoculum in 5 of the animals so challenged. Two animals did develop transient erythema which lasted for less than 24 hours. At the $10^4$ inoculum, all animals developed some erythema, and 4 of the animals developed induration from 4 to 6 cm in diameter. The erythema and induration lasted for approximately 72 to 96 hours.

At the $10^5$ inoculum all animals developed erythema and induration. Appearing at 24 hours, the induration usually reached its maximum extent by 72 to 96 hours and then slowly resolved. Two animals demonstrated induration of greater than 1 cm in diameter. Three had induration approximating 0.7 to 1.0 cm, and 2 had induration of less than 0.5 cm.

At the $10^6$ viable units inoculum, 2 animals experienced sloughing, 4 of the remainder

TABLE II. Effects of Intradermal Injections of Tetracycline-Resistant Staphylococci on Guinea Pigs Treated with Perorally Administered Tetracycline.

| GUINEA PIG NO. | INFECTIOUS CHARGE (VIABLE UNITS/0.1ml) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $10^3$ | | $10^4$ | | $10^5$ | | $10^6$ | | | $10^7$ | | |
| | REACTION (PEAK)** | | | | | | | | | | | |
| | E | I | E | I | E | I | E | I | N | E | I | N |
| 1A* | O | O | + | O | ++ | + | +++ | ++ | O | ++++ | ++++ | ++ |
| 2.A | O | O | + | O | +++ | ++ | ++++ | ++++ | + | ++++ | ++++ | +++ |
| 3.A | O | O | ++ | + | +++ | +++ | ++++ | ++++ | + | ++++ | ++++ | ++++ |
| 4.A | + | O | ++ | ++ | +++ | +++ | ++++ | +++ | O | ++++ | ++++ | ++++ |
| 5. | O | O | ++ | + | +++ | ++ | +++ | +++ | O | ++++ | ++++ | + |
| 6. | + | O | ++ | ++ | +++ | ++ | +++ | +++ | O | ++++ | ++++ | + |
| 7. | O | O | + | O | ++ | + | +++ | +++ | O | ++++ | +++ | O |

A* = EXPOSED TO AEROSOL.
** = IN mm.
E = ERYTHEMA
I = INDURATION
N = NECROSIS

KEY TO SYMBOLS

| mm | |
|---|---|
| 1 - 5 | + |
| 6 - 10 | ++ |
| 11 - 20 | +++ |
| 20 | ++++ |

demonstrated induration of greater than 1 cm in diameter, and one had induration of less than 1 cm.

At the $10^7$ inoculum, the 2 animals which had experienced sloughing at the $10^6$ site manifested a greater area of necrosis and slough eventually requiring their sacrifice. Another animal developed a necrotic area greater than 2 cm in diameter and was also sacrificed. Three other animals had smaller areas of necrosis which eventually healed. The 7th animal had induration slightly greater than 1 cm in diameter but never demonstrated a break in the skin.

*Effect of perorally administered tetracycline.* The above experiment was repeated, this time utilizing the peroral route of tetracycline administration and a 12-hour fast before each dose of the antimicrobial (Table III). Except for diarrhea, which occurred in all animals so treated, the inoculum-related cutaneous reactions were very similar to those encountered following parenteral administration of tetracycline.

Induration appeared in 2 of 8 animals at the $10^4$ viable units inoculum; 4 animals developed induration of greater than 1.0 cm in diameter with $10^5$ viable units and the other 4 demonstrated induration of between 0.5 and 1.0 cm; 3 animals had small areas of sloughing at the $10^6$ inoculum, 2 others had induration almost 2 cm in diameter but without slough, and 3 demonstrated induration approximately 1.5 cm in diameter. At the $10^7$ viable units inoculum, 3 animals manifested areas of slough so great they eventually had to be sacrificed, 2 had necrosis and slough but eventually healed, and 3 demonstrated induration of almost 2 cm in diameter but never developed breaks in the skin.

TABLE III. Effect of Intradermal Injections of Tetracycline-Resistant Staphylococci on Guinea Pigs Treated with Parenterally Administered Tetracycline.

| GUINEA PIG NO | INFECTIOUS CHARGE (VIABLE UNITS/0.1ml) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $10^3$ | | $10^4$ | | $10^5$ | | $10^6$ | | | $10^7$ | | |
| | REACTION (PEAK)** | | | | | | | | | | | |
| | E | I | E | I | E | I | E | I | N | E | I | N |
| 1.A* | O | O | ++ | O | +++ | ++ | +++ | ++ | O | ++++ | +++ | O |
| 2.A | O | O | + | O | +++ | ++ | +++ | +++ | O | ++++ | ++++ | ++ |
| 3.A | O | O | ++ | O | +++ | +++ | ++++ | ++++ | + | ++++ | ++++ | ++++ |
| 4.A | O | O | + | O | +++ | +++ | ++++ | ++++ | + | ++++ | ++++ | ++++ |
| 5. | + | O | ++ | + | +++ | +++ | ++++ | ++++ | + | ++++ | ++++ | ++++ |
| 6 | O | O | ++ | O | ++ | ++ | +++ | +++ | O | ++++ | +++ | O |
| 7. | O | O | ++ | + | +++ | +++ | ++++ | +++ | O | ++++ | ++++ | ++ |
| 8. | O | O | + | O | +++ | ++ | +++ | +++ | O | ++++ | +++ | O |

A* = EXPOSED TO AEROSOL
** = IN mm
E = ERYTHEMA
I = INDURATION
N = NECROSIS

KEY TO SYMBOLS (mm)
1 - 5 = +
6 - 10 = ++
11 - 20 = +++
> 20 = ++++

Similar experiments utilizing the other tetracycline resistant strain of staphylococcus failed to reveal any significant differences from the results observed with strain 4974. Experiments utilizing a tetracycline *susceptible* strain of staphylococcus were also carried out, but failed to yield consistent results. All of the lesions were suppressed in some of the animals receiving tetracycline whereas other animals developed erythema and mild induration at the $10^6$ or $10^7$ viable units inoculum.

*Discussion.* The experiments reported here were undertaken as a part of a more extensive study concerning the influence of tetracycline on an induced attenuated staphylococcal infection in guinea pigs(1). The results indicate that tetracycline administration significantly decreased the charge of tetracycline resistant staphylococci required to initiate a standardized intracutaneous lesion. The results were independent of route of administration. Efforts were made to control and minimize those changes in host physiology which have been shown to influence phagocytosis(2).

Somewhat similar and still unexplained experimental findings have been reported. For instance, Tacking(3) found that penicillin administration to rabbits infected with penicillin resistant staphylococci resulted in a 37.5% mortality whereas all infected but untreated animals survived.

Other studies have been concerned with the possible growth-enhancing or virulence-increasing effects of antimicrobials on antimicrobial resistant bacteria. Thus, Miller *et al.*(4) found that small concentrations of penicillin-G enhanced the growth of penicillin-G resistant staphylococci. Additionally, Weaver and Middlebrook(5) demonstrated that prior, *in vitro* exposure to penicillin enhanced the capability of penicillin resistant staphylococci

to initiate an infection despite the use of prophylactic penicillin.

The mechanisms of resistance to penicillin and tetracycline, their mode of action on bacteria, and their effects on mammalian hosts are fundamentally different. Nevertheless, situations analogous to those described in the present communication have been described; *i.e.*, apparent increases in virulence seem to be mediated through an antimicrobial when the infecting agent is resistant to that antimicrobial.

We have reported(1) that neither tetracycline-dependence nor enhancement of growth was found under a variety of *in vitro* conditions.

Other possibilities concerning the mechanism of action of tetracycline in this experiment include induced changes in the indigenous microflora of the animals and direct influences of tetracycline on host metabolism. Antimicrobial-induced(6) or environmentally-induced(7) alterations among the indigenous microflora of laboratory animals have resulted in increased susceptibility to staphylococcal infections. Although such changes were noted in the above experiments, the precise mechanisms whereby such changes alter susceptibility are not understood(6,7,8).

Tetracycline may exert profound effects on host metabolism(9,10) and may thereby increase susceptibility directly or through some impairment of the inflammatory reaction. Aside from the negative results discussed above, however, further work is needed to define precisely the infection-enhancing effect of tetracycline in this experimental model.

*Summary.* Administration of tetracycline markedly lowered the charge of tetracycline-resistant staphylococci required to initiate a standardized intracutaneous lesion in guinea pigs. The results were independent of the route of antimicrobial administration. Tetracycline administered before and during the course of an infection with tetracycline susceptible staphylococci appeared inconstantly to increase the infectious charge required to establish the lesion. The mechanisms underlying these observations are not understood.

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Received February 25, 1963. P.S.E.B.M., 1963, v113.

## Abnormalities in Brain Esterases in Multiple Sclerosis.* (28415)

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It has been shown that lipolytic enzymes can cause demyelination both *in vitro* and *in vivo*(1-3). That "ali-esterase" may be concerned in myelin degradation during the later stages of Wallerian degeneration has been suggested(4). Since multiple sclerosis is a disease characterized by demyelination, an investigation of the esterases of nervous tissues from cases of this disorder might be justified on the basis that abnormalities, if demonstrable, could be correlated with the

* Supported by grants from Public Health Service, and from Nat. Multiple Sclerosis Soc.