

as 1.2 mg/g. Fatty changes, deposits of bile pigment and erythropoiesis in the liver were additional features of the pathological picture encountered only in infected embryos.

Having established that reproducible malarial infections of the duck embryo could be obtained with inoculations of erythrocytic stages of *P. cathemerium*, we undertook a preliminary study of the possibility of producing infections using the ground organs of infected mosquitoes (*Culex pipiens*).[†] Though no infections have been produced as yet some embryos can survive at least 14 days after such inoculation when incubated at 40°C. No attempt was made to produce sterile inocula

[†] The strain of *Culex pipiens* was kindly supplied by Dr. F. Wolfson.

beyond the use of completely sterile equipment.

Summary. Intraembryonic inoculation of duck embryos with the 3T strain of *P. cathemerium* causes reproducible fatal infections with a high level of parasitemia 6-11 days after inoculation. Splenomegaly occurs in these infected embryos. Intraembryonic inoculation of *P. lophurae* produces much more variable results. Infection requires a considerably longer period to develop. This may depend upon the maturity of the erythrocyte in the embryo. Both species of plasmodia can develop in the embryo at either 37°C or 40°C. Duck embryos can survive the intraembryonic inoculation of ground organs of malaria-infected mosquitoes if they are incubated at 40°C.

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Relative Susceptibility of Staphylococci to the Bacteriostatic Action of Antibiotics.

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During the last few years many antibiotics have been studied experimentally and several, notably penicillin, tyrothricin, streptothricin, and streptomycin, have been employed successfully in the treatment of human infections or are being investigated at the present time regarding their clinical usefulness. It is well known that the spectra of activity vary from compound to compound. Certain antibiotics, however, are more effective than others against some species of microorganisms. Herrell and Heilman¹ found penicillin superior to gramicidin in bactericidal activity toward staphylococcus, pneumococcus, and hemolytic streptococcus. The question presents itself as to whether or not various strains belonging to one and the same species differ in their susceptibility to the action of antibiotics. Crowe

and Ward² state that certain strains of staphylococci are insensitive to tyrothricin or crude penicillin. On the other hand, Rammelkamp and Maxon³ reported that 29 different strains of staphylococci varied only slightly in their resistance to penicillin. The results of an investigation into the relative activity of penicillin, tyrothricin, and streptothricin on various strains of staphylococci are embodied in the ensuing report.

Material and Methods. Strains of *Staphylococcus aureus*, isolated from patients at this hospital, were used in this investigation. In addition, several strains (Nos. 3, 20, 209) were obtained through the kindness of Dr. Jean V. Cooke, Washington University, St.

¹ Herrell, W. E., and Heilman, D., *Am. J. Med. Sci.*, 1943, **206**, 221.

² Crowe, S. J., and Ward, A. T., *N. Y. State J. Med.*, 1945, **45**, 61.

³ Rammelkamp, C. H., and Maxon, T., *Proc. Soc. Exp. Biol. and Med.*, 1942, **51**, 386.

Louis. The strains were kept on brain veal agar. For the experiments, they were grown in brain heart infusion for 18 hours at 37°C. Suspensions, containing approximately 10,000,000 organisms per ml, were prepared for seeding purposes.

All antibiotics were dissolved in brain heart infusion. Sodium penicillin-broth was freshly prepared for each experiment from a stock solution containing 100,000 U per 10 ml. This stock solution was kept in the refrigerator for 3-4 days. Streptothricin was supplied through the courtesy of Merck & Co. A stock solution containing 100 mg% of this compound was prepared and sterilized by filtration; it was kept in the refrigerator and dilutions thereof were prepared freshly for each experiment. Brain heart infusion containing various concentrations of tyrothricin was made from a stock solution containing 25 mg per ml of this compound.

The experiments were set up as follows: To 2 ml of broth containing various amounts of antibiotics, was added 0.2 ml of the staphylococcal suspension. Broth, used as a control, was seeded likewise. The broths were then incubated at 37°C. The resulting growth was noted grossly at various intervals.

Results. Experiments with various strains of coagulase-positive and coagulase-negative staphylococci, revealed that the relative susceptibility to the bacteriostatic action of penicillin, streptothricin, and tyrothricin varied from strain to strain. The results of the illustrative experiment carried out with four selected coagulase-positive strains are presented in Table I.

It may be noted that strain No. 1725 was less susceptible to penicillin and more susceptible to tyrothricin than strain CH. The former strain was more resistant to penicillin than all others studied. Strain No. 3 was less susceptible to streptothricin and more susceptible to penicillin than strain No. 1725. Strain No. 2019 was inhibited in its growth by all three antibiotics in the concentrations employed and thus differed from the other 3 strains. These findings were corroborated in repeated experiments. It is evident, therefore, that the 4 strains of *Staphylococcus aureus* differ in their relative susceptibility to

TABLE I.
Bacteriostatic Action of Various Antibiotics on Staphylococci.

Antibiotic substance		No. 1725		No. CH		Strains of Staphylococci No. 2019		No. 3	
		a	b	a	b	a	b	a	b
Penicillin	1 U/ml	—	++++	—	—	—	—	—	—
	0.1 " "	++++	++++	—	—	—	—	—	—
Streptothricin	20 mg%	—	—	—	—	—	—	—	—
	10 " "	—	++++	++++	++++	—	—	++++	++++
Tyrothricin	2.5 " "	—	—	—	—	—	—	—	—
	0.8 " "	—	—	++++	++++	—	—	—	—
Control		++++	++++	++++	++++	++++	++++	++++	++++

a = 24 hr at 37°C. b = 96 hr at 37°C.

— = No visible growth. + to +++++ = Various degrees of visible growth.

these antibiotics.

Similar results were obtained with other strains of staphylococci. For instance, strain No. C proved to be less susceptible to tyrothricin and markedly more susceptible to both penicillin and streptothricin than strain No. 1725. The growth of strain No. 1725 was not inhibited in the presence of 1 U of penicillin, whereas that of strain No. 2535 was prevented by 0.1 U of this antibiotic. In contrast, tyrothricin in a concentration of 0.25 mg% prevented the growth of strain No. 1725 for 24 hours and failed to do so with strain No. 2535 in a concentration of 0.6 mg%. Of 3 selected coagulase-negative strains No. 2320 was more susceptible to the action of penicillin than Nos. 2203 and 2204.

Quantitatively, a certain strain of staphylococcus may grow in the presence of an antibiotic in concentrations from 2 to 10 times greater than that required to prevent visible growth of another strain. It is evident that according to the results of these experiments, the relative susceptibility or insusceptibility of a certain antibiotic does not necessarily parallel a similar susceptibility or insusceptibility to another antibiotic.

Discussion. The observations reported here seem to indicate that the mode of action of the antibiotics tested is not alike. As yet it is not possible to state which properties of different strains of staphylococci determine the relative susceptibility or insusceptibility to the action of these drugs. Furthermore, it is conceivable that the findings may have clinical implications, provided that the difference in susceptibility of various strains observed *in vitro* manifest themselves also *in vivo*. If this be the case, it may be advisable under certain circumstances to determine *in vitro* the relative susceptibility of microorganisms to various antibiotics as the basis for the selection of the most suitable chemotherapeutic agent.

Summary. Various strains of staphylococci differ in their relative susceptibility to the bacteriostatic action of penicillin, tyrothricin, and streptothricin *in vitro*. The relative susceptibility to one antibiotic is not necessarily paralleled by a similar susceptibility to another antibiotic. The implications of these observations are discussed.

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2(Naphthyl-(1')-Methyl)-Imidazoline Hydrochloride (Privine).^{*} I. Potency in Modifying Cocaine Hydrochloride-Induced Convulsions.

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The early work of Braun,¹ confirmed by Maurel,² Custer,³ and others, seemed to

^{*} This report is part of a project which involves a complete pharmacologic investigation of Privine and related amines and is supported by Ciba Pharmaceutical Products of Summit, N.J.

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¹ Braun, H., *Arch. f. klin. Chir.*, 1903, **69**, 541.

² Maurel, E., *Bul. gen. de Therap. Paris*, 1892, **122**, 201.

³ Custer, *Kokain and Infiltrations Anästhesie*, Basel, 1898, p. 34.

establish that epinephrine simultaneously injected subcutaneously with cocaine exerted its protective action against cocaine-induced convulsions in a mechanical fashion by constricting the vicinal vessels and so delaying the absorption of the cocaine that a convulsive level was never attained in the blood stream. It occurred to us that advantage might be taken of this phenomenon to compare the effectiveness of different vasoconstrictors. As the first phase of an extended study of the vasoconstrictor properties of Privine we employed this method of comparing its effectiveness with that of epinephrine.