the high hydrolysis rates of tributyrin and butyrylcholine compared to the low rate for benzoylcholine, and exhibited maximal hydrolyzing powers at high substrate concentrations. When the lung was incubated *in vitro* for 8 days, the acetylcholinesterase activity increased with the growth of the cells and the non-specific esterase activity decreased. Thus, the acetylcholinesterase activity would tend to become "unmasked," explaining the optimal substrate concentrations observed for acetylcholine and propionylcholine. Addition of acetylcholine to the medium increased the activity (concentration) of acetylcholinesterase only, explaining why the enzymatic hydrolysis of tributyrin and butyrylcholine was not affected. This suggested explanation is compatable with the data and indicates that, at least under the experimental conditions of the reported study, a physiological substrate of an enzyme has a role in the formation of that enzyme.

Summary. Addition of 0.02 Molar acetylcholine to the medium of 15-day embryo chick lung cultivated *in vitro* for 8 days caused a 2- to 6-fold increase in the cholinesterase activity of that tissue. The increase in enzyme activity was maximal 4 days after addition of acetylcholine to the medium, and was maintained only by the continued addition of acetylcholine to the medium. Neither choline nor acetate alone nor choline and acetate together produced an increase in enzyme activity comparable to that produced by acetylcholine. Results of substrate specificity studies on the cholinesterases of chick embryo lung suggested that there was both an acetylcholinesterase and a non-specific esterase in the lung cells. Only the acetylcholinesterase was affected by the added acetylcholine. The results indicated that acetylcholine was an inducing agent for the formation of acetylcholinesterase in the embryo chick lung cells cultivated *in vitro*.

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Relationship Between Penicillin Susceptibility and Virulence of *Staphylococcus aureus*. (23598)

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Penicillin susceptibility and virulence as measured by intraperitoneal mouse pathogenicity with mucin(1) of 80 recently isolated strains of staphylococci were determined and compared to ascertain whether or not any relationship exists between both characteristics. A good degree of reproducibility for a biological procedure has been observed with the animal virulence test for staphylococcal pathogenicity provided young Swiss mice and a constant, moderate-sized inoculum of microorganisms are employed. To further elucidate this problem, 4 penicillin-sensitive staphylococcal strains were assessed for virulence before and after induction of penicillin resistance *in vitro*. Since repeated artificial passage on media containing graded concentrations of penicillin might conceivably result in loss of virulence by itself, each strain was also transferred on antibiotic-free media an equal number of times as that required to induce resistance and also tested for virulence.

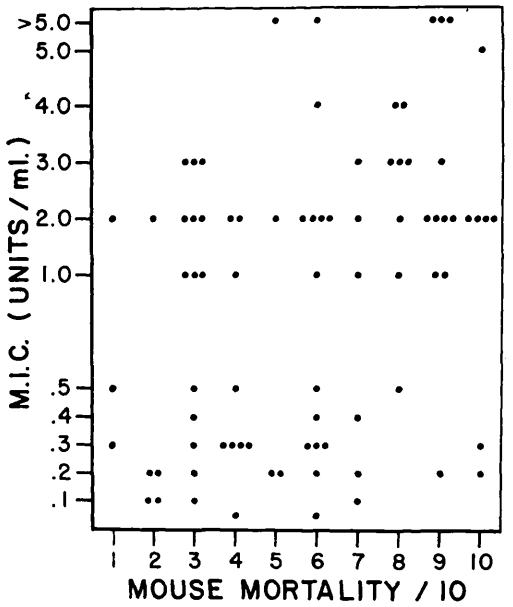

FIG. 1. Comparison between penicillin susceptibility and intraperitoneal mouse pathogenicity with mucin of 80 coagulase positive, hemolytic, mannitol fermenting strains of *Staphylococcus aureus.*

To establish a uniform basis for comparison, only coagulase positive, hemolytic, distinctly pigmented, mannitol fermenting strains were employed.

Methods. The tube dilution method was used to determine penicillin sensitivity. Medium was fresh veal extract broth, pH 7.2-7.4. Test was performed in 2 ml amounts, by adding 1 ml of a 10^{-6} dilution of a 6 hour broth culture of the test strain to a series of tubes containing varying concentrations of standard penicillin in 1 ml of broth. The minimal inhibitory concentration is defined as the smallest amount of antibiotic which completely inhibited growth after 18 hours of incubation at 37°C. A growth control tube containing the test inoculum without antibiotic was used with each determination. Ten albino Swiss mice, weighing approximately 15-20 g, were used to assess the virulence of each strain. 0.5 ml of 5% hog gastric mucin, prepared according to Miller(2), was injected intraperitoneally into each mouse followed within 15-20 minutes by 0.25 ml of a 16-20 hour fresh veal extract broth culture of the strain under test adjusted to an optical density of 0.05 on a Photoelectric Lumetron colorimeter, using a red filter (650 mμ) and which contained approximately 150 million organisms. Animals were observed 48 hours and deaths recorded. Penicillin resistance was induced by serial subculture of each strain on agar plates containing progressively increasing concentrations of antibiotic. Inoculum for each passage was obtained from preceding growth on plates containing the highest concentration of penicillin. Each strain was also serially transferred on antibiotic-free agar slants an equal number of times as that required to induce resistance.

Results. Mortality in mice produced by each strain following intraperitoneal injection with mucin in relation to its penicillin sensitivity is shown in Fig. 1.

Considerable dispersion of mouse pathogenicity may be observed among strains of the same antibiotic susceptibility. Although no distinct correlation is found between both factors, a greater concentration of strains producing a higher mortality is noted among those requiring larger amounts of penicillin to effect growth inhibition as compared to those strains inhibited by smaller amounts where mouse mortality is fairly evenly distributed throughout. The status of all 80 strains with regard to antibiotic sensitivity and virulence is summarized in Table I. As may be noted from the table, the 42 penicillin-sensitive strains were almost evenly divided between those eliciting a mortality of up to 50% (5/10) and over 50% (6/10), 23 and 19 respectively. On the other hand, of the 38 antibiotic-resistant strains only 11 or 28.9% proved avirulent by the above criterion while 27 or 71.1% were virulent. When these data

TABLE I. Mouse Mortality of Staphylococcal Strains in Relation to Their Penicillin Susceptibility.

Penicillin susceptibility	Total No. strains	Mouse mortality: Up to 50% No.	%	Greater than 50% No.	%
Sensitive*	42	23	54.8	19	45.2
Resistant	38	11	28.9	27	71.1

* Strains inhibited by 1.0 unit of penicillin/ml or less are classified as sensitive, and those requiring larger amounts as resistant.

TABLE II. Intraperitoneal Mouse Pathogenicity with Gastric Mucin of Penicillin-Sensitive Strains of *Staphylococcus aureus* before and after Artificial Induction of Resistance to Penicillin.

Strain	Mouse mortality/10		
	Original isolate	Passage control on antibiotic-free medium	After induction of penicillin resistance
25	2	1	0
88	2	4	1
89	4	4	9
90	7	4	0

were subjected to statistical analysis by means of the chi square test.* a chi square of 5.44 and a P value of 0.02 was obtained thereby indicating that the observed differences were definitely significant. Thus based upon the above findings with the series of staphylococci investigated it may be concluded that although no direct relationship is evident between penicillin susceptibility and virulence, penicillin-resistant strains are more apt to be virulent than are those sensitive to the antibiotic.

Mouse pathogenicity of 4 penicillin-sensitive strains before and after induction of resistance as well as the mortality produced after passage on antibiotic-free agar is recorded in Table II.

After serial transfer on graded concentrations of antibiotic, strains #25, 88, 89, and 90 were able to grow on agar plates containing 80, 100, 1,000, and 400 units of penicillin per ml whereas their original isolates as well as their subcultures on antibiotic-free media were inhibited by 0.05, 0.1, 0.5, and 0.05 unit of penicillin per ml respectively. No consistent pattern with respect to change in virulence following induction resistance is discernible. Thus, strains #25 and 88 exhibited a slight decrease, strain #89 a marked increase, and strain #90 a marked decrease in virulence after being made resistant.

Summary. No distinct correlation was established between penicillin susceptibility and virulence of 80 coagulase positive, hemolytic, mannitol fermenting strains of *Staphylococcus aureus*. Both penicillin-resistant and penicillin-sensitive strains vary considerably in their mouse pathogenicity but a significantly higher proportion of the former proved to be virulent as compared to the latter which were fairly evenly divided between virulent and avirulent strains. No definite pattern with respect to change in virulence was observed with 4 originally penicillin-sensitive strains following artificial induction of resistance to the antibiotic.

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Establishment of Human Adult Tonsil Cells in Continuous Culture and Their Virus Susceptibilities.* (23599)

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This paper describes the development in tissue culture of a continual line of epithelial-like cells from adult human tonsil tissue and compares the susceptibility of these cells to a number of viruses with that of a human carcinoma cell line (strain HeLa). The tonsil line has been subcultured serially over 40 times in the course of a year and has been maintained from the start in a medium free from human serum.

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