

also be considered. Further study is necessary to elucidate the nature of this interesting phenomenon.

Summary. 1. Thirteen strains of Group A streptococci were transferred serially on medium containing penicillin in an effort to induce resistance. Two strains developed a 17-fold increase in resistance. Eleven strains either remained unchanged or showed an increase up to only 6-fold at the end of 60 subcultures.

2. No increased resistance was induced in the same organism by serial transfers on control medium.

3. Strains which had acquired resistance were restored to their original level of penicillin sensitivity by serial subculture on

penicillin-free medium or by serial intracerebral passages in mice.

4. A marked loss of virulence for mice was shown by all resistant strains of streptococci. The virulence was restored in 2 strains by passage in normal mice.

5. In about one-half of the resistant strains the group specific antigen could not be demonstrated.

6. Transient changes in the colonial appearance, and changes in hemolysis from beta to alpha or gamma were shown by most strains when growing on maximal concentrations of penicillin.

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Antibiotic Studies on Beta Hemolytic Streptococci: II. Penicillin Resistance Acquired by Group B Organisms.

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Most strains of group B beta hemolytic streptococci possess a higher degree of initial penicillin resistance than group A organisms. The range reported by 3 groups of investigators^{1,2,3} was 0.0625 to 0.25 units of penicillin per ml of medium. We have found the upper and lower limits of sensitivity to extend from 0.01 to 0.10 unit of penicillin per ml of medium by the Fleming ditch-plate method⁴ for 16 freshly isolated strains of group B streptococci.

The present study was carried out in an effort to determine the rate of development of penicillin resistance *in vitro*. In addition we

were interested in learning whether the antigenic, hemolytic, colonial, and mouse virulence changes found in resistant group A organisms⁵ occurred in group B streptococci.

Results. *A. Acquired Resistance on Medium Containing Penicillin.* Ten strains of group B streptococci were transferred on increasing concentrations of penicillin. The results are given in Table I. A straight line curve results when the mean of fifteen transfers is plotted on semi-logarithmic paper. The graph of the daily values shows the same general trend but with many irregularities in the curve. These variations can be explained by slight differences in the amount of medium per plate, daily loss of potency of penicillin in stock solution, and errors in judgment in estimating the proper range of penicillin concentrations needed for each transfer.

The maximum change occurred in the R₁

¹ Watson, R. F., *Proc. Soc. Exp. Biol. and Med.*, 1944, **57**, 65.

² Simmons, R. T., and Christie, R., *M. J. Australia*, 1946, **1**, 349.

³ Rutherford, R. H., Marquardt, G. A., and Van Ravenswaay, A. C., *J. Missouri State Med. Assn.*, 1946, 535.

⁴ Fleming, A., *Proc. Roy. Soc. Med.*, 1941, **34**, 342.

⁵ Gezon, H. M., *Proc. Soc. Exp. Biol. and Med.*, 1948, **67**, 207.

TABLE I.
Induced Penicillin Resistance in Group B Beta Hemolytic Streptococci.

Strain	Number of passages	Initial penicillin sensitivity,* u/ml	Final penicillin sensitivity,* u/ml	Fold change
T ₁	60	.03	.34	11
T ₂	60	.03	.65	22
T ₃	60	.03	.65	22
T ₄	60	.03	.55	18
T ₅	60	.03	1.35	45
T ₆	60	.03	.65	22
T ₇	60	.03	.65	22
A ₅	60	.015	.90	60
A ₆	60	.015	.80	53
R ₁	56	.01	1.90	190

* Penicillin sensitivity is defined as the highest concentration of penicillin in the medium which permits colonies to grow.

strain which developed a 190-fold increase in resistance in 56 transfers. However, between each transfer of this organism on penicillin medium it was subcultured in penicillin-free blood broth. With the other nine bacteria, the increase in penicillin resistance by the plate to plate technic varied from 11 to 60-fold in 60 serial transfers.

B. Acquired Resistance on Control Media. Seven strains subcultured from the original parent stock were transferred serially on penicillin-free blood agar plates. None of the group B organisms studied showed a change in penicillin sensitivity after 60 transfers, nor did they acquire resistance more rapidly than the parent strain when cultured subsequently on penicillin medium for the 61st to 75th passages. The results are given in Table II. This same behavior was described for Group A streptococci in the preceding communication.⁵

C. Loss of Acquired Resistance. Three strains of streptococci, after acquiring penicillin resistance, were subcultured on plain blood agar. The R₁ strain was transferred 25 times, and the A₅ and A₆ strains 50 times. At the end of these serial subcultures there was no loss of the acquired resistance in any of the 3 organisms studied.

Three resistant organisms were passed serially by intracerebral inoculation in white mice for 12 passages. Again there was no loss of acquired resistance to penicillin.

Change in Virulence. Using the method described in the preceding communication,⁵ the mouse virulence studies were performed

TABLE II.
Comparison of the Rates of Development of Penicillin Resistance of Parent and Control Strains of Streptococci.

Strain	Mean of first 15 transfers of parent* strain on penicillin medium u/ml	Mean of first 15 transfers of control† strain on penicillin medium u/ml
T ₁	.088	.072
T ₂	.083	.050
T ₃	.120	.051
T ₄	.099	.070
T ₅	.105	.076
T ₆	.094	.059
T ₇	.100	.070

* Parent strain is a freshly isolated organism.

† Control strain is the parent strain after 60 transfers on control medium.

on all 10 strains. The results are summarized in Table III. As with the group A organisms, all strains lost virulence after serial transfers on penicillin medium. The majority showed relatively little change after serial transfers on control medium.

Three resistant strains were passed 12 times through mice to restore the lost pathogenicity. The virulence reappeared in one strain. In 2 strains no significant change occurred.

Antigenic, Hemolytic, and Pigment Changes. The results of the serological grouping of the streptococci before and after serial transfers on penicillin and control media are summarized in Table IV. In 2 resistant and one control strain, it was not possible to demonstrate the presence of the group specific precipitinogen. A majority of the organisms in each classification remained groupable.

TABLE III.
Mouse Virulence of Group B Streptococci with Acquired Penicillin Resistance.

Strain	Initial LD ₅₀ [*]	LD ₅₀ [*] after 60 transfers on		LD ₅₀ [*] of resistant strain after 12 mouse passages
		control medium	penicillin medium	
T ₁	.000048 ml	.0000015 ml	.0096 ml	
T ₂	<.0000030	.000012	>.03	
T ₃	<.0000030	.000060	>.03	
T ₄	.000096	.00030	.015	>.03 ml
T ₅	<.0000030	.0000000015	>.03	
T ₆	<.0000030	.000075	>.03	
T ₇	<.0000030	>.03	.003	.0048
A ₅	.0015	.00096	>.03	
A ₆	.012	.0048	.0096	
R ₁	.00030		>.03	.00048

*LD₅₀ is stated as the amount of undiluted bacterial suspension in normal saline that will kill 50% of the mice injected. This suspension has a density equal to McFarland No. 1 standard and contains approximately 2×10^9 organisms per ml.

TABLE IV.
Group Specificity of Streptococci after 56 to 60 Serial Transfers on Penicillin and Penicillin-Free Media.

	Strain										
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	A ₅	A ₆	R ₁	
Parent culture	B	B	B	B	B	B	B	B	B	B	
Final control culture	B	B	B	B	B	B	B	N	B	—	
Final penicillin-resistant culture	N	B	B	B	B	B	B	B	B	N	

B = group B.

N = not groups A, B, or C.

— = grouping not performed.

The alteration in the character of the hemolysis described for group A organisms in the preceding report⁵ was even more striking and more permanent for group B streptococci. All 10 strains showed varying degrees of change from beta to alpha or gamma colonies at maximal concentrations of penicillin. Single colonies of either the gamma or alpha types when subcultured onto plain blood agar produced only beta hemolysis. Small colony forms occurred frequently on penicillin medium. These reverted back to the normal forms when transferred to control medium.

Several of the streptococci of Group B showed the presence of the reddish pigment described by Lancefield.⁶ No modification in this pigmentation appeared after the organisms acquired penicillin resistance.

Discussion and Conclusions. All strains of Group B streptococci studied showed a rapid development of penicillin resistance as compared with Group A organisms. The 190-fold

increase demonstrated by one organism after 60 transfers and the 107-fold rise observed in another after 80 subcultures⁷ indicate that great penicillin resistance may be induced in Group B streptococci. We have not yet attained the upper limit of resistance for any of the strains tested.

The greater ease in acquiring penicillin resistance in Group B than in Group A streptococci is probably of clinical importance. An example of this phenomenon was seen recently in a patient who died of subacute bacterial endocarditis at the Albert Merritt Billings Hospital of the University of Chicago. Shortly before death a strain of Group B streptococci was obtained from his pharynx, and at autopsy from his blood. He had received up to 5 million units of penicillin a day for the previous 30 days.

The loss of mouse virulence, changes in hemolytic characteristics, variations in the colonial appearance, and disappearance of

⁶ Lancefield, R. C., *J. Exp. Med.*, 1934, **59**, 459.

⁷ Unpublished data.

group specificity in some strains after serial transfers on penicillin medium remain unexplained.

Summary. 1. Ten strains of Group B streptococci were transferred serially on media containing increasing quantities of penicillin in an effort to produce resistance. All 10 organisms showed a significant increase in resistance varying from 11 to 190-fold over the parent strains.

2. No increased resistance was induced by serial transfers on control medium.

3. In contrast to Group A organisms, Group B streptococci did not lose resistance on subculture on blood agar or on serial

passage through mice.

4. Virulence decreased in all resistant strains of streptococci. The pathogenicity was partially restored in one organism but remained unchanged in two after passage in normal mice.

5. In only 2 resistant strains was the group specificity lost.

6. Transient alterations in the colonial appearance, and changes in hemolysis from beta to alpha or gamma were demonstrated by all strains when grown on maximal concentrations of penicillin. These colonies reverted to the parent type when subcultured on blood agar.

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Antibiotic Studies on Beta Hemolytic Streptococci: III. Penicillin Resistance Acquired by Group C Organisms.

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Streptococci of Lancefield Group A or B present group differences as well as strain variations within a group, when transferred serially on media containing penicillin. These characteristics are described in the preceding reports.^{1,2} In general, Group A differ from Group B streptococci in having a lower initial penicillin sensitivity. They show less tendency to acquire penicillin resistance *in vitro*, lose acquired resistance more rapidly on passage in mice or plain medium, and develop colonial and hemolytic changes less quickly when growing on penicillin medium.

The initial penicillin sensitivity of Group C organisms is reported³⁻⁵ to vary from 0.0078 to

0.0313 units of penicillin per ml of medium. We have studied 24 recently isolated strains by the Fleming⁶ ditch-plate method and have found their sensitivity range to be 0.007 to 0.03 units of penicillin per ml of medium.

The present study was undertaken to determine the similarities and differences between streptococci of Groups A, B, and C as regards their ability to acquire penicillin resistance.

Results. Acquired Resistance on Medium Containing Penicillin. Five strains of Group C streptococci underwent serial transfer on blood agar containing penicillin. Three of the 5 organisms after 60 subcultures demonstrated a moderate increase in penicillin resistance varying from 11- to 16-fold. Two forms, U₃ and R₃, after 60 and 56 transfers respectively, developed only a slightly increased resistance. The results are given in Table I.

Acquired Resistance on Control Medium. Four strains of streptococci subcultured from

¹ Gezon, H. M., *Proc. Soc. Exp. Biol. and Med.*, 1948, **67**, 208.

² Gezon, H. M., *Proc. Soc. Exp. Biol. and Med.*, 1948, **67**, 212.

³ Watson, R. F., *Proc. Soc. Exp. Biol. and Med.*, 1944, **57**, 65.

⁴ Simmons, R. T., and Christie, R., *M. J. Australia*, 1946, **1**, 349.

⁵ Rutherford, R. H., Marquardt, G. A., and Van Ravenswaay, A. C., *J. Missouri State Med. Assn.*, 1946, 535.

⁶ Fleming, A., *Proc. Roy. Soc. Med.*, 1941, **34**, 342.