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Resistance of *Staphylococcus aureus* to the Action of Penicillin.*

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During the course of a study of the effect of penicillin therapy in human infections it was found that a favorable response was not always obtained in those patients with infections caused by *Staphylococcus aureus*. The present study was undertaken to determine some of the factors involved in the resistance of *Staphylococcus aureus* to the action of penicillin.

Methods. The sodium salt of penicillin† was dissolved in 0.85% sodium chloride so that the final concentration was 20 Florey units per cubic centimeter. This standard solution of penicillin was used in all *in vitro* tests. In patients with infections the penicillin was administered in a similar solution but the concentration was increased to 1000 Florey units per cc.

The strains of *Staphylococcus aureus* used in this study were all isolated from patients with clinical infections and were stored on blood agar slants until needed.

The method used to determine the sensitivity of staphylococci to the action of penicillin is a modification of a test previously described.[1] In brief, in a series of 11 tubes serial dilutions of 0.2 cc of the standard solution of penicillin was made with 0.2 cc of veal infusion broth. To each tube in the series 0.5 cc of the proper dilution of a 12-hr culture of the test organism was added. Pour plates of each inoculum were made and the number of organisms was found to vary from 1000 to 30,000 per 0.5 cc. The cultures containing penicillin were then incubated at 37° C for 18-24 hr, following which they were examined for turbidity. Subcultures on blood agar plates were made from those tubes near the end point.

Resistant strains were developed *in vitro* by growing the test organism in gradually increasing concentrations of penicillin, A series of 11 cultures tubes containing decreasing amounts of penicillin were set up as described above. The test culture containing from 1000 to 100,000 organisms was added in 0.5 cc of veal infusion broth. At 24-hr intervals transfers were made from the tube showing full growth and containing the greatest concentration of penicillin. At intervals comparative tests using the parent strain of the test organism were run.

Results. The lowest concentration of penicillin required to kill 1000 to 30,000 organisms of each of 29 strains of *Staphylococcus aureus* was determined. It was found that all strains were killed in concentrations between 0.02 and 0.35 Florey units per cc of broth. Two strains were killed by 0.02, 13 by 0.04, 12 by 0.08, 1 by 0.17 and 1 by 0.35 Florey units per cc. These results indicate, then, that different strains of staphylococci vary only slightly in their resistance to penicillin.

Production of Resistant Strains. The variation in the susceptibility of strains of *Staphylococcus aureus* isolated from 14 patients with active infections could not be correlated with the clinical and bacteriological response to penicillin therapy. It therefore was important to study the possibility of acquired resistance to penicillin following prolonged exposure to the antibacterial substance. *In vitro* it was demonstrated in 2 strains that the *Staphylococcus aureus* may so adapt itself that growth takes place in broth containing relatively large amounts of penicillin. The rapidity with which the staphylococci acquired resistance is shown in Table I. Strain Aurice showed an increased resistance of 64-fold after 54 days' exposure

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1 Rammelkamp, C. H., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 95.

TABLE I.
Development of Penicillin Resistant Strains of *Staphylococcus aureus in vitro*.
In all strains there was visible subculture growth.

Strain	Days exposure to penicillin	Concentration of Penicillin in Florey units per cc									
		5.71	2.85	1.42	.71	.35	.17	.08	.04	.02	.01
Aurice	0	0	0	0	0	0	0	0	0	+	+
							0	0	+	+	+
	24	0	0	0	0	+	+	+	+	+	+
			0	0	0	+	+	+			
	38	0	0	+	+	+	+	+	+	+	+
		0	0	+	+	+	+				
	54	0	+	+	+	+	+	+	+	+	+
			+	+	+	+					
Ram	0	0	0	0	0	0	0	0	0	+	+
							0	0	0	+	
	30	0	0	0	0	0	+	+	+	+	+
					0	+	+	+	+		
	41	0	0	0	+	+	+	+	+	+	+
		0	0	0	0	+	+	+			
	49	0	0	+	+	+	+	+	+	+	+
		0	0	+	+	+	+	+			

TABLE II.
Development of Resistant Strains of *Staphylococcus aureus* During Penicillin Therapy.
In all strains there was visible subculture growth.

Case	Diagnosis	No. days treated	Total Penicillin administered (Florey units)	Concentration of Penicillin in Florey units per cc									
				5.71	2.85	1.42	.71	.35	.17	.08	.04	.02	.01
J.B.	Chronic osteomyelitis	0	0	0	0	0	0	0	0	0	+	+	+
									0	0	+		
		18	540,000	0	0	0	+	+	+	+	+	+	+
				0	+	+	+	+	+				
J.R.	Empyema	4	50,000	0	0	0	0	+	+	+	+	+	+
					0	+	+	+	+				
H.H.	”	0	0	0	0	0	0	0	0	0	0	+	+
									0	0	0	+	
		62	111,000	0	+	+	+	+	+	+	+	+	+
				0	+	+	+						
A.P	Axillary abscess	0	0	0	0	0	0	0	0	0	0	0	+
									0	0	0	0	+
		2	10,000	0	0	0	0	+	+	+	+	+	+
						0	0	0	+	+	+	+	+

to penicillin. Strain Ram showed a similar degree of increased resistance. It is of some interest that a strain of hemolytic streptococcus exposed to penicillin for a period of 45 days did not become "penicillin-fast."

Of importance are the observations made on the strains of staphylococci isolated from 14 patients during the course of penicillin therapy.‡ In only 4 subjects was a change in the susceptibility of the organism demonstrated. The results of these observations are recorded in Table II.

J.B., a 47-year-old male with chronic osteomyelitis of the left femur was treated by intravenous injections of penicillin. Cultures of the draining sinus were taken daily, and after 18 days of therapy the resistance of the staphylococcus to the action of penicillin had increased 64-fold. In this subject the local lesion was not sterilized by penicillin therapy.

Patient J.R., a male aged 16 years, de-

‡ Details of the results obtained with penicillin therapy will be reported at a later date.

veloped empyema following a left lower lobectomy. In this subject the strain of *Staphylococcus aureus* isolated prior to treatment was not tested; however, the organism isolated 4 days after the institution of local penicillin therapy was found to be markedly resistant. A concentration of 2.85 Florey units per cc was necessary to sterilize a culture of this strain.

H.H., a male aged 14 years, received 18 injections of penicillin in 62 days into a chronic empyema cavity. The original strain of *Staphylococcus aureus* was killed in broth cultures by a concentration of 0.04 Florey unit per cc, whereas the organism isolated on the 62nd day required 5.71 Florey units to effect killing. It was possible to keep the empyema cavity sterile by frequent injections of penicillin.

In one subject (A.F.) with a deep axillary abscess the local injection of penicillin into the infected area did not result in sterilization. In this instance the strain of staphylococcus increased its resistance to penicillin about 16-fold in 2 days.

Discussion. Previous reports have shown that the *Staphylococcus aureus* may be rendered resistant to the action of the sulfonamide drugs[2] and to tyrothricin[3] by growing the organism in increasing concentrations of the two substances. There is also evidence that this organism may acquire resistance in patients with staphylococcal infections receiving either sulfonamide or tyrothricin therapy.[3,4] It has been suggested that the adaptation of the infecting bacteria to the antibacterial agent may explain certain therapeutic failures.[3,4]

In this study 29 strains of *Staphylococcus aureus* were found to vary only slightly in their susceptibility to the action of penicillin. Twenty-five of the 29 strains were killed in broth cultures by a concentration of 0.04 to 0.08 Florey unit per cc. This suggests that the variation in susceptibility of different strains of *Staphylococcus aureus* does not play an important role in the therapeutic effectiveness of penicillin therapy in man.

Florey *et al.*[5] were able to produce penicillin-"fast" strains of *Staphylococcus aureus* by cultivation in broth in the presence of increasing concentrations of penicillin. A 30-fold resistance was produced after 9 weeks' exposure to penicillin. These *in vitro* observations were confirmed by the present studies. Two strains were rendered resistant 64-fold in a period of 54 and 49 days. It is of interest that the adaptation of *Staphylococcus aureus* to the antibacterial effect of penicillin takes place only after a prolonged exposure whereas sulfonamide resistance is produced much more readily.[2]

The nature of the acquired resistance of *Staphylococcus aureus* is not known. Florey showed that there was a reduction in the growth-velocity of the resistant strains.[5] No penicillin-destroying enzyme was demonstrated in the resistant strains.[5] Gramicidin-resistant and non-resistant staphylococcic strains were found to be equally susceptible to the action of penicillin.[6] Powell and Jamieson[7] observed that sulfonamide-fast pneumococci were susceptible to the antibacterial effects of penicillin.

In the present studies 4 relatively resistant strains of *Staphylococcus aureus* were isolated from patients during the course of penicillin therapy. In 3 subjects the organism isolated before the institution of therapy was compared with a later strain and found to be much less resistant. This shows, therefore, that in certain subjects penicillin therapy may result in the development of resistant strains. In one subject (J.B.) such resistance was believed to be a factor in the poor results obtained.

Conclusions. Strains of *Staphylococcus aureus* vary only slightly in their susceptibility to the antibacterial action of penicillin. By growing the organism in increasing concentrations of penicillin over a long period it was

[2] Strauss, E., Dingle, J. H., and Finland, M., *J. Immunol.*, 1941, **42**, 331.

[3] Rammelkamp, C. H., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 346.

[4] Vivino, J. J., and Spink, W. W., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 336.

[5] Florey, H. W., Abraham, E. P., Chain, E., Fletcher, C. M., Gardner, A. D., Heatley, N. G., and Jennings, M. A., *Lancet*, 1941, **2**, 177.

[6] Rammelkamp, C. H., unpublished observations.

[7] Powell, H. M., and Jamieson, W. A., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 387.

possible to render the organism resistant to penicillin.

Similar degrees of increased resistance were found in 4 strains of staphylococci isolated during the course of penicillin therapy for localized infections.

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Prolonged Adrenalin Hypertension and Subsequent Circulatory Failure.*

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Previous reports from this laboratory[1] supported the view that a state of shock which cannot be reversed by substantial infusions of the animal's own blood and which is characterized by pathological changes in the mucosa of the upper intestine can follow hemorrhage; but this only occurs when arterial pressures have been reduced severely for significant periods of time. Diminished blood flow through tissues dominantly due to hypotension seems to be the effective agent. The question remains whether the blood flow through tissues can be throttled to a similar degree of intensive vasoconstriction, while arterial pressures remain essentially normal or even become elevated.

Experimental evidence for this possibility has been sought by two types of investigation, (a) prolonged stimulation of afferent pressor nerves and (b) continued administration of potent vasoconstrictor drugs, notably adrenalin. Results from the use of the former have been consistently negative; those realized by the latter procedure remain contradictory.[2] This is due in part to the fact that prolonged administration of adrenalin causes diverse direct and secondary effects on the heart, circulation and respiration, from which an animal may expire during or following its administration. Among these accidents are pulmonary edema, myocardial insufficiency, ventricular fibrillation and respiratory failure. The possibility also exists that some investigators used doses which were too large and others, doses which were too small.

Methods. In order to compare results with those of hemorrhagic shock, dogs were anesthetized with morphine followed by "just adequate" doses of sodium barbital. Respiration, mean femoral, right atrial and intrathoracic pressures were recorded. Adrenalin chloride (Parke, Davis and Co.) was slowly and continuously infused as a 1:5000 solution into a cannulated femoral vein by means of a Mariotte burette. Table I gives the data from separate experiments. The rate of administration ranged from .0069 to .0398 mg per kilo per minute. The total dose depended partly on the weight of the animal. The duration of the infusions varied from 51 to 120 min.

After cessation of the infusion the animals were observed for 3-5 hr. As a rule, mean arterial pressure declined to, or somewhat below, control levels within 5 to 15 min. Thereafter, three types of sequence were observed: (1) Mean pressure remained above 80 min for 4 to 5 hr. (2) While mean pressure remained reasonably normal, the animal expired suddenly, due to respiratory failure. (3) Mean arterial pressure fell progressively to 40 or 20 mm and the animal expired. Only the latter sequence was regarded as a reasonable criterion of progressive circulatory failure, which was probably irreversible. These are designated by the term, "shock," in Table I. A survey of 10 experiments shows that 2 animals (not included in Table I) died during administration of adrenalin due to causes which had no relation to shock. Two

* This investigation was supported by a grant from the Commonwealth Fund.

1 Werle, J. M., Cosby, R. S., and Wiggers, C. J., *Am. J. Physiol.*, 1942, **136**, 401.

2 Wiggers, C. J., *Physiol. Rev.*, 1942, **22**, 74.