TABLE II.
Sterilizing Dosage of Penicillin in Eggs Infected in the Chorio-Allantoic Space with Streptococci.

Group	Strain	Daily dosage in units Mean of 5 passages				
		1-5	6-10	11-15	16-20	21-25
A	S_2	0.19	0.13	0.17	0.24	0.28
A	S_3	0.34	0.32	0.32	0.34	0.30
B	T_3	1.70	2.80	1.76	3.60	5.60
B	T_5	0.42	0.66	1.52	3.80	6.60
C	U_1	0.25	0.18	0.54	0.52	0.66
C	U_2	0.30	0.22	0.36	0.60	2.18

but we failed to demonstrate it through limitations of the method. The increasing penicillin resistance *in vivo* of the group B and C streptococci was not associated with increasing resistance *in vitro*.

16705

Drug Sensitivity of Hemolytic Streptococci Isolated from Cases of Scarlet Fever Treated with Penicillin.*

THOMAS L. HARTMAN AND LOUIS WEINSTEIN.

From the Haynes Memorial and Evans Memorial, Massachusetts Memorial Hospitals, and the Department of Medicine, Boston University School of Medicine, Boston, Mass.

The development of resistance to penicillin has been observed to occur *in vivo* in strains of *Streptococcus viridans* and *Staphylococcus aureus* during therapy with this agent.[1,2]

Although penicillin has had very wide use in the treatment of group A hemolytic streptococcus infections there have been no reports of the development of strains with lack of sensitivity to this drug which constituted therapeutic problems.

Available information[3-5] would seem to indicate that *Strep. pyogenes* (group A) as encountered in the general population is almost uniformly highly susceptible to penicillin. Hirsch *et al.*,[6] however, have reported the isolation of a strain of this organism which was susceptible only to 0.625 units of penicillin from a patient ill with scarlet fever who had not received any antibiotic therapy. Recently, Milzer *et al.*,[7] reported the occurrence of penicillin-resistant beta hemolytic streptococci in the throat cultures of children with rheumatic fever treated prophylactically with 100,000 units of penicillin orally each day for at least 4 months. Although these strains were resistant to 10 units of penicillin following the period of prophylaxis, none of the streptococci present prior to chemotherapy were available for comparison; nor was the serologic group (group specific carbo-

* This study was aided by a grant from Schenley Laboratories, New York City.

1 Clark, W. H., Bryner, S., and Rantz, L. A., *Am. J. Med.*, 1948, **4**, 671.

2 Spink, W. W., and Ferris, V., *J. Clin. Invest.*, 1947, **26**, 379.

3 Watson, R. F., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 65.

4 Meads, M., Ory, E. M., Wilcox, C., and Finland, M., *J. Lab. and Clin. Med.*, 1945, **30**, 725.

5 Rantz, L. A., Randall, E., Spink, W. W., and Boisvert, P. J., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 54.

6 Hirsh, H. L., Rotman-Kavka, G., Dowling, H. F., and Sweet, L. K., *J.A.M.A.*, 1947, **133**, 657.

7 Milzer, A., Kohn, K. H., and Mae Sears, R., *J.A.M.A.*, 1948, **136**, 536.

hydrate) of the "resistant" strains isolated after treatment determined.

The present report is concerned with a study of the penicillin sensitivity of strains of group A hemolytic streptococci isolated from scarlet fever patients before, during, and following therapy in order to determine whether or not resistance to the antibacterial agent developed during short periods of administration of the drug by either the oral or parenteral route.

Materials and methods. The patients from whom the strains hemolytic streptococci described in this paper were obtained had clinical evidence of scarlet fever and were started on penicillin therapy† on admission to the hospital. The total dose of the drug ranged from 1,200,000 to 3,000,000 units intramuscularly or 3,000,000 to 15,000,000 units orally given over a period of 10 days, the interval between each dose being either 3 or 8 hours depending on the schedule employed. Blood levels of penicillin were determined on most patients twice (2nd + 9th days) during treatment and were therapeutically adequate in every instance.

Cultures of the throat and nasopharynx of all patients were made at the time of admission, every day during the 10 days of treatment, and every other day after therapy was discontinued (usually 11 days). If beta-hemolytic streptococci were found in the nasopharynx after the penicillin was stopped, daily culturing was resumed. Characteristic colonies of beta-hemolytic streptococci were isolated from blood-heart infusion-yeast-agar, reinoculated on the same medium and stored at 4°C. All of the strains were grouped by the precipitin technic,[8] and their penicillin sensitivity determined by the method of Rammelkamp[9] using a known penicillin sensitive strain of *strep. pyogene* (R-98) as control.

Results. 195 strains of group A beta-hemolytic streptococci isolated from patients before they were treated with penicillin were tested for sensitivity to the drug. Two of the strains were inhibited by 0.0313 unit of penicillin, 75 by 0.0156 unit, 115 by 0.0078 unit, and 3 by 0.0039 unit. This range of sensitivity is essentially in agreement with that previously reported for this organism.[3]

Although penicillin eradicated the beta-hemolytic streptococci from the nasopharynx of most patients with scarlet fever in 48 hours, positive cultures were obtained during treatment on 37 different occasions. The number of hemolytic streptococcal isolations, the day on which the organisms were recovered and their penicillin sensitivity are shown on Table I. These strains had all been in contact with penicillin at the time when their drug sensitivity was determined since they were isolated from cases in which the streptococci failed to disappear despite treatment or from individuals in whom they had disappeared and subsequently recurred probably as the result of exposure to other patients in the ward who still harbored the organisms in the nasopharynx. None of the strains showed a greater tolerance to penicillin than that of the cultures isolated at the time of admission to the hospital before any antibiotic therapy was administered.

Beta-hemolytic streptococci belonging to group A were isolated in 24 instances after penicillin was stopped, the organisms being recovered 1 to 34 days after therapy was halted. Six of these strains were inhibited by 0.0156 unit of penicillin, 16 by 0.0078 unit and 2 by 0.0039 unit. None were penicillin resistant and their presence in the nose or pharynx following a full course of antibiotic administration can probably be accounted for best on the basis of contamination resulting from intimate or remote contact with individuals who were in the early phase of treatment or who had just entered the hospital and had not yet received any penicillin.

Discussion. The development of resistant strains of bacteria during contact with various antibiotic agents in use at present is well-

† The penicillin used in this study was supplied by the Schenley Laboratories, Inc., New York City. The intramuscular penicillin used was the potassium salt of crystalline penicillin G. The oral tablets were crystalline penicillin G potassium salt buffered with calcium carbonate, 50,000 or 100,000 units each.

8 Swift, H. F., Wilson, A. T., and Lancefield, R. C., *J. Exp. Med.*, 1943, **78**, 127.

9 Rammelkamp, C. H., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 54.

TABLE I.
Penicillin Sensitivity of Group A Beta-hemolytic Streptococci Isolated During Penicillin Therapy.

Penicillin sensitivity*	Route of penicillin† administration	Day of treatment and No. of strains of strep.									Total isolations
		2	3	4	5	6	7	8	9	10	
.0156	IM	3	1	1	1	3	1	2	2	1	15
	PO	1	0	0	0	0	0	0	0	0	1
.0078	IM	3	1	2	0	0	0	1	1	2	10
	PO	2	1	2	0	1	2	1	1	0	10
.0039	IM	1	0	0	0	0	0	0	0	0	1
	PO	0	0	0	0	0	0	0	0	0	0
Total		10	3	5	1	4	3	4	4	3	37

* Quantity which inhibited growth.
† IM—intramuscular; P.O.—oral.

known. The circumstances of the appearance of sulfonamide resistant group A beta-hemolytic streptococci in the Armed Forces has been reviewed elsewhere.[10] The possibility that a similar situation might arise as a result of the extensive use of penicillin has been anticipated but not observed clinically.

Despite the failure of group A beta-hemolytic streptococci to develop proved penicillin resistance *in vivo* during treatment, there is good evidence that this phenomenon can be produced with ease *in vitro*. Weinstein and Tsao[11] made a number of strains of this organism 4 to 32 times more resistant to penicillin by frequent subculture in media containing increasing amounts of the drug; this resistance was only temporary, however, and was made to disappear by frequent subculture in antibiotic-free media. Gezon,[12] using a slightly different technic, reported similar results.

Summary. (1) Studies of the penicillin sensitivity of group A hemolytic streptococci isolated from scarlet fever patients before, during, and following penicillin therapy indicate that, with the usual "short" courses of treatment, drug-resistant strains of the organism do not appear.

(2) The likelihood of the development of penicillin resistance in cases of streptococcal infection treated with this agent appears to be very remote.

(3) No penicillin-resistant strains of *Strep. pyogenes* (group A) were recovered in any instance before antibiotic therapy was started.

10 Hartman, T. L., and Weinstein, L., *New Eng. J. Med.*, 1948, **238**, 560.

11 Weinstein, L., and Tsao, C. C. L., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 598.

12 Gezon, H. M., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 208.

16706

Oxygen Saturation of Sternal Marrow Blood with Special Reference to Pathogenesis of Polycythemia Vera.*

LIONEL BERK,† JOSEPH H. BURCHENAL, THEO WOOD, AND WILLIAM B. CASTLE.
From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School.

There are two main theories of the pathogenesis of polycythemia vera. The older theory considers that the disease is neoplastic in nature. The other theory supposes that polycythemia vera, like most forms of experimental and clinical polycythemia, is due in

* The expenses of this investigation were defrayed in part by the J. K. Lilly gift to the Harvard Medical School.

† Deceased.