illustrates the retarding influence of both heat and cold on recovery rate. The far more drastic effect of heat is in accord with the decreased survival time and increased mortality rate reported[5,6] under similar conditions in hemorrhagic shock. The retarding influence of cold indicates that the explanation for the increased survival time resulting from exposure to cold in hemorrhagic shock[5,6] must be sought elsewhere (it is possible that the effect of cold may depend on intensity, and that milder degrees of cooling may exert a beneficial effect).

Work in progress is designed to quantitate the influence of environmental temperature, anesthesia, anoxia, fluid balance, liver damage, and similar accessory factors on the time course of plasma volume recovery after standard hemorrhage.

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Sensitivity of Various Serological (Lancefield) Groups of Streptococci to Penicillin.*

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(Introduced by Homer F. Swift.)

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Penicillin is now being made available in increasing quantities for the treatment of patients. This agent has been demonstrated to be of unquestionable value in the treatment of a number of human infections and to be active against an even larger number of infectious agents in experimental animals and *in vitro*. In general the effectiveness of penicillin in the treatment of infections *in vivo* is closely paralleled by its activity against the same infectious agents *in vitro*.

In previous work[1-5] dealing with the action of penicillin on streptococci *in vitro* these microörganisms have generally been classified either by their fermentation reactions or by the appearance of their colonies on blood agar. The first method is long, difficult, and often yields inconclusive results; and the second is inadequate in that each group contains a large number of antigenically unrelated strains. The Lancefield method of classifying streptococci into serological groups, based on antigenic structure, is now widely used; and groups A through N include most of the strains which have been shown to be pathogenic for man and lower animals.[6,7] In a recent report Dawson *et al.*[8] found strains of streptococci belonging

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to groups B, C, D, F and H to vary in their sensitivity to penicillin and to be equally as sensitive to 1/8th as sensitive as a group A strain (C 203MV).

Although strains belonging to group A account for perhaps 90 to 95% of all human streptococcal infections, groups other than A are occasionally responsible for infections in man; and group B and C strains comprise the chief pathogens for animals. It is also well known that strains belonging to certain groups, namely, B, C, D, H, K, and N are often nonhemolytic or produce only alpha hemolysis; and at times even some group A strains are found to be indifferent or methemoglobin producers under aerobic conditions.

In the present study representative strains of serological groups A through N have been tested *in vitro* for their relative sensitivity to penicillin; and in addition, attempts have been made to demonstrate the presence of a penicillinase or inhibitor for penicillin in a resistant group D strain.

Materials and Methods. Many of the strains of streptococci used had been recently isolated from human infections; the remainder were from stock cultures which had been preserved in the dried state.†

For testing the sensitivity of the various strains to penicillin the dilution method described by Rammelkamp[9] was employed. The penicillin was diluted in broth; and to each dilution, 0.5 cc of an appropriate suspension, generally containing between 1,000 and 10,000 streptococci, was added. After 15 to 18 hours incubation at 37°C subcultures were made on blood agar plates which were then incubated overnight. The highest dilution which completely inhibited growth was taken as the end point. This technic is obviously subject to the usual error involved in dilution methods.

The acetone-ether extraction method described by Harper[10] was used to test for the presence of a penicillin inhibitor. The acetone-ether extracted cells were suspended in broth so that each 0.3 cc of the suspension contained 2 mg of dried bacteria. This amount was then added to serial dilutions of penicillin and incubated at 37°C for one hour; then 0.5 cc of culture containing 1,000 to 5,000 cells of a group A strain, C 716, was added to test for the presence of penicillin. The filtrate of a broth culture was also tested for the presence of an inhibitor. A strain of *E. coli* was used as a positive control.

Results. The results of the tests for sensitivity to penicillin of representative strains belonging to the different serological groups (A through N) are presented in Table I. There was very little variation in sensitivity among the strains within any single group. The size of the inoculum, moreover, could be varied within wide limits (100 to 1,000-fold) without changing the amount of penicillin required to inhibit growth completely. Group M strains were the most sensitive and required only 0.0039 Oxford units to sterilize an inoculum containing 25,000 to 35,000 cells. Strains belonging to groups A, C, G, H, and L required 0.0078 to 0.0313 units to kill 1,000 to 10,000 bacteria. Growth of all the group A strains and most of those belonging to the other members of these groups were completely inhibited by 0.0156 units. Strains belonging to groups B, E, F, K, and N were slightly more resistant and generally required 0.0625 units to sterilize an inoculum containing 1,000 to 10,000 cells. Group D strains, which required 2.0 to 5.0 units to kill a similar number of bacteria, were most resistant to the action of penicillin.

Since group D strains were relatively resistant to penicillin, it was of interest to determine whether they produce a substance inhibitory to the action of the drug. The results of tests made with acetone-ether extracted cells and filtrates of broth cultures of strain H 69D5 and a control strain of *E. coli* are recorded in Table II. With this technic a penicillin inhibitor could not be demonstrated in either the cells or filtrate of the culture H 69D5. On the other hand, an inhibitor for penicillin, which was inactivated or destroyed at a temperature of 65°C, was demonstrated in both cells and filtrate of the broth culture of *E. coli*.

Summary and Discussion. The tests re-

† The author acknowledges his indebtedness to Dr. R. C. Lancefield for many of these strains.

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TABLE I.

Results of Tests for Sensitivity of the Various Serological Groups of Streptococci to Penicillin.

Strain	Group	Type	Source	Inoculum No. bacterial cells	O.U.* inhibiting growth
C 716	A	Undet.†	Dr. C. S. Keefer	100-200	0.0156
"	A	"	" " " "	1,000-2,000	0.0156
"	A	"	" " " " "	10,000-20,000	0.0156
1RSC169	A	30	Rockefeller Hospital, Rheumatic Fever, throat	1,000-2,000	0.0156
" 173	A	36	Rockefeller Hospital, Rheumatic Fever, throat	5,000-8,000	0.0156
" 174	A	19	Rockefeller Hospital, Rheumatic Fever, throat	3,000-5,000	0.0156
" 176	A	17	Rockefeller Hospital, Rheumatic Fever, throat	3,000-5,000	0.0156
" 178	A	19	Rockefeller Hospital, Rheumatic Fever, throat	2,000-4,000	0.0156
" 165	A	24	Rockefeller Hospital, Rheumatic Fever, throat	1,000-2,000	0.0156
" 181	A	10-12	Rockefeller Hospital, Acute Pharyngitis and Mastoiditis	3,000-5,000	0.0156
C 203	A	3	Scarlet Fever, Dr. A. R. Dochez	5,000-8,000	0.0078
T 1	A	1	Strain SF 130/2, Dr. F. Griffith	3,000-5,000	0.0156
T 1/72/0	A	1	" " " after 72 mouse passages	4,000-6,000	0.0156
T 12	A	12	" " 42, Dr. F. Griffith	3,000-5,000	0.0156
T 12/36/0	A	12	" " " after 36 mouse passages	3,000-5,000	0.0078
22RS72	A	24	Rockefeller Hospital, Rheumatic Fever, throat	4,000-6,000	0.0156
C 98/88/0	A	24	Strain 22RS72 after 88 mouse passages	2,000-4,000	0.0078
1RSC116	A	3	Rockefeller Hospital, Scarlet Fever	2,000-4,000	0.0078
D 136 A	B	III	Black 771, Subacute Bact. Endocardtis, Dr. R. M. Fry	4,000-5,000	0.0625
" " B	B	II	Brownett 96, Puerperal Sepsis, Fatal, Dr. R. M. Fry	500-1,000	0.0625
" " "	B	"	Brownett 96, Puerperal Sepsis, Fatal, Dr. R. M. Fry	5,000-10,000	0.0625
" " "	B	"	Brownett 96, Puerperal Sepsis, Fatal, Dr. R. M. Fry	50,000-100,000	0.0625
" " C	B	III	Cole 106, Puerperal Sepsis, Fatal, Dr. R. M. Fry	5,000-10,000	0.0625
" " D	B	Ia	SHI Shipley, Puerperal Sepsis, Fatal, Dr. R. M. Fry	5,000-10,000	0.0625
V 9	B	II	Bovine	5,000-10,000	0.0625
16RS72	C		Rockefeller Hospital, Rheumatic Fever, throat	2,000-3,000	0.0156
1RSC73	C		Rockefeller Hospital, Rheumatic Fever, throat	1,000-2,000	0.0156
D 181	C		Strain No. 4 Guinea Pig, Dr. C. V. Seastone	100-200	0.0313
"	C		" " " " " " " " "	1,000-2,000	0.0313
"	C		" " " " " " " " " "	10,000-20,000	0.0313
C 519	C		" " 1890, Human throat, Dr. F. Schwentker	5,000-10,000	0.0078
K 150 B	C		Bovine Mastitis, Dr. P. R. Edwards	4,000-8,000	0.0156
J 148 D	C		Monkey, Normal throat, Dr. E. E. Terrell	3,000-6,000	0.0078
D 178 A	D		Strain Andrews, Subacute Bact. Endocarditis, Dr. D. Skinner	12,000-14,000	5.0
D 173	D		Strain Osakow, Subacute Bact. Endocarditis, Dr. D. Skinner	1,000-2,000	2.0
C 745	D		Meningitis, Dr. J. M. Neill	6,000-10,000	2.5
H 69D5	D		Strain Heazman, Normal Human Vagina, Dr. R. Hare	800-1,000	5.0
"	D		Strain Heazman, Normal Human Vagina, Dr. R. Hare	8,000-12,000	5.0
"	D		Strain Heazman, Normal Human Vagina, Dr. R. Hare	80,000-120,000	5.0‡
"	D		Strain Heazman, Normal Human Vagina, Dr. R. Hare	800,000-1,200,000	5.0‡

Strain	Group	Type	Source	Inoculum No. bacterial cells	O.U.* inhibiting growth
C 1	D		Cheese, Dr. O. T. Avery	1,000-2,000	5.0
"	D		" " " " "	10,000-20,000	5.0
K 129	E		Milk, Dr. J. H. Brown	7,000-10,000	0.0625
C 24A	E		Swine, lymphadenitis, Dr. H. J. Stafseth	10,000-12,000	0.0313
F 67	F		Strain Forrester II, Human Sinus Infection, Dr. E. A. Bliss	5,000-6,000	0.0625
C 41F	F		Rockefeller Hospital, Empyema, Dr. G. S. Mirick	1,000-2,000	0.0625
H 127	F		Rockefeller Hospital, Tonsil	500-600	0.0313
1RSC161	G		Rockefeller Hospital, Acute Pharyngitis, ? Scar. Fever	2,000-3,000	0.0156
4RS72	G		Rockefeller Hospital, Acute Pharyngitis	3,000-4,000	0.0156
F 78B	G	(Minute)	Normal pharynx, Dr. Helen Plummer	3,000-4,000	0.0313
C 518	G		Strain 3702, human throat, Dr. F. Schwentker	7,000-10,000	0.0078
Monk. 37	G		Monkey, Pneumonia, Dr. Thomas Francis	5,000-10,000	0.0156
F 90A	H		Strain Perryer, Normal human throat, Dr. R. Hare	6,000-7,000	0.0156
K 208	H		Rockefeller Hospital, Alveolar abscess	6,000-7,000	0.0156
D 34F	K		Normal pharynx, Dr. R. Hare	5,000-6,000	0.0625
K 130	L		Certified Milk, Dr. J. H. Brown	5,000-6,000	0.0156
D 167A	L		Strain SCH 16, Dog (Vagina), Dr. R. M. Fry	3,000-4,000	0.0313
D 168AX	M		" " 192, Dog or Fox, Dr. R. M. Fry	25-35	0.0019
"	M		" " " " " " " " " "	250-350	0.0019
"	M		" " " " " " " " " "	2,500-3,500	0.0039
"	M		" " " " " " " " " "	25,000-35,000	0.0039
D 168BX	M		" " 200 " " " " " " "	600-700	0.0039
C 559	N		*S. lactis*, Strain O. J., Milk, Phyllis M. F. Shattock	1,000-2,000	0.0625

* O.U.—Oxford Unit.
† Undet.—Undetermined.
‡ An occasional colony appeared on subculture.

TABLE II.

Results of Tests for Penicillin Inhibitor in Broth Filtrates and Acetone-Ether Extracted Cells of a Group D and *E. coli* Strain.

System 10–5 dilutions Strain C716 plus	Dilutions of penicillin									
	1:2*	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
0.3 cc broth (control)	—	—	—	—	—	—	—	+	+	+
2 mg extracted H 69D5 cells	—	—	—	—	—	—	—	+	+	+
0.3 cc H69D5 broth filtrate	—	—	—	—	—	—	—	+	+	+
2 mg extracted *E. coli* cells	+	+	+	+	+	+	+	+	+	+
2 mg extracted *E. coli* cells (heated to 56°C 30 min)	+	+	+	+	+	+	+	+	+	+
2 mg extracted *E. coli* cells (heated to 65°C 30 min)	—	—	—	—	—	—	—	+	+	+
0.3 cc *E. coli* broth filtrate	+	+	+	+	+	+	+	+	+	+
0.3 cc *E. coli* broth filtrate (heated to 65°C 30 min)	—	—	—	—	—	—	—	+	+	+

* First tube contained 2.0 Oxford Units of penicillin.
+ indicates growth of test strain, C 716.
— indicates no growth of test strain, C 716.

ported here show that penicillin in amounts varying between 0.0039 and 0.0625 Oxford units sterilized an inoculum containing 1,000 to 10,000 cells of all the strains tested belonging to serological groups A through N with the exception of group D strains which required 2.0 to 5.0 units to inhibit completely the growth of a similar sized inoculum. Group M strains were the most sensitive; group A, C, G, H, and L strains were next; and group B, E, F, K, and N strains were the least sensitive of those found susceptible to the action of penicillin in small amounts.

From our experience as well as that of others,[11,12] it appears that with the doses and methods of administration currently used in patients, concentrations of more than 1 Oxford unit per cc of blood would be difficult to maintain for an appreciable length of time. It, therefore, seems unlikely that human infections with group D streptococci that require systemic treatment would respond readily to penicillin therapy in amounts commonly used now. Possibly localized infections, where higher concentrations of penicillin could be maintained, might respond favorably to this drug. On the other hand, infections with strains of groups A, B, C, E, F, G, H, L, K, M, and N would be expected to respond favorably to penicillin since they are susceptible in concentrations that can be readily maintained in either the circulation or locally.

By the methods used, no penicillinase or inhibitor of penicillin could be demonstrated in either the extracted cells or filtrate of a broth culture of a resistant group D strain, although with similar methods an inhibitor which was destroyed or inactivated by heat (65°C for 30 min.) was demonstrated in both the cells and filtrate of a broth culture of a strain of *E. coli*. No attempts were made to further identify this inhibitor. If group D streptococci do not produce substances which destroy or inactivate penicillin, they probably will not interfere with the action of the drug on other penicillin-susceptible microörganisms when found in mixed infections.

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14701

Effect of Ether Extract of the Thymus and Pancreas on Synthesis of Acetylcholine.*

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It was found that less acetylcholine was synthesized in the presence of serum[1,2] and spinal fluid[3] from patients with myasthenia gravis than from healthy persons or patients with diseases other than myasthenia gravis. The pathological changes demonstrable at autopsy may be an enlarged thymus and lymphocytic infiltration of certain tissues. Patients with myasthenia gravis may benefit from removal or irradiation of the thymus. It was ascertained in the following that the synthesis of acetylcholine is modified by the presence of thymus extracts.

Experimental. Series I. The effect of minced thymus on the synthesis of acetylcholine *in vitro* was studied in a few experiments, but the results were inconstant. Since the various constituents of the minced thymus

* This study was aided by a grant from the Josiah Macy, Jr., Foundation.

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