THE BACTERICIDAL ACTION OF HUMAN SERUM ON HEMOLYTIC STREPTOCOCCI

II. Factors Which Influence the Phenomenon in Vitro

By WILLIAM S. TILLETT, M.D.

(*From the Biological Division, Department of Medicine, Johns Hopkins Medical School and Hospital, Baltimore*)

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The observations described in the preceding article (1) have demonstrated that sera, obtained from patients during acute active infection, are lethal for *Streptococcus hemolyticus* of the *beta* type. In addition, it was shown by contrast that subsequent samples of serum taken from the same group of patients very soon after they had recovered, and that specimens from normal adults, were essentially devoid of the streptococcidal property, when measured by comparable tests. Consistent results were obtained by an experimental method which took into consideration the following factors: (*a*) the amount of serum used for the test; (*b*) number of bacterial cells inoculated into serum; (*c*) selection of test strains of hemolytic streptococci; (*d*) periods of exposure of organisms to the poisoning effect of serum. The results indicate that the streptococcidal property of serum is demonstrable early in acute infection, that it is maintained during the period of active disease, but returns to the normal level very soon after the illness has been overcome.

The investigation has been extended in an attempt to gain some information concerning the processes involved in the serological phenomenon. It is the purpose of this article to describe certain conditions which play an important rôle in determining the results of tests, *in vitro*, and to indicate the possible application of the findings to an interpretation of the underlying mechanism. Additional observations, which aid in the characterization of streptococcidal sera, are also contained in this report.

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The same experimental technique which was described in detail in the preceding article (1) has been used throughout the investigation.

Effect of Anaerobic Conditions on the Streptococcidal Action of Serum

In addition to the routine method of carrying out the experiments, sera, containing the regular inoculum of culture, have been incubated in the partial anaerobic state afforded by a vaseline seal and also under the more completely anaerobic conditions of an anaerobic jar.

1. Vaseline Seal.—Technique: 1.0 cc. of serum was inoculated with a platinum loopful of culture, as in the usual test (1). Strain Sc was employed. The tubes were shaken in order to mix the contents well, but care was taken to avoid the formation of foam. 1.0 to 1.5 cc. of sterile melted vaseline was layered on top of the serum in a Wassermann tube as soon as possible after the organisms were introduced.

Because of the simplicity of the procedure, almost every sample of serum from patients was tested both with and without a vaseline seal. Representative results from a large number of tests are given in Table I. The data selected for tabulation were obtained, in most instances, with portions of the same sera used in the previous study (1). To facilitate comparisons, Table I of this article follows, with respect to the listing of patients, the order of Tables I and II of the preceding article.

Observations have been repeatedly made with normal serum. Since colonies, too numerous to count, were obtained in both aerobic and anaerobic tests, no attempt was made to compare the relative abundance of organisms in normal serum under the two conditions.

The thirty observations recorded in Table I, were made with sera from twenty different patients, twelve of whom had pneumococcus pneumonia and the remaining eight had different kinds of infections. They were all acutely ill at the time at which the blood was taken. The contrast between the sterility, on the one hand, of subcultures made after 24 hours of aerobic incubation of the tests, and the abundance of streptococci, on the other hand, in subcultures of the sealed specimens, is the striking feature of the results. With twenty-four of the thirty specimens of serum, the profound influence of anaerobiosis on the phenomenon is evidenced by the difference between the figures in the column designated aerobic, 24 hours and the indices of bacterial

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survival in the column which is headed vaseline seal, 24 hours. Under the conditions of the experiment, the death or survival of the organisms in potentially streptococcidal sera appeared to be determined, to a

TABLE I

Effect of Anaerobic Conditions (Vaseline Seal) on Streptococcidal Tests with Patients' Sera

Number of colonies present in subcultures made at beginning of experiments was always 1000S to ∞

Patient	Disease	Sample* of serum	Aerobic		Vaseline seal 24 hrs.†	Patient	Disease	Sample of serum	Aerobic		Vaseline seal 24 hrs.
			6 hrs.†	24 hrs.†					6 hrs.	24 hrs.	
M. F.	Pneumonia	1	–‡	–	∞	A. F.	Pneumonia	1	2	–	∞
W. S.	"	1	–	–	–	E. P.	"	2	–	–	∞
"	"	2	150	–	800	"	"	3	–	–	34
G. J.	"	1	–	–	∞	"	"	4	2	–	∞
R. C.	"	1	–	–	∞	"	"	5	–	–	7
C. S.	"	1	3	–	10	L. B.	"	1	4	–	∞
"	"	2	2	–	500	R. B.	Acute tonsillitis	1	1	–	1000
"	"	3	22	–	∞	G. C.	Peritonsillar abscess	1	–	–	1000S
O. T.	"	1	1	–	∞	"	" "	2	16	–	∞
F. R.	"	1	1	–	24	R. J.	Meningitis	1	18	2	∞
"	"	2		6	∞	A. M.	Pleurisy	1	83	–	1000S
J. M.	"	1	1	–	∞	T. D.	Acute respiratory infection	1	–	–	∞
"	"	2	71	9	∞	H. S.	Malaria	2	54	–	∞
J. A.	"	1	1	–	62	W. F.	"	1	19	–	∞
"	"	2	400	–	∞	C. T.	"	2	52	–	∞

* Identifies sample of serum from individual patients. 1 for first, 2 for second, etc.

† Indicates number of hours of incubation before subculture was made.

‡ Sign – indicates sterile subculture. Numerals indicate number of colonies in subcultures. 1000S and ∞ are approximate estimations when actual counts were impractical.

large extent, by factors which were effective under conditions of oxidation but were inactive under circumstances which induced reduction.

It should be noted also that, with six of the specimens of serum,

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exclusion of air modified the killing action only slightly, or not at all. Final explanation of the exceptions is not yet available. However, a probable interpretation is suggested by the outcome of some of the experiments which immediately follow.

2. *Anaerobic Jar.*—In order to obtain more complete anaerobiosis than that afforded by a layer of vaseline, additional tests have been carried out in a Brown anaerobic jar (2). In this apparatus, which employs the same principle as

TABLE II

Comparison of Streptococcidal Action of Patients' Sera under Aerobic Conditions, with Vaseline Seal, and in Anaerobic Jar

Number of colonies present in subculture at beginning of experiment was always 1000S to ∞

Patient	Disease	Aerobic				Vaseline seal		Anaerobic jar	
		Sc*		Co*		Sc	Co	Sc	Co
		6 hrs.†	24 hrs.†	6 hrs.	24 hrs.	24 hrs.	24 hrs.	24 hrs.	24 hrs.
B.	Pneumonia	125‡	4			∞		∞	
M.	"	1	—	114	3	∞	∞	∞	∞
C.	"	25	2	350	6	∞	∞	∞	∞
T.	Malaria	160	—			∞		∞	
S.	"	54	—	1000	—	∞	∞	∞	∞
C.	"	125	3	800	15	∞	∞	∞	∞
F.	"	121	—			∞		∞	
V.	"	68	—			∞		∞	
H.	Lung abscess	142	—			∞		∞	
W.	Pneumonia	—	—	26	1	10	1000S	41	1000S
S.	"	2	—	120	—	86	400	—	1000S
R.	"	1	—	4	—	24	∞	94	∞
C.	Tuberculosis	72	—	210	—	—	1000	—	780

* Designates strain of hemolytic streptococcus used in tests.

†, ‡ See Table I.

the McIntosh and Fildes jar, oxygen is exhausted in the formation of water and an excess of hydrogen remains. The sera to be tested were placed in the jar immediately after inoculation with culture. A tube of sterile dextrose broth, containing methylene blue, was added as an indicator of anaerobiosis. During the 24 hours of incubation the methylene blue regularly changed to methylene white.

The findings recorded in Table II afford a comparison of the streptococcidal effect of sera under aerobic conditions, under partial anaerobic state obtained with a vaseline seal, and in an anaerobic jar.

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From the table it may be noted that, with nine specimens of sera—listed in order at the top of Table II—streptococci survived and perhaps multiplied, when either of the two anaerobic methods was employed in the tests. By contrast, progressive bacterial destruction occurred aerobically. A comparison of the results obtained with the anaerobic jar and vaseline seal demonstrate the inactivation of the lethal process by both procedures. However, the observations do not indicate the superiority of one method over the other in accomplishing the particular purpose for which the experiment was designed. For example, in four tests listed at the bottom of Table II, strain Sc was destroyed almost to the same extent anaerobically as aerobically; even the complete anaerobiosis of the jar was not more effective than the vaseline seal. However, by substituting the somewhat less sensitive strain Co for strain Sc and repeating the test with the same four specimens of sera, the inactivating effect of anaerobiosis on the streptococcidal process became evident. The results of these observations indicate, therefore, that the outcome of some of the experiments may be conditioned by a balance between the potency of the serum in streptococcidal activity and the vulnerability of the test strain.

In connection with the observations just described, it is interesting to call attention to an article by Schottmüller and Barfurth (3). They reported results concerning the bactericidal action of defibrinated blood on streptococci of the hemolytic, anhemolytic, and *viridans* types. They asserted that the bactericidin in *sauerstoffhaltigen* blood was greater than in *kohlensäurehaltigen* blood, and that factors, such as increased water content of the blood or decreased number and resistance of erythrocytes, lessened the bactericidal power of the blood.

Effect of Reversing the Aerobic-Anaerobic System

Tests have been performed in which the aerobic and anaerobic conditions were altered during incubation of sera inoculated with streptococci. The purpose of the experiments was to determine: (*a*) whether the streptococcidal process could be interrupted by anaerobiosis if tests were allowed to proceed aerobically for varying lengths of time before excluding air; and (*b*) whether the destructive action was permanently inactivated by short periods of anaerobiosis before exposure to air.

The method consisted in placing 1.0 cc. of serum into a series of tubes and inoculating each with a loopful of culture. Strain Sc was used in all the experi-

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ments. Subcultures from each tube were made immediately; then, half of the series was layered with vaseline. All of the tubes were incubated continuously for 24 hours; at the end of this time a final subculture was made and the experiment was terminated. At selected intervals, ranging from 2 to 12 hours, vaseline was added in succession to each of the aerobic tubes; subcultures were made just before sealing. From each tube of the anaerobic set, vaseline was carefully removed in series, after periods ranging from 2 to 12 hours; subcultures were made promptly and, then, incubation was continued aerobically until the termination of the experiment. Additional subcultures from the tubes incubating aerobically could be made as desired. When anaerobiosis was continuous, the interruption of the tests to obtain subcultures necessitated the removal and replacement of a vaseline seal. When this procedure was used, it was carried out as rapidly as possible, in order to minimize the period of transient exposure to air. The unavoidable error in technique has not significantly affected the results.

Table III contains four examples of the effect which reversing the aerobic or anaerobic status of individual tests had upon the survival or destruction of hemolytic streptococci in single samples of serum.

From the data derived from tests made with sera from three of the patients (V., L., and C.), it may be noted that when a vaseline seal was added to an aerobic test within 2 hours after the serum had been inoculated with organisms (tubes 2, Table III), the destruction of streptococci was checked, as evidenced by the innumerable colonies present in subcultures made after the subsequent 22 hours of anaerobic cultivation. When aerobic conditions prevailed for 4 hours before adding a layer of vaseline (tubes 3, Table III), the surviving streptococci in one specimen of serum (patient L.) multiplied under anaerobiosis, but with the other two sera, similarly tested, the number of viable organisms recovered after the subsequent 20 hours of anaerobic incubation was not significantly increased over the few present when air was first excluded. By prolonging the aerobic phase of the experiment for 6 hours before excluding air (tubes 4, Table III), the lethal process was inactivated very slightly, if at all, by adding a vaseline seal. Finally, when aerobic incubation proceeded for 12 hours, the subsequent exclusion of air did not alter the final result.

The tabulated results derived from the tests made with serum from patient B. indicate that the interchange of aerobic and anaerobic conditions modified the outcome only to a limited extent. Comparable results have been obtained with some other specimens of sera.

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TABLE III

Reversibility of Effect of Aerobic and Anaerobic Conditions on Streptococcidal Reaction of Patients' Sera

Number of colonies present in subculture at beginning of experiment was always 1000S to ∞

Tube No.	Aerobic and anaerobic conditions	Duration of incubation before subculture					Tube No.	Duration of incubation before subculture				
		2 hrs.†	4 hrs.	6 hrs.	12 hrs.	24 hrs.		2 hrs.	4 hrs.	6 hrs.	12 hrs.	24 hrs.
	Patient V. (induced malaria)							Patient L. (broncho-pneumonia)				
1	Aerobic 24 hrs.	600‡	140	68	4	—	1	200	41	6		—
2	Aerobic 2 hrs. Anaerobic 22 hrs.	510			650	∞	2	160				1000S
3	Aerobic 4 hrs. Anaerobic 20 hrs.	550	86		2	61	3		34			∞
4	Aerobic 6 hrs. Anaerobic 18 hrs.			82	7	22	4			10		27
5	Aerobic 12 hrs. Anaerobic 12 hrs.			51	4	—	5				—	—
6	Anaerobic 24 hrs.	710		1000S	∞	∞	6					∞
7	Anaerobic 2 hrs. Aerobic 22 hrs.	580	90		3	—	7	240				—
8	Anaerobic 4 hrs. Aerobic 20 hrs.		960		2	—	8		340			—
9	Anaerobic 6 hrs. Aerobic 18 hrs.			∞	∞	1000	9			600		6
10	Anaerobic 12 hrs. Aerobic 12 hrs.			∞	∞	∞	10				1000S	800
	Patient C. (tuberculosis)							Patient B. (lobar pneumonia)				
1	Aerobic 24 hrs.	750	54	6	5	1	1	140	45	5	1	—
2	Aerobic 2 hrs. Anaerobic 22 hrs.	600				1000S	2	104				16
3	Aerobic 4 hrs. Anaerobic 20 hrs.		115			300	3		16			8
4	Aerobic 6 hrs. Anaerobic 18 hrs.			11	—	220	4					
5	Aerobic 12 hrs. Anaerobic 12 hrs.				15	18	5				—	—
6	Anaerobic 24 hrs.					∞	6					∞
7	Anaerobic 2 hrs. Aerobic 22 hrs.	∞		1000S		160	7	91		2		—
8	Anaerobic 4 hrs. Aerobic 20 hrs.		∞			1000	8		47		10	3
9	Anaerobic 6 hrs. Aerobic 18 hrs.			∞	∞	1000S	9					
10	Anaerobic 12 hrs. Aerobic 12 hrs.				∞	∞	10				200	350

†, †† See Table I.

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In these instances, inactivation of the streptococcidal process was completely successful only in the tests maintained in continuous anaerobiosis for 24 hours, although a few organisms ultimately survived short periods of aerobic incubation. Sera, yielding results such as that just described, have been found, by other tests, to possess an unusually high degree of killing power. From a large number of observations a consistent relationship has been noted between the streptococcidal potency of individual specimens of serum and the critical period of incubation which determines the reversibility of the reaction. Additional evidence that the quantitative factor is important has been obtained by experiments in which the amount of inoculum of culture introduced into highly potent sera, was increased severalfold. The results, under these circumstances, are comparable to those obtained when the regular amount of culture was added to other sera.

In spite, therefore, of limitations which surround the experimental procedure just described, the results demonstrate that, if the progressive killing of streptococci is not allowed to continue too long, the streptococcidal effect, which occurs aerobically, may be altered by introducing a state of anaerobiosis.

In the second part of the experiment, relating to reversibility, the tests were begun under anaerobic conditions and subsequently changed to aerobiosis (tubes 6 to 10, Table III). In three instances, the reactivation of the lethal effect following exposure to air was demonstrable provided the vaseline seal was not allowed to remain in place longer than 6 hours before being permanently removed. When 12 hours were allowed to intervene between the change from anaerobic to aerobic state, the final subculture usually contained large numbers of colonies. (In the last tests in this group, however, (tubes 9, Table III) the period of aerobic incubation may have been so short that the lethal process, if active, was not demonstrable.) As previously stated, the ultimate survival of streptococci in the highly potent serum from one patient (B.) required uninterrupted anaerobic incubation.

It is interesting to call attention to the fact that, in the tests initiated under anaerobiosis, subcultures after 2 to 4 hours incubation often revealed a temporary decrease in bacterial population. That the killing action occurring in these instances was, however, not

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sustained, is evidenced by the large numbers of viable organisms which were ultimately demonstrable when anaerobiosis was continued for 24 hours. The result indicates that the lethal action may proceed for a period of time even under anaerobic conditions. The probable explanation of the transient streptococcidal effect occurring anaerobically rests upon a period of lag between the addition of vaseline and the ultimate induction of a sufficient degree of anaerobiosis to inactivate the process. The simple methods, which have been employed in the present study, do not offer the means for a detailed analysis of this phase of the problem. Preliminary trials with chemical reductants have, therefore, been made. The results, although incomplete, suggest that the use of reducing agents or a control of oxidation-reduction potentials by appropriate reagents will afford a more accurate assay of the aerobic-anaerobic conditions involved in the phenomenon. At the present time, however, even with the methods which have been employed, it may be concluded that the anaerobic as well as the aerobic effect on the streptococcidal reaction is, under proper conditions, reversible.

Effect of Heat on the Streptococcidal Action of Patients' Sera

Twelve specimens of sera, each from a different patient, have been heated at 56°C. and 60°C. respectively, for 1 hour. The results are given in Table IV.

From the data it may be seen that the results are not uniform. In nine of the twelve instances, heating at 60°C. either destroyed the lethal property of sera or definitely impaired it. With three specimens, each derived from a patient severely ill with pneumonia, the effect of heating even at 60°C. was not striking. When exposure to 56°C. was used, the streptococcidal action of half the specimens was either completely or partially inactivated, but the remaining six samples retained the capacity to destroy streptococci. Although there were exceptions, heat was less deleterious for sera which were considered to be highly potent in lethal properties than for specimens which were deemed to be less active.

Maintenance of Streptococcidal Action by Sera Kept in the Ice Box

In the former article (1) it was stated that serum was usually tested within 24 hours of the time that it was obtained from the patient. In

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order, however, to gain information as to the lability or stability of the streptococcidal property, some of the specimens of sera have been retested after being kept in the ice box for as long as 4 weeks. The limited number of observations indicate the destructive action is retained unimpaired for 3 weeks and that there is only slight decrease even after standing for 4 weeks in the ice box.

TABLE IV

Effect of Heat on Streptococcidal Property of Patients' Sera

Number of colonies present in subcultures at beginning of experiment was always 1000S to ∞

Patient	Disease	Unheated		Heated 56°C., 1 hr.		Heated 60°C., 1 hr.	
		6 hrs.†	24 hrs.†	6 hrs.	24 hrs.	6 hrs.	24 hrs.
P.	Pneumonia	2‡	–	–	–	1000S	8
T.	"	15	–	–	–	1000S	500
C.	"	–	–	–	–	41	–
M.	"	46	–	∞	∞	∞	∞
S.	"	120	–	∞	1000	∞	∞
L.	"	6	–	48	–	52	–
B.	"	5	–	8	–	48	2
D.	"	100	–	∞	∞	∞	∞
L. S.	"	22	–	65	–	1000	14
S.	Bronchopneumonia	360	–	1000S	8	∞	∞
R.	"	1	–	800	9	∞	1000S
V.	Malaria	68	–	1000S	800	∞	∞

†, ‡ See Table I.

Consideration of Agglutination

In order to determine whether or not agglutination may be a factor in the bactericidal tests employed in this study, observations have been made concerning both true agglutination and pseudo-agglutination of hemolytic streptococci by patients' sera.

A procedure, commonly used in determining the presence of agglutinins in serum for hemolytic streptococci, consists in adding to appropriate dilutions of serum, organisms which have been killed by heat. The test is then incubated at 56°C. for 2 hours followed by overnight exposure to ice box temperature. Many of the sera used in the streptococcidal experiments have been tested in the manner just described. Either the results have been negative, or a doubtful positive reaction was noted in 1–10 dilution of serum. Additional evidence of

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the improbability of agglutinins participating in the lethal action of the sera is furnished by the diverse types of infection which evoked the appearance in patients' blood of a common serological property.

In previous reports from this laboratory (4) an agglutination phenomenon obtained with hemolytic streptococci in patients' sera was reported which had several conditions differentiating it from true antibacterial agglutination, but which had certain factors in common with the streptococcidal action of sera with which the present articles deal. The clumping of streptococci, just mentioned, occurred with serum from patients acutely ill but was not demonstrable after recovery. It was also noted that some strains of hemolytic streptococci formed aggregates under the conditions just described, but the majority of strains were not visibly affected. In order to determine whether the pseudo-agglutination reaction and killing power of patients' sera were causally related, comparable tests were carried out with strains of streptococci suitable for the two purposes. Since the results fail to reveal any clear cut relationship, it is unnecessary to present detailed protocols. It may be briefly stated that none of the three strains (Sc, Co, and Ba) most commonly used in the streptococcidal tests and differing in sensitiveness to the lethal action of sera, belong to the so called agglutinable group. Furthermore, two additional strains, which are susceptible to pseudo-agglutination under proper conditions, did not exhibit in streptococcidal tests any special characteristics which separated them from other strains. Further investigation of this phase of the problem is now in progress.

DISCUSSION

In the first article of this series, results were presented which indicated that factors, *in vivo*, associated with acute illness are significant in determining the presence of a demonstrable streptococcidal property in the serum of patients. In this, the second report, it is demonstrated that additional circumstances, *in vitro*, may be introduced which further affect the phenomenon. The conditioning elements may be contributed to some extent by the particular strain of hemolytic streptococcus employed in the test, but to a much more striking degree by the aerobic or anaerobic state of the environment in which the experiments were performed.

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Information concerning differences in susceptibility among the strains is, at the present time, incomplete. A few strains have been found to be highly sensitive to the poisoning effect of patients' sera; a few others have been consistently the most resistant; the majority, however, have occupied a broad intermediate position between the two extremes. In view of the fact that the presence or absence of air has been found to be so significant in determining the death or survival of hemolytic streptococci in patients' sera, ones attention is directed toward the physiological processes of the organisms in which the availability or exclusion of oxygen may be concerned. Callow (5) found that, among twelve aerobic bacterial species, only streptococci were devoid of catalase; one of the strains used was *Streptococcus hemolyticus*. Farrell (6) confirmed this finding and, in addition, reported that neither cytochrome nor indophenol-oxidase could be demonstrated in streptococci. A thermostable peroxidase, having special characteristics, was, however, found to be present. McLeod and Gordon (7), in classifying bacteria according to sensitiveness to hydrogen peroxide, found the susceptibility of streptococci to be relatively high as compared with other aerobes, and there was also some difference between the strains of streptococci. Stevens (8) recently reported that irradiated cod liver oil and oil of pine are bactericidal for hemolytic streptococci. He ascribed the killing effect to peroxides which were formed in the oils as a result of ultraviolet radiation. He also reported that reduction of irradiated oil with cysteine hydrochloride inactivated the bactericidal process. When the observations reported in this article are considered in connection with the findings of others, which have just been outlined, it seems possible that the special biological reactions of *Streptococcus hemolyticus* pertaining to its respiratory mechanism and interrelated activities may be significant in the present study.

The fact that the streptococcidal process in sera was inactivated by exclusion of air does not, however, answer the question of whether the effect of the anaerobic state is on the bacterial cells or on the bactericidal element in the serum. If the former interpretation is correct, then it must be presumed that biological processes of hemolytic streptococci proceed differently, depending on the presence or absence of air. Farrell (6) found that the uptake of atmospheric oxygen by

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washed streptococci was negligible as measured by the Warburg apparatus. Unpublished observations[1] indicate that hemolytic streptococci live and multiply in atmospheres ranging from complete anaerobiosis to that of 95 per cent oxygen. Even though atmospheric oxygen does not appear to exert a direct effect on the viability of streptococci, indirect influences may be involved. For example, it is well known that some facultative anaerobic bacterial species utilize different metabolic processes under aerobic and anaerobic conditions and that the products of metabolism may also be different, depending on the availability or exclusion of air. It becomes necessary, therefore, to take the factors just mentioned into consideration in attempting to analyze the basic mechanism of the streptococcidal reaction.

As suggested earlier, the other possible effect of anaerobiosis is upon the serum. If the differences in the outcome of experiments carried out under aerobic and anaerobic conditions are due to the effect of environment upon the streptococcidal property of serum, then it may be surmised that the element in serum responsible for the death of streptococci is active in an oxidized form but seriously impaired when reduced. It is impossible at the present time to offer a correct interpretation of the results. However, in spite of the present limitations, the findings suggest that the killing power of patients' sera depends upon the presence of a property which interferes with some phase of streptococcal physiology necessary for the aerobic life of the bacterial cell. The results also emphasize the difficulty of attempting to correlate the streptococcidal power of patients' sera *in vitro*, with activities *in vivo*, where factors pertaining to oxidative and reductive states in an infected individual are incompletely understood.

SUMMARY

Although sera derived from patients at the time of acute, active infection were found to be capable of destroying hemolytic streptococci under aerobic conditions, the organisms retained viability when the tests were performed in the environment of anaerobiosis afforded by a vaseline seal or an anaerobic jar.

Within the limitations of the experimental procedures which were

[1] Made in this laboratory by Dr. R. L. Garner and the author.

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employed, the aerobic or anaerobic effect was found to be a reversible reaction.

Heating sera at 60°C. for 1 hour inactivated the streptococcidal element in most instances, but not in every case; heating at 56°C. for 1 hour impaired the killing power of half of the specimens which were tested.

Sera retained the capacity to destroy hemolytic streptococci when kept in the ice box for 3 weeks; a slight diminution in killing power was noted after 4 weeks.

By the methods which were employed, the streptococcidal property of sera could not be correlated with either true antibacterial agglutination or with pseudo-agglutination.

The significance of the findings as a basis for analyzing the mechanism of the streptococcidal phenomenon is discussed.

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