

A STUDY OF THE HEMOLYTIC STREPTOCOCCI IN THE THROAT AND IN EMPYEMA

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In the winter of 1917 and 1918, infections with hemolytic streptococci were one of the most serious medical problems at this camp as well as at other army camps. At first these infections were intercurrent with measles; later they seemed to constitute an independent epidemic. The respiratory and auditory tracts were chiefly involved, the lesions being "sore throat," bronchopneumonia frequently complicated by empyema, pericarditis and peritonitis and otitis and mastoiditis occasionally complicated by meningitis. Of these various affections, bronchopneumonia was the most important, especially since in over 30% of cases it was accompanied by empyema.

Hamburger and Fox¹ have shown that the epidemic was composed of three variables—measles, streptococcus disease, and pneumococcus disease—and indicated their mutual relations. H. L. Alexander² demonstrated that the streptococcus was a highly responsible factor in this epidemic and widely distributed in the camp, following this up by work in association with Levy³ which went to prove that a streptococcus carrier state was an indication of the probability of complications in measles. Lucke⁴ noted that the pathologic anatomy differed from that of the usual pre-war measles and bronchopneumonia, and presented the results of his postmortem work during the fall and winter before the Camp Taylor Medical Society in April, 1918. These findings were coincident with and confirmed by McCallum.⁵ Gay⁶ has recently summarized the studies from various army camps and clarified the atmosphere about the position of the streptococcus.

The occurrence of hemolytic streptococci in the normal throat, its presence in the throat of measles patients and its relation to acute respiratory diseases have been the subject of numerous studies. In this camp, Levy and Alexander² found the incidence of hemolytic streptococcus carriers among 489 new recruits, as they stepped from the train, to be 14.8%; in 388 measles patients, 77.1%,

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¹ Medical Clinics of North America, December, 1918, p. 322; Jour. Am. Med. Assn., 1918, 70, p. 1758.

² Jour. Am. Med. Assn., 1918, 70, p. 775.

³ Jour. Am. Med. Assn., 1918, 70, p. 1827.

⁴ Jour. Am. Med. Assn., 1918, 70, p. 2005.

⁵ Cole, R., and MacCallum, W. G.: Jour. Am. Med. Assn., 1918, 70, p. 1146. MacCallum, W. G.: The Medical Clinics of North America, December, 1918.

⁶ Jour. Lab. and Clin. Med., 1918, 3, p. 721.

and in 95 apparently healthy men from an organization which had furnished a large number of measles patients, 83.2%.

It is the purpose of this study to ascertain whether the streptococci from such various sources are biologically similar or dissimilar, and whether they are identical with strains isolated from empyema fluids. Kinsella⁷ has shown all hemolytic streptococci to be immunologically identical by the complement fixation test. To further study their relationship, the following criteria have been employed in this work: growth in serum peptone broth, morphology, hemolysin production on blood agar, quantitative hemolysin estimation, carbohydrate reaction, and virulence for rats.

Our present knowledge of the streptococcus group has been so thoroughly recently reviewed by Holman,⁸ Blake,⁹ Gay,⁶ and others, that citations and discussions are here omitted as unnecessary.

MATERIAL AND METHOD OF STUDY

Organisms were isolated from the throats of patients belonging to five disease groups: (1) acute infections of the upper respiratory tract; (2) noninfectious diseases (apparently normal throats); (3) uncomplicated measles; (4) from the pus of patients suffering with empyema preceded by measles, and (5) empyema not preceded by measles.

The throat swab or the pleural pus was streaked on blood-agar plates, colonies picked therefrom, and transferred to blood-agar slants. All studies were made from these as stock cultures. All mediums were placed in the thermostat at 37 C. for 1 hour before using. As far as possible the various tests were made on the same date so as to permit checking up. The general routine was to inoculate horse serum peptone broth and blood plates; record the appearance of growth in both after 18 hours; use the supernatant fluid from the horse serum peptone broth for hemolysin titration and the sediment for morphologic studies. Then carbohydrate mediums were inoculated and rats injected. Practically all tests were repeated to determine any variation in results.

ISOLATION OF STREPTOCOCCI

We had no difficulty in isolating streptococci in pure cultures because of their general occurrence. Throat swabbings on blood-agar plates often gave a pure culture and the pleural fluids contained in every instance a pure growth. After isolation from the original

⁷ Kinsella, R. A., and Swift, H. F.: *Jour. Exp. Med.*, 1918, 28, p. 169.

⁸ *Jour. Med. Research*, 1916, 34, p. 377; *Jour. Infec. Dis.*, 1914, 15, p. 209, and 1914, 15, p. 227.

⁹ *Jour. Med. Research*, 1917, 36, p. 99.

material, cultures were repeatedly plated to insure their purity. It is noteworthy here that smears from the pleural fluids showed such a dense growth of short and long chain streptococci as to resemble the growth in an artificial culture medium. In several instances the empyema patient had received treatment with gentian violet irrigation. The work done in association with the surgical department would seem to indicate that no profit was experienced by the patient from the use of gentian violet, and it was perfectly clear that this dye exerted no inhibitive action on the organisms in the pus, for smears and cultures from such cases presented no variation, and we have kept streptococci for several months in fluids heavily tinged with gentian violet. The pleural fluids were always retained, since we found they sustained the life of the organisms admirably in the icebox. If contamination of any of the stock cultures occurred, we have been able to recover the pure strain by growing it according to Holman's excellent method in horse serum broth, in which the streptococci would outgrow many of the contaminating organisms; the sediment was then streaked on blood-agar plates and typical colonies picked.

Blood Agar.—Heat was applied to 1.5% plain agar (0.4 +) made from beef infusion and sterilized for 3 days by steam, and to each 90 cc, 10 cc of defibrinated human blood was added after cooling the agar to 50 C. After mixture by rotation, plates and tubes were poured therefrom. (The plates were inoculated by streaking a loopful of material along 3-5 parallel lines. After 24 hours' incubation the plates are examined by holding against the light. Typically the line of incubation is marked as a ribbon, consisting of discrete, minute colonies, 3-6 deep, bounded on either side by a rim of hemolysis. This rim may be described as narrow, medium, or wide. Blood-agar slants were inoculated by dipping the loop into the water of condensation and streaking it in a zigzag manner over the slant. On blood agar, pure cultures, after 24 hours' incubation, present typically minute, barely visible, evenly distributed, colorless, discrete colonies. When held against the light hemolysis in the thinner parts of the slant may be seen. Large, easily visible colonies usually indicate contamination. The water of condensation is usually slightly turbid; dense turbidity indicates contamination).

Horse Serum Peptone Broth.—Peptone meat infusion, 2%, was boiled down to 80% its original volume, adjusted to 0.4 +, tubed and sterilized for 3 days in steam. Horse serum was obtained aseptically and heated to 60 C. for 1 hour in the water-bath to destroy antihemolysins. Two cc of this was added to each tube containing 8 cc of medium, and incubated for 48 hours to insure sterility. We have found this an admirable medium, easily made, of uniform composition and very rarely contaminated. Typically, streptococci produce in this medium a heavy, white sediment consisting of albumin with entangled chains of organisms; older cultures are apt to present a larger amount of sediment. Yellowish, orange or smoky color of supernatant fluid, or viscid stringy consistency of sediment denote contamination.

TABLE 1
SOURCE: THROAT. DISEASE GROUP: ACUTE INFECTIONS UPPER RESPIRATORY TRACT

No.	Disease and Final Outcome*	Date of Cul- tures	Date of Tests	Bouil- lon	Mor- phology	Blood Plate	Erythro- cyte Suspen- sion	Lac- tose	Man- nite	Sali- cin	Virulence for Rats				
											Date	Age	Weight in Gm.	Amount Injected, C C	Results
1	7/1—Tonsillitis, acute. D 7/18	7/1 7/4	7/12	S-D	L-M-R	F-W	0.20 (1 hr.) 0.075 (2 hr.)	+	—	+					
2	7/15—Pharyngitis, acute. D 7/17	7/15	8/15 8/16 9/1	S S-D S-D	L-L-R L-L-R L-L-R	F-M F-M	0.10 0.012 0.014	± + +	— — —	++ ++ ++	9/1 9/6	young young	35 31	1.0 0.5P	Died 9½ hours Died 21½ hours
3	7/21—Bronchitis, acute. D 7/25	7/21	8/16 8/26	S	L-L-R M-M-R	F-M	0.15	± ±	— —	± ±					
4	7/11—Pharyngitis, acute. D 7/15	7/15	8/16 8/25	S S	M-M-R L-M-R	F-N F-M	0.05 N.H.	— +	— —	++ ++	8/25	young	36	1.0	Survived
5	5/25—Pharyngitis, acute. fracture of tibia. S.C.D. 7/25	5/30	7/12 8/20 9/4	D-S D-S	B-M-R L-M-R	F-W F-N	N.H. 0.012 0.02	+	—	++ ++ ++	9/4 9/6	young young	30 45	1.0 0.5P	Died 9 hours Died 10 hours
6	6/28—Streptococcus car-rier. D 6/28	6/28	8/16 8/26	S	M-L-R B-L-R	O-N	0.4	± ±	— —	++ ++					
7	7/17—Tonsillitis, acute. D 7/22	7/17	8/16 8/26	S S-D	L-L-R B-N-R	F-W	0.05 0.014	± +	— —	— ++					
8	7/15—Tonsillitis, acute. D 7/25	7/15	8/15 8/26	S	L-L-R L-L-R	F-M	0.075	± ±	± ±	— ++					
9	6/29—Tonsillitis, acute. 7/3—Bronchitis, acute. D 7/7	6/29	7/12 8/26	S	L-L-R B-M-R	F-W	N.H.	++ +	— —	++ ++					
10	6/4—Tonsillitis, acute. D 6/19	6/19	7/23	D-S	L-M-R	0.0125	+	+	+					

11	6/29—Tonsillitis, acute. D 7/8	6/29 7/4	7/12 8/6 9/4	D-S S-D	L-N-E L-M-R	F-W	0.5 0.3 0.3	+	+	+	+	9/4 young 9/6 young 9/6 adult	62 42 185	1.0 1.0 0.5P 2.0P	Died 6 hours Died 8 hours Died 6½ hours Survived
12	6/28—Bronchitis	6/28 7/3	7/15 7/16	S-D	M-M-R	F-W	0.2 (1 m.) 0.1 (2 m.) 0.05 (1 m.) 0.01 (2 m.)	±	±	+	+				
13	7/9—Tonsillitis, acute. D 7/13	7/10	8/15 8/16 9/14	S S	M-M-R M-M-R	F-M F-M	N.H. 0.3	±	+	+	+				
14	6/28—Tonsillitis, acute. D 7/3	6/30	7/15 8/29 9/14	S-D D-S	M-L-E	M-M	0.075 (1 m.) 0.05 (2 m.)	+	+	+	+				
15	7/29—Streptococcus car- rier. D 7/29	7/29	8/15 8/25	S S-D	L-M-R L-L-R	M-M C-M	0.025 0.025	±	±	+	+	8/25 young	40	1.0	Died 12 hours

* Disease and Final Outcome: The disease is preceded by the date of development; the date following is that of final disposition. D = indicated return to duty; S.C.D. = discharged from army on Surgeon's certificate of disability; D.I. = died; T.G.H. = transferred to general hospitalization; S = sedimentation; S-D = sedimentation with slight diffuse turbidity; D-S = diffuse turbidity with slight sedimentation. Rouillon: The first letter indicates length of chain; L = longus, M = medium, S = small. The third letter indicates size of individual cocci: L = large; M = medium, S = small. The third letter indicates shape of individual cocci: R = round; E = elongated. Morphology: The first letter indicates size of colony: F = fine (minute); C = coarse. The second letter indicates width of hemolytic rim: N = narrow; M = medium; W = wide. Carbhydrate fermentations: + indicates fermentation; —, no fermentation; ±, doubtful fermentation.

Morphology.—Smears were made from 18-hour horse serum peptone broth culture, stained by Gram's method, and the length of chain and size and shape of the individual cocci recorded.

Carbohydrate Broth.—Peptone meat infusion (2%) 0.4 + containing 1% of mannite, lactose, and salicin, respectively, with Andrade's indicator were used. To each tube 0.2 cc of an 18-hour horse serum broth culture was added and the tubes incubated for 5 days. In many instances smears were made from the sediment to detect contamination. Doubtful reactions were repeated.

Streptolysin.—Hemolytic streptococci produce certain soluble toxins of which a hemolysin (streptolysin) is the most important. Its properties may be summarized as follows: Streptolysin production takes place best in serum containing liquid mediums, beginning a few hours after incubation, reaching its maximum in from 16-18 hours, gradually decreasing, and disappearing after 48 hours. It is thermolabile, heating for 30 minutes at 55 C. destroying it. After centrifugating a streptococcus culture, the supernatant clear fluid is as potent as the bacterial suspension. Filtration through a Berkefeld, or similar filter, slightly decreases its potency. McLeod¹⁰ attributes much importance to streptolysin and believes that the amount produced by a given strain stands in direct relation to its virulence. Quantitative estimation of hemolysin has been used by McLeod,¹⁰ Lyall,¹¹ and others, adding to a standard erythrocyte suspension increasing quantities of streptolysin and incubating the mixture for a certain time. The smallest amount of streptolysin which produces complete solution of the red corpuscles is taken as the unit and called by McLeod the "minimal hemolytic dose." Since practically all the components of this test are of variable nature, we have aimed to standardize each substance so as to permit comparison of the results.

The supernatant fluids from 18-hour cultures in horse serum peptone broth were used ("streptolysin solution"). We found that the amount of streptolysin produced does not depend on the number of bacteria used for seeding the serum broth, provided that a sufficient number be inoculated to cause growth. Thus, we introduced from 1-10 drops, respectively, of a suspension of streptococci into a series of 10 serum broth tubes. After 18 hours' incubation, practically an identical hemolytic titer was found in each tube. One loopful of streptococci from a blood-agar slant is sufficient to produce maximum growth in horse serum broth. Maximum hemolysin production occurred invariably in mediums affording suitable conditions for luxuriant growth provided carbohydrates were not present. In ordinary peptone broth, hydrocele fluid, or broth of low serum content, streptococci grow poorly and a corresponding low feeble hemolysin production obtained.

The sheep corpuscle suspension was made so that each 1 cc would contain 300,000 erythrocytes (this is approximately a 2.5% suspension). A measured quantity of sheep blood was collected with a syringe previously rinsed with sodium citrate solution, and washed 3 times with normal salt solution. A suspension in salt solution approximating 3% was then made, and the cells counted with a white blood pipet. By a simple calculation and the addition of salt solution the standard suspension was obtained and checked up by another cell count. This suspension was always used on the same date.

The "streptolysin solution" in amounts ranging from 0.01-1 cc was introduced into a series of test tubes, 1 cc of the erythrocyte suspension added to

¹⁰ Jour. Path. and Bacteriol., 1915, 19, p. 392.

¹¹ Jour. Med. Research, 1914, 30, pp. 487 and 515.

TABLE 2
SOURCE: THROAT. DISEASE GROUP: NONINFECTIOUS DISEASES

No.	Disease and Final Outcome	Date of Cul- tures	Date of Tests	Bouil- lion	Mor- phology	Blood Plate	Erythro- cyte Suspen- sion	Lac- tose	Man- nite	Sali- cin	Virulence for Rats				
											Date	Age	Weight In Gm.	Amount Injected, O O	Results
1	7/8—Myalgia. D 7/14	7/ 8	7/23	D-S	L-M-R	F-N	0.2	+	—	+					
2	7/2—Ulcer of nasal sep- tum. D 7/10	7/ 2	7/23 9/23	D-S	M-S-E	F-W	N.H.	+	—	+					
3	6/13—Pulm. tuberculosis, chronic. T.G.H.	7/ 3	7/ 9	S-D	M-M-R	F-N	0.012	+	—	+					
4	7/13—Reaction to ty- phoid vaccine. D 7/17	7/14	8/13 8/26 9/24	D-S	L-L-R M-L-R	F-N	0.0125	±	+	+					
5	7/16—Neurocirculatory asthenia. D 7/20	7/16	8/13 9/ 1	S-D S-D	L-M-F L-L-R	F-M	0.0125 0.01	+	—	+	9/1	young	30	1.0	Died 35½ hours
6	7/18—Reaction typhoid vaccine. D 7/19	7/ 8	8/15 8/26	S	L-L-R M-M-R	F-M	0.025	±	—	+	9/2	young	45	1.0	Died 20 hours
7	7/15—Cardiac disorder. D 7/25	7/15	8/15 8/20	S	M-M-R	F-M	0.05	±	—	+			15	0.2	Died 26 hours
8	7/3—Gastric ulcer. S.C.D. 8/8	7/15	7/15 7/16	S-D D-S	M-M-R	M-W	N.H. 0.15 (1 hr.) 0.05 (2 hr.)	+	+	+					
9	7/14—Gastric ulcer. S.C.D. 8/25	7/14	8/15 9/ 4	S	M-L-R	C-M	0.3 0.08	+	—	+	9/4 9/6 9/6 9/6	young young young young	46 37 180	1.0 1.0 0.5P 2.0P	Died 5½ hours Died 5 hours Died 4½ hours Died 6 hours
10	7/12—Synovitis, acute, of ankles. D 8/9	7/13	8/15 9/14	S	L-L-R	F-M	0.012	±	—	±					
11	7/8—Epilepsy S.C.D. 8/14	7/ 8	8/15 9/17	S	L-L-R	C-M	0.05	—	—	+					
12	6/13—Malaria D 7/12	6/30	8/11 9/ 4	S-D	M-M-R	F-W	0.075 (1 hr.) 0.025 (2 hr.) 0.03	+	—	+	9/4 9/6 9/6	young young young	59 42	1.0 1.0 0.5P	Died 6 hours Died 5½ hours Died 6½ hours
13	5/9—Injury to cervical vertebral. S.C.D. 7/22	7/ 3	7/11 7/13	D-S	M-M-R	F-W	0.2 (1 hr.) 0.025 (2 hr.) 0.3	+	—	+					

each tube, gently shaken, and the result read after 1 hour's incubation in the water-bath at 37 C. In several instances second readings were taken after 2 hours; as a general rule the hemolysis was doubled in this time. Usually the dilution was not carried below 0.01 c.c. Streptolysin solutions which were not hemolytic in quantities of 1 c.c. were designated N H.

Virulence for Rats.—Virulence observations were made on cultures younger than the 4th generation. Small white rats, 6 weeks old, approximately of the same weight, and large rats 3 months old, were used. All injections were made intraperitoneally with a well shaken 18-hour horse serum broth culture. A necropsy was made usually within an hour after death, smears and cultures taken from the peritoneal exudate and heart blood; streptococci in pure culture were recovered in every instance. Hemolytic titrations were conducted with these "passage" cultures in several instances.

RESULTS

The results are summarized in tables 1-5 and are here discussed collectively.

Growth in Horse Serum Peptone Broth.—As a general rule, the growth was a heavy, white, flocculent sediment with faintly opalescent supernatant fluid. Less often the supernatant fluid was cloudy; diffuse clouding with slight sediment occurred least frequently. No differences were noted in the strains from the various sources or disease groups. The appearance of the culture often varied at different tests; thus, a strain which usually presented a distinct sediment without any clouding, would occasionally exhibit distinct clouding and only slight sedimentation.

Morphology.—The length of the chains and the size of the individual cocci did not present many characteristic features and often varied from week to week. This variation usually accompanied changes in the appearances of the broth culture. The shape of the individual cocci remained constantly as a slightly laterally compressed oval. Elongated forms were only rarely met with.

The tables illustrate well that no reliance can be placed on morphologic features. No essential difference was noted in the various strains.

Growth on Blood Plates.—The width of the hemolytic rim appeared to depend on the consistency and amount of the culture used for inoculating, on size and shape of loop, on consistency of the culture medium, the pressure of the loop in inoculating the medium and the luxuriance of the growth. Contaminating organisms, such as staphylococci, are usually much larger than the streptococcic colonies, and

TABLE 3
SOURCE: THROAT. DISEASE GROUP: MEASLES UNCOMPLICATED

No.	Disease and Final Outcome	Date of Cul- tures	Date of Tests	Bouil- lon	Mor- phology	Blood Plate	Erythro- cyte Susten- sion	Lac- tose	Man- nite	Sali- cin	Virulence for Rats				
											Date	Age	Weight in Gm.	Amount Injected, C C	Results
1	5/25—Measles. D 7/16	7/ 2 7/ 9	8/13 8/26 9/14	D-S	L-M-R L-M-R	M-M F-M	0.0125	± ± ±	++ ++ ++	++ ++ ++					
2	6/4—Epilepsy; 7/8—Measles. S.C.D. 7/28	7/16	8/30	S	L-L-R	F-M	0.4	+	—	+					
3	6/25—Measles. D 7/10	7/10	8/15 8/26	S	L-L-R M-L-R	F-W	0.012	± ±	— —	++ ++					
4	7/10—Measles. D 8/7	7/10 7/16	8/14 9/12 9/17	S	L-L-R	F-M	0.4	— — —	— — —	± ++ ++					
5	7/13—Measles. D 8/13	7/13	8/15 8/27	S	L-L-R L-L-R	F-M F-W	0.4	— +	— —	— +					

TABLE 4
SOURCE: PLEURAL FLUID. DISEASE GROUP: EMPYEMA, PRECEDED BY MEASLES

No.	Disease and Final Outcome	Date of Cul- tures	Date of Tests	Bouil- lon	Mor- phology	Blood Plate	Erythro- cyte Susten- sion	Lac- tose	Man- nitol	Sali- cin	Virulence for Rats						
											Date	Age	Weight in Gm.	Amount Injected, C C	Results		
1	4/20—Measles; 5/18—Bronchopneum.; 5/22—Empyema; 5/27—Thoracotomy. H 9/1	5/13 5/25	8/19 9/14 9/20	S	L-S-R	F-M	0.025	± — +	— + +	+	+	+	9/4 9/6	young young	54 ..	1.0 1.0	Died 6 hours Died 5½ hours
2	4/19—German measles; 5/1—Bronchopneum.; 5/7—Empyema; 6/3—Thoracotomy. H 9/1 H 9/1	5/5 5/9	7/16 8/19 9/1	D-S S-D S-D	M-M-R L-M-R B-L-R	F-M	N.H. 0.025 0.028	+	— .. .	+	+	+	9/1 9/6 9/6	young young adult	42 40 187	1.0 0.5P 2.0P	Died 9 hours Died 11 hours Died 10½ hours
3	3/29—Measles; 4/18—Tonsillitis; 4/19—Bronchopneum.; 4/21—Empyema; 4/24—Thoracotomy. Dd. 5/4	4/24 4/25	7/23 8/19 9/1 9/4	D-S S	L-M-R B-M-R	F-M C-M	0.025 0.007 0.01 (P)	+	— — .	+	+	+	9/1 9/6	young young	40 32	1.0 0.5P	Died 10 hours Died 30 hours
4	3/29—Measles; 4/12—Bronchopneum.; 4/24—Empyema; 5/2—Thoracotomy. S.C.D. 8/11	4/24	8/6 9/3 9/17	S S-D	L-L-R L-M-R	F-W	N.H. 0.02	— ± ±	— + —	+	+	+					

of an opaque, white color. Sometimes, however, individual streptococcic colonies are larger than usual, broad and flat. This is commonly met with in cultures which have stood for a long period of time in the icebox, or which have been overincubated. Hazy, ground glass hemolysis is produced when incubation is carried on for less than 24 hours, at a lower temperature than 37 C. on unsuitable mediums or in old cultures. The size of the colonies was usually small, the rim of hemolysis generally medium in size. No constant uniform variation in hemolysis occurred on blood-agar plates such as could not be explained on technical grounds. There was also no constant variation in the size or type or quality of colony in the five groups discussed.

Quantitative Hemolysin Determination.—All strains exhibited a high hemolytic power. This, while slightly varying at times, was as a rule fairly constant for the individual strains. Variations of hemolytic potency were usually associated with variations in bouillon growth and morphology, although no definite law could be formulated. Cultures, after passage through one animal, exhibited practically no change in titers from those of the original cultures; however, further passage was not performed. In a general way it would seem to us that hemolysin production depended on favorable conditions for growth and that any given strain, having a low hemolytic degree, exhibited decided increase in hemolysin production on reculture and repetition of the test. This was equally true of throat and pleural strains. Thus, for instance, there are several strains that proved nonhemolytic in quantities of 1 c c which later on showed a high degree of hemolysis. No essential changes were noticed among the various strains, so that if the hemolytic property of a strain of streptococcus stands in any relation to virulence, it would seem that the organisms isolated from the throat were as virulent and as capable of cellular destruction as the strains from the pleural exudate.

Carbohydrate Fermentation Reaction.—The tables show, in several instances, decided variation in carbohydrate reaction. This may be due to accidental contamination or to the presence of more than one strain of streptococcus. We urge, therefore, repetition of all carbohydrate tests preferably after an interval. This we have done in many instances; in conflicting readings, the last result obtained being the one recorded. The results are summarized in table 6. It shows that according to Holman's classification *Streptococcus pyogenes* pre-

TABLE 5
SOURCE: PLEURAL FLUID. DISEASE GROUP: EMPYEMA, NOT PRECEDED BY MEASLES

No.	Disease and Final Outcome	Date of Cul- tures	Date of Tests	Bouil- lon	Mor- phology	Blood Plate	Erythro- cyte Suspen- sion	Lac- tose	Man- nite	Sali- cin	Virulence for Rats				
											Date	Age	Weight in Gm.	Amount Injected, C O	Results
1	Pneumonia lobar; empyema. S.C.D. 3/5	12/17	7/25	S-D	L-L-R	F-W	N.H.	+	—	±					
2	4/16—Empyema; thoracotomy. D 8/15	4/8 4/11	7/26 8/20 9/4	S-D S	B-M-R L-L-R	N-W C-W	0.075 0.012 0.03	+	—	+	9/4 9/6	young young	53 ..	1.0 1.0	Died 7½ hours Died 6½ hours
3	5/5—Bronchopneum.; 5/7—Empyema; 5/29—Thoracotomy. H 9/1	5/9 5/12	8/9 8/20	L-M-R L-L-R	F-M	0.05 0.10	+	—	+					
4	4/23—Arthritis, acute; 5/3—Pleurisy; 5/5—Empyema. H 9/1	5/5 5/20 5/26	7/16 7/22 8/18 8/20	S S-D S-D S-D	L-M-R L-M-R L-L-R L-M-R	F-M F-M F-M F-M	0.05 0.025 0.025	— — — +	+	+					
5	4/8—Bronchitis; 4/17—Empyema; 4/25—Thoracotomy. S.C.D. 8/25	5/15 5/18	8/6 9/1	D-S S-D	L-M-R M-L-R	F-M F-M	0.01 0.05	+	—	+	9/1 9/4	young young	45 40	1.0 0.1	Died 12 hours Survived
6	4/8—Bronchitis; 4/17—Empyema; 4/25—Thoracotomy. S.C.D. 8/15	4/17 5/18 5/21	8/19	S	L-S-R	F-M	N.H.	+	—	+	9/20	mouse	17	0.2	Died 7½ hours

dominated in all disease groups; *Streptococcus infrequens* occurring next; *Streptococcus equi* occurred only twice and that in the throat; *Streptococcus hemolyticus* II was found once in a pleural exudate. The other four types of Holman—*Streptococcus hemolyticus* I and III, *subacidus*, and *anginosus*—did not occur in our series. There was no essential difference in hemolysin production by the strains of the four types found, nor did they exhibit any cultural or morphologic distinctions.

Virulence for Rats.—No difference in the virulence for rats of the various disease groups was apparent. In practically all instances the animals died within 24 hours, the majority within 6-12 hours, thus

TABLE 6
SUMMARY OF CARBOHYDRATE FERMENTATION TESTS

Source and Disease Group	<i>Streptococcus</i> <i>Infrequens</i>	<i>Streptococcus</i> <i>Pyogenes</i>	<i>Streptococcus</i> <i>Hemolyticus</i> <i>II</i>	<i>Streptococcus</i> <i>Equi</i>
Throat:				
Acute infectious diseases.....	2	13	—	—
Noninfectious diseases.....	1	11	—	1
Measles.....	1	3	—	1
Empyema:				
Preceded by measles.....	1	4	—	—
Not preceded by measles.....	—	12	1	—
Total.....	5	43	1	2

showing our strains to be highly virulent. If hemolysin production stands in intimate relation to virulence, this high virulence to rats would confirm our findings of a uniform high hemolytic titer of the various strains.

CONCLUSIONS

Streptococci isolated from the throat and from empyema exudate during the epidemic of the winter of 1917 and 1918, appeared biologically identical and highly virulent as based on the criteria mentioned in the early part of the paper. Our study confirms the observations of others, using immunological procedures, and gives additional support to the belief that the streptococcus carrier state is an indication of the possibility of complications in respiratory tract diseases.