Bacteriologic, Etiologic, and Serologic Studies in Epilepsy and Schizophrenia I.

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In studies on the etiology of various diseases it was found, in agreement with other investigators, that the aerobic methods usually employed in bacteriology do not suffice for the consistent isolation of causative organisms. By the use of special mediums which afforded a gradient of oxygen tension and other properties favorable for the growth of fastidious organisms, specific types of streptococci which had elective localizing power on injection into animals were isolated consistently in studies of various diseases.[1,2,3,4]

The results obtained in some epidemic[5,6,7,8] and nonepidemic diseases[9,10] have been reported. Those obtained in studies of epilepsy and schizophrenia over a period of a dozen years as opportunity was afforded, although highly suggestive,[11] have been withheld until results of a more intensive study became available. These it is felt are now at hand.

The results of studies on the isolation of alpha streptococci and of experiments in animals in which spasms and convulsive seizures and disorientation and strange symptoms in behavior were produced with the respective strains will be reported elsewhere. Those obtained in agglutination, agglutinin absorption, and precipitation experiments with the antiserums prepared in horses and rabbits and produced in vitro from the respective streptococci and with the serum of persons ill are to be reported in this paper.

This is first of a series of three papers by Dr. Rosenow in which are recorded the results of bacteriologic and experimental studies on the etiology of epilepsy and schizophrenia. Papers II and III will appear in later issues of *Postgraduate Medicine*.

METHODS AND MATERIAL

Realizing the difficulties that have been encountered in the isolation of specific types of alpha streptococci from persons suffering from various diseases and in experiments on agglutination[12] and precipitation, we have used special methods to eliminate these in so far as possible. The diagnoses for the most part were made by examining physicians in charge of patients in hospitals and private practice. Cultures were made from nasopharynx, infected teeth, blood, spinal fluid, urine, and feces.

Nasopharyngeal swabbings were made with swabs wrapped on aluminum wire and bent to a suitable angle without touching the tongue. The nasopharyngeal swabbings and the material obtained from pyorrhea pockets and the severed apexes of pulpless teeth, drawn in a sterile manner, were suspended in 2 ml. of a 0.2 gelatin-Locke or isotonic sodium chloride

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solution for cultures, precipitation tests, and inoculation of animals.

Routinely, the surface of horse blood agar plates were inoculated and serial dilution cultures[13] were made in mediums affording a gradient of oxygen tension, viz. in dextrose brain broth or alternately dextrose brain broth (0.2 per cent dextrose) and soft dextrose brain agar (0.2 per cent dextrose and 0.2 per cent agar) in tall (12 cm.) columns in test tubes. The dextrose brain broth and dextrose brain agar were prepared by adding, before autoclaving, approximately 1 part by volume of pieces of fresh or frozen calf or young beef brain to 6 or 7 volumes of the mediums previously adjusted to *p*H 7.2 and contained in ⅝ x 6- or 8-inch test tubes in columns 12 cm. in height.

THE amount of inoculum in the first tube and degree of serial dilution, usually at steps of 1–100 or 10,000, was determined by the number and kind of organism found in stained films of the material under study. The brain-containing mediums used were freshly prepared or were boiled to drive off dissolved oxygen and cooled to 40°C. immediately before they were inoculated.

Pure cultures for inoculation of animals, and for serologic and other studies were obtained not from blood agar plates but from the end point of growth of usually young primary serial dilution cultures in dextrose brain broth or dextrose brain agar. Blood agar plates were made of these to determine the type of streptococcus and to be sure of the purity of the young cultures.

Pure cultures of streptococci obtained from patients and from animals were routinely preserved in dehydrated form by placing the centrifugated sedimented organisms from young dextrose brain broth or dextrose broth cultures of streptococci after decanting the supernatant broth in very dense suspension (300,000,000,000 per ml.) of glycerol 2 parts and saturated sodium chloride solution 1 part. Moreover, selected strains of streptococci were inoculated into previously warmed 0.2 per cent dextrose broth in gallon bottles containing 3500 ml. each, and the organisms of young cultures from a continuous feed centrifuge bowl were likewise preserved at 10°C. in dehydrated form in dense suspension of the glycerol–sodium chloride solution menstruum (1,000,000,000,000 organisms per ml.) for agglutination and precipitation tests, for immunization of horses and rabbits, and for the preparation of solutions of specific polysaccharide and thermal antibodies.[14]

The agglutination tests were made by placing 0.2 ml. of fivefold dilutions of 1–20 to 1–2500 of the serum or thermal antibodies into test tubes ⅜ x 3 inches and adding to each tube 0.2 ml. of the respective suspensions of streptococci containing approximately 5,000,000,000 organisms per ml. The suspensions were allowed to stand at room temperature or were lightly centrifuged to eliminate clumped organisms. The setups were thoroughly shaken and placed in the incubator at 47°C. to 50°C. for eighteen to twenty-four hours.[12] Readings were made in a dark room under the edge of a shaded light

TABLE 1

ILLUSTRATIVE EXPERIMENT ON THE AGGLUTINATION OF STREPTOCOCCI BY THE SERUM OF PATIENTS

Source of Streptococcal Pools	Dilutions of Serums and Per Cent of Agglutination	Degree (0-4) and Percentage of Total Possible Agglutination at Fivefold Dilutions of Pools of 5 Serums Each of Well Persons and of Persons Suffering from:			
		Epilepsy	Schizophrenia	Arthritis	Control well persons
Epilepsy (11)*	1-20	**2**	3	2	2
	1-100	**2**	3	2	2
	1-500	**3**	0	0	0
	1-2500	**3**	0	0	0
	Per cent	**63**	38	25	25
Schizophrenia (10)	1-20	3	**3**	2	2
	1-100	3	**3**	2	2
	1-500	2	**3**	0	0
	1-2500	0	**2**	0	0
	Per cent	50	**69**	25	25
Arthritis (30)	1-20	2	2	**2**	2
	1-100	2	2	**3**	2
	1-500	2	0	**3**	0
	1-2500	0	0	**3**	0
	Per cent	38	25	**69**	25
Control pneumococci (9)	1-20	2	2	2	2
	1-100	0	2	0	0
	1-500	0	0	0	0
	1-2500	0	0	0	0
	Per cent	13	25	13	13

*The figures in parentheses indicate the number of strains in pools.

from a 100-watt electric light bulb against a nonreflecting background of black velvet cloth. The degree of agglutination was recorded without regard to the source or type of antibody according to the arbitrary scale of 0 to 4 plus.

Two types of thermal antibodies were used in agglutination and precipitation tests. The one designated simply as thermal antibody represented the bacteria-free supernatant of suspensions in sodium chloride solution containing 10,000,000,000 streptococci per ml. after autoclaving at 15 to 17 pounds pressure for ninety-six hours.[14] The other designated as thermal hydrogen peroxide antibody represented the bacteria-free supernatant of suspensions in sodium chloride solution containing 10,000,000,000 streptococci per ml. which were autoclaved for one hour after adding 1.5 per cent H_2O_2 (initial).[15] The respective streptococci in both instances represented dilutions of the partially dehydrated organisms from dense suspensions in a mixture of glycerol and saturated sodium chloride solution.

Agglutinin absorption experiments were done by adding approximately 5,000,000,000 of the washed organisms per ml. from the dense suspensions in glycerol–sodium chloride solution to the respective undiluted serums and thermal hydrogen peroxide antibody. The first two absorptions were done by incubating for one and one-half hours at 35°C. and the third absorption at 35°C. for one and one-half hours and at 10°C. for eighteen hours. The respective suspensions were centrifuged, the serum or antibody was decanted, and the agglutinin titer of the absorbed and unabsorbed serums determined in parallel manner.

For the sake of brevity and in order that the degree of agglutination of the different strains by the four dilutions of the different materials

TABLE 2

AGGLUTINATION OF ALPHA STREPTOCOCCI ISOLATED FROM THE NASOPHARYNX OR BLOOD OF PERSONS SUFFERING FROM EPILEPSY, SCHIZOPHRENIA AND ARTHRITIS BY THE ANTISERUMS* PREPARED IN HORSES WITH THE RESPECTIVE STRAINS OF STREPTOCOCCI.

Source of Streptococci	Streptococcal		Agglutination Experiments	Percentage of Total Possible Agglutination at Fivefold Dilutions of 1-20 to 1-2,500 of Antiserums Prepared in Horses with Streptococci Isolated in Studies of:		
	Pools	Strains		Epilepsy	Schizophrenia	Arthritis
Idiopathic Epilepsy	9	105	13	**68**	47	35
Schizophrenia	7	63	11	49	**72**	38
Chronic Arthritis	7	76	12	28	30	**55**

*The antiserums used were prepared in parallel manner by repeated intravenous and subcutaneous injection of appropriate dilutions of the respective heat killed (70°C. 1 hour) streptococci whose antigenic specificity was preserved throughout the long period of immunization (one and one-half years) in dense suspension (1,000,000,000,000 streptococci per ml.) in glycerine 2 parts and 25 per cent sodium chloride solution 1 part.

TABLE 3

AGGLUTINATION OF STREPTOCOCCI ISOLATED IN STUDIES OF EPILEPSY, SCHIZOPHRENIA AND ARTHRITIS BY THE UNABSORBED AND ABSORBED SERUM OF HORSES IMMUNIZED WITH THE RESPECTIVE STREPTOCOCCI.

Source of Streptococci	Streptococcal		Experiments	Percentage of total possible agglutination at fivefold dilutions of 1-20 to 1-2,500 of the unabsorbed and absorbed serums of horses immunized with streptococci isolated in studies of											
				Epilepsy				Schizophrenia				Arthritis			
				Absorbed with streptococci isolated in studies of				Absorbed with streptococci isolated in studies of				Absorbed with streptococci isolated in studies of			
	Pools	Strains		Unabsorbed	Epilepsy	Schizophrenia	Arthritis	Unabsorbed	Schizophrenia	Epilepsy	Arthritis	Unabsorbed	Arthritis	Schizophrenia	Epilepsy
Epilepsy	1	11	1	**75**	**38**	56	44	31	25	25	25	31	38	38	38
	1	12	1	**75**	**31**	50	49	31	31	31	38	56	56	56	25
Schizophrenia	1	10	1	31	25	25	25	**69**	**31**	44	38	44	25	25	25
	1	12	1	38	31	19	25	**63**	**25**	44	31	44	38	44	44
Arthritis	1	30	1	44	31	44	25	31	31	31	13	**69**	**0**	44	38
	1	42	1	25	13	25	6	19	13	13	6	**63**	**0**	25	25

containing antibodies may be readily visualized and compared in the tables and text, the percentages of total possible agglutination in the four dilutions are given. A 4-plus agglutination in each of the four dilutions, or a total of 16, would be 100 per cent; a total of 5 pluses for the four dilutions would be 5/16, or 31 per cent, and so forth. This method was found to express more accurately the agglutinin titer of serums and of antibody solutions than did the end point at which agglutination occurred.

Interface precipitation tests were made in small glass tubes (5 x 25 mm.) by superimposing the solutions of specific polysaccharide of the respective streptococci obtained by the Lancefield method and the cleared NaCl solution washings of nasopharyngeal swabbings to which 0.2 per cent phenol had been added onto the undiluted serum of patients, on the serum of horses and rabbits that had been immunized with the respective streptococci, and on the solutions of the thermal antibodies to which 0.1

per cent agar or 1 per cent gelatin were added. The degree of clouding or precipitation at the interface, after thirty minutes at 35°C. and eighteen hours at 10°C., or after eighteen to twenty-four hours at 35°C. was recorded according to the arbitrary scale of 0 to 4 plus.

RESULTS OF CULTURES

Cultures on blood agar plates and some dilutions of cultures in dextrose brain broth or soft dextrose brain agar were made of nasopharyngeal swabbings from 181 persons suffering from epilepsy, from 258 persons suffering from schizophrenia, 85 persons suffering from either chronic or subacute arthritis, and, as a control, from 78 well persons. Green-producing colonies of alpha streptococci grew in greatly predominating numbers in most of the cultures and indifferent colonies in a few instances, never in predominating numbers. Variable numbers of colonies of Micrococcus catarrhalis and of staphylococci grew in most instances, and in no instance did Hemophilus influenzae grow in large numbers. There was no distinctive difference between the type of colonies that grew in the different groups. In general, however, the number of colonies was greater, often far greater, in the persons who were ill than in those who were well.

Shake cultures in blood agar and serial dilution cultures in dextrose brain broth or alternately in dextrose brain broth and dextrose brain agar were made from the spinal fluid in 24 individuals, from the urine in 10, from the apexes of pulpless teeth extracted in a sterile manner in 24, and from the stool in 23 persons suffering from schizophrenia. The spinal fluids proved sterile; greening streptococci were isolated in small numbers from the urine, and in large numbers from the teeth; and greening or indifferent streptococci were isolated in 19 of the 23 stools cultured.

Cultures in the dextrose brain broth of the partially macerated blood clot yielded highly pleomorphic greening or indifferent streptococci to blood agar in 49, or 29 per cent, of 161 persons suffering from epilepsy, in only 5, or

TABLE 4

AGGLUTINATION OF ALPHA STREPTOCOCCI ISOLATED IN STUDIES OF EPILEPSY, SCHIZOPHRENIA AND ARTHRITIS BY THE SERUM OF RABBITS BEFORE AND AFTER IMMUNIZATION WITH THE RESPECTIVE STRAINS OF STREPTOCOCCI.*

Source of Streptococci	Streptococcal		Percentage of total possible agglutination by the serum of rabbits at fivefold dilutions, 1-20 to 1-2,500 before and after immunization with streptococci isolated in studies of							
			Epilepsy		Schizophrenia				Arthritis	
			rabbit 8		rabbit 6		rabbit 7		rabbit 9	
	Pools	Strains	Normal	Immune	Normal	Immune	Normal	Immune	Normal	Immune
Epilepsy	1	15	0	**56**	13	19	0	25	0	19
	1	16	19	**81**	25	**50**	25	50	13	38
	1	17	0	**56**	6	13	19	25	0	0
Total	3	43	6	**64**	15	27	15	33	4	19
Schizophrenia	1	10	19	38	25	**81**	25	**63**	19	25
	1	8	25	44	25	**88**	25	**88**	25	38
	1	18	13	25	25	**56**	19	**63**	13	25
Total	3	36	19	36	25	**75**	23	**71**	19	29
Arthritis	1	31	13	25	19	31	6	25	13	**56**
	1	31	19	25	13	19	6	19	0	**63**
	1	65	13	0	0	19	0	6	6	**56**
Total	3	96	15	17	11	23	4	17	6	**58**

*The antiserums used were prepared in parallel manner by three daily intravenous and subcutaneous injections per week for four weeks of the respective heat killed (65°C. 1 hour) streptococci. The rabbits were bled before the first and ten days after the last injection and the agglutination tests were done ten days after the immunized rabbits were bled.

TABLE 5

AGGLUTINATION OF STREPTOCOCCI ISOLATED IN STUDIES OF EPILEPSY, SCHIZOPHRENIA AND ARTHRITIS BY THE UNABSORBED AND ABSORBED SERUMS OF RABBITS THAT HAD BEEN IMMUNIZED WITH THE RESPECTIVE STREPTOCOCCI.

Source of Streptococci	Streptococcal		Percentage of total possible agglutination of respective streptococci by the serums of rabbits at fivefold dilutions of 1-20 to 1-2,500 immunized with the streptococci isolated in studies of								
			Epilepsy			Schizophrenia			Arthritis		
			Rabbit 8			Rabbit 6			Rabbit 9		
	Pools	Strains	Unabsorbed	Absorbed with streptococci isolated in studies of		Unabsorbed	Absorbed with streptococci isolated in studies of		Unabsorbed	Absorbed with streptococci isolated in studies of	
				Epilepsy	Arthritis		Schizophrenia	Arthritis		Arthritis	Epilepsy
Epilepsy	1	15	**75**	**31**	44	25	25	25	31	31	31
	1	16	**69**	**44**	63	50	50	50	50	38	31
Schizophrenia	1	10	50	44	44	**69**	**38**	56	31	25	25
	1	8	50	38	44	**69**	**38**	63	50	50	44
Arthritis	1	31	25	19	19	25	19	19	**50**	**6**	44
	1	65	25	19	19	25	19	19	**50**	**0**	31

4 per cent, of 125 persons suffering from schizophrenia, in 7, or 14 per cent, of 69 persons having chronic arthritis, and in not one of 62 well persons or persons suffering from noninfectious ailments. The incidence of isolation of streptococci from the blood in persons having epilepsy was highest shortly before or during seizures, and most of the 49 persons from whose blood the streptococcus was isolated were taking phenobarbital or dilantin or both at the time the blood was drawn.

RESULTS OF AGGLUTINATION EXPERIMENTS

The type of agglutination experiments which were done, the way results were recorded, and the method of determining the percentage of agglutination and of indicating specificity are shown in *Table 1*. The results of agglutination of a large number of the respective strains by the different antiserums, by thermal antibodies. and by the serum of patients are summarized in *Tables 2, 4, 6, 7, and 8*. A high degree of respective specific agglutination was obtained with

TABLE 6

AGGLUTINATION OF ALPHA STREPTOCOCCI ISOLATED IN STUDIES OF EPILEPSY, SCHIZOPHRENIA AND ARTHRITIS BY THERMAL ANTIBODY PREPARED FROM THE RESPECTIVE STRAINS OF STREPTOCOCCI, 20,000,000,000 PER ml. IN NaCl SOLUTION, AUTOCLAVED FOR 96 HOURS.

Source of Streptococci	Streptococcal		Agglutination Experiments		Antibody preparations	Percentage of total possible agglutination at fivefold dilutions of 1-20 to 1-2,500 of thermal antibody prepared from streptococci isolated in studies of		
	Pools	Strains	Number	Time		Epilepsy	Schizophrenia	Arthritis
Epilepsy	1	11	8	1945	4	**50**	30	0
	1	12	6	1946	5	**44**	25	0
Schizophrenia	1	10	9	1945	4	38	**50**	0
	1	15	7	1946	5	38	**63**	13
Arthritis	1	30	7	1945	4	38	38	**50**
	1	12	8	1946	5	31	38	**56**

TABLE 7

AGGLUTINATION OF ALPHA STREPTOCOCCI ISOLATED IN STUDIES OF EPILEPSY, SCHIZOPHRENIA, ARTHRITIS AND RESPIRATORY INFECTIONS BY THERMAL ANTIBODY PREPARED FROM THE RESPECTIVE STRAINS OF STREPTOCOCCI, 10,000,000,000 PER ml. IN NaCl SOLUTION PLUS 1.5% H_2O_2 AND AUTOCLAVED FOR ONE HOUR.

SOURCE OF STREPTOCOCCI	STREPTOCOCCAL		PERCENTAGE OF TOTAL POSSIBLE AGGLUTINATION AT FIVEFOLD DILUTIONS OF 1-20 TO 1-2,500 OF THERMAL ANTIBODY PREPARED IN VITRO FROM STREPTOCOCCI ISOLATED IN STUDIES OF							
			EPILEPSY (10)*		SCHIZOPHRENIA (14)		ARTHRITIS (5)		RESPIRATORY INFECTIONS (8)	
	POOLS	STRAINS	Tests	Per cent Agglutination	Tests	Per cent Agglutination	Tests	Per cent Agglutination	Tests	Per cent Agglutination
EPILEPSY	13	83	39	**63**	48	53	6	38	14	52
SCHIZOPHRENIA	13	118	42	47	67	**68**	6	45	19	47
ARTHRITIS	3	44	21	40	28	35	8	**73**	12	50
RESPIRATORY INFECTIONS	8	175	23	48	26	51	7	38	17	**73**

*Figures in ( ) indicate the number of preparations of thermal antibody used in the agglutination tests.

TABLE 8

AGGLUTINATION OF STREPTOCOCCI ISOLATED IN STUDIES OF EPILEPSY, SCHIZOPHRENIA, ARTHRITIS AND RESPIRATORY INFECTIONS BY THE SERUMS OF PERSONS SUFFERING FROM EPILEPSY, SCHIZOPHRENIA OR ARTHRITIS AND OF WELL PERSONS.

SOURCE OF STREPTOCOCCI	STREPTOCOCCAL		EXPERIMENTS		PERCENTAGE OF TOTAL POSSIBLE AGGLUTINATION OF RESPECTIVE STREPTOCOCCI AT FIVEFOLD DILUTIONS OF 1-20 TO 1-2,500 OF THE SERUM OF PERSONS SUFFERING FROM			
	POOLS	STRAINS	NUMBER	TIME OF	EPILEPSY (137)*	SCHIZOPHRENIA (108)	ARTHRITIS (53)	CONTROL WELL PERSONS (59)
EPILEPSY	2	23	9	1945	**64**	45	19	20
	10	133	30	1946	**58**	35	12	15
SCHIZOPHRENIA	2	22	9	1945	45	**68**	26	19
	6	58	23	1946	42	**60**	25	13
ARTHRITIS	2	43	9	1945	14	19	**53**	16
	4	43	14	1946	10	10	**37**	13
RESPIRATORY INFECTIONS	3	115	4	1945	22	18	27	36

*The figures in ( ) indicate the number of patients whose serum was used. About one-half were tested in 1945 and 1946 respectively. However, the agglutinating action of the former group was determined in pools of 4-6 while that of the serums of the latter group was determined separately.

TABLE 9

AGGLUTINATION OF STREPTOCOCCI ISOLATED IN STUDIES OF EPILEPSY, SCHIZOPHRENIA AND ARTHRITIS BY THE UNABSORBED AND ABSORBED SERUMS OF RESPECTIVE PATIENTS AND BY THERMAL ANTIBODY.

SOURCE OF STREPTOCOCCI	STREPTOCOCCI		EXPERIMENTS		PERCENTAGE OF TOTAL POSSIBLE AGGLUTINATION AT FIVEFOLD DILUTIONS OF 1-20 TO 1-2,500 OF SERUM OF PATIENTS SUFFERING FROM											
					EPILEPSY			SCHIZOPHRENIA			ARTHRITIS			THERMAL ANTIBODY PREPARED FROM STREPTOCOCCI ISOLATED IN STUDIES OF EPILEPSY		
	POOLS	STRAINS	NO.	TIME	UNABSORBED	ABSORBED WITH STREPTOCOCCI ISOLATED IN STUDIES OF		UNABSORBED	ABSORBED WITH STREPTOCOCCI ISOLATED IN STUDIES OF		UNABSORBED	ABSORBED WITH STREPTOCOCCI ISOLATED IN STUDIES OF		UNABSORBED	ABSORBED WITH STREPTOCOCCI ISOLATED IN STUDIES OF	
						Epilepsy	Arthritis		Schizophrenia	Arthritis		Arthritis	Epilepsy		Epilepsy	Arthritis
EPILEPSY	2	23	2	1945	**56**	**13**	38	38	13	25	25	13	13	**63**	**44**	50
	1	11	1	1946	**69**	**13**	38	50	38	25	.....	.....	.....	**69**	**56**	.....
SCHIZOPHRENIA	2	21	2	1945	25	13	25	**54**	**0**	38	0	13	13	50	44	31
	1	10	1	1946	38	25	25	**57**	**19**	38	.....	.....	.....	31	31	.....
ARTHRITIS	2	43	2	1945	19	25	19	19	25	13	**56**	**13**	38	44	**44**	**31**
	1	30	1	1946	25	25	13	38	25	13	.....	.....	.....	44	31	.....

TABLE 10

AGGLUTINATION OF STREPTOCOCCI AND PRECIPITATION OF STREPTOCOCCAL POLYSACCHARIDE BY THE SERUM OF PERSONS SUFFERING FROM EPILEPSY IN RELATION TO GRAND MAL SEIZURES.

Source of Streptococci	Streptococcal		Percentage of total possible agglutination of the respective streptococci at fivefold dilutions of 1-20 to 1-2,500 of the serum of persons suffering from epilepsy obtained shortly before and after grand mal seizures.									
			Case 1		Case 2		Case 3		Case 4		Case 5	
	Pools	Strains	B*	A*	B	A	B	A	B	A	B	A
Epilepsy	1	11	44	**69**	56	**63**	44	**56**	38	**63**	56	**81**
Schizophrenia	1	10	31	50	25	38	31	44	38	50	38	50
Arthritis	1	30	38	31	0	0	25	13	13	25	25	25
Encephalitis	1	24	38	25	0	0	38	25	13	38	31	31
Respiratory Infections	1	95	31	38	25	13	25	13	0	13	31	31
Peptic Ulcer	1	12	31	13	0	0	13	25	0	13	13	13
			Percentage of precipitation of respective streptococcal polysaccharide.									
Epilepsy	1	11	25	**50**	50	**50**	25	**50**	25	**50**	50	**75**
Schizophrenia	1	10	0	25	25	25	25	25	0	25	25	25
Arthritis	1	30	0	0	25	25	0	25	0	25	25	25

*B➤1–3 hours before seizures. A➤1–3 hours after seizures. The degree of precipitation was recorded according to the arbitrary scale of 0–4 plus, each plus representing 25 per cent.

each of the different types of antiserums, with the thermal antibody, and with the serum of the patients.

The results of experiments on specificity of agglutinins by absorption from the respective serums and thermal antibody with homologous and heterologous streptococci are summarized in *Tables 3, 5, and 9.* It will be seen that absorptions with the homologous strains consistently caused a far greater reduction of agglutinins than heterologous strains. Moreover, the reduction was usually relatively greater by the more closely related heterologous strains isolated in studies of epilepsy and schizophrenia, respectively, than by more distantly related strains isolated in studies of arthritis.

Thermal antibody, the serums of horses and rabbits that had been immunized with the respective streptococci, and the serum of persons all agglutinated specifically the streptococci isolated from nasopharynx, apexes of pulpless teeth, and blood, but did not agglutinate the streptococci isolated from the urine and feces.

The agglutinin titers of the serums of patients suffering from epilepsy shortly before and shortly after grand mal seizures over streptococci isolated in studies of different diseases and precipitation of polysaccharide obtained from streptococci isolated in studies of epilepsy, schizophrenia, and arthritis are summarized in *Table 10.* It will be seen that the agglutinin and precipitin titers were consistently higher with the serum obtained shortly after seizures than with the serum obtained shortly before seizures. This was associated with a sharp drop in specific streptococcal antigen in skin or blood determined by intradermal injection of antibody (to be reported elsewhere).

RESULTS OF PRECIPITATION EXPERIMENTS

The results of precipitation experiments with the antiserums prepared in rabbits and horses, the serum of patients, and the two types of thermal antibodies over different dilutions of polysaccharide of the respective streptococci are summarized in *Tables 11, 12, and 13,* and those obtained with antiserum of horses, rabbits, and serum of patients over cleared sodium chloride solution washings of nasopharyngeal swabbings are shown in *Table 14.* A high degree of specificity was consistently obtained with each of the materials containing antibody.

COMMENTS AND SUMMARY

The results of agglutination and precipitation experiments with suspensions, extracts,

and solutions of polysaccharide of alpha streptococci isolated in studies of idiopathic epilepsy, schizophrenia, and as controls in arthritis, and the serum of respective patients, the serum of horses and rabbits that had been immunized with the respective streptococci, and thermal antibodies prepared in vitro from streptococci isolated in studies of epilepsy, schizophrenia, and arthritis are reported.

A consistently high degree of specific agglutination of the respective streptococci and of specific agglutinin absorption and a correspondingly high degree of specific precipitation of extracts of the streptococci, of extracts of nasopharyngeal swabbings, and of solutions of polysaccharide by the serums of patients, by the serums of immunized horses and rabbits, and by thermal antibody were obtained.

The streptococci subjected to the serologic tests were isolated chiefly during studies of the past two years, but some were taken as long as twelve years before from nasopharyngeal swabbings, from infected teeth, and from the blood of widely separated groups of persons suffering from the respective diseases.

Success in separating the specific types of alpha streptococci from saprophytic types normally present in nasopharynx of human

TABLE 11

PRECIPITATION REACTION BETWEEN THE SERUM OF RABBITS IMMUNIZED WITH STREPTOCOCCI ISOLATED IN STUDIES OF EPILEPSY, SCHIZOPHRENIA AND ARTHRITIS AND THE POLYSACCHARIDE OF THE RESPECTIVE STREPTOCOCCI.

Source of Streptococci	Solution of polysaccharide from 50 billion streptococci per ml. of following pools of streptococci			Precipitation (0–4) at the interface between solutions of polysaccharide of streptococci and the serum of rabbits immunized with streptococci isolated in studies of							
				Epilepsy		Schizophrenia				Arthritis	
				Rabbit 8		Rabbit 6		Rabbit 7		Rabbit 9	
	Pools*	Strains	Dilution	Normal	Immunized	Normal	Immunized	Normal	Immunized	Normal	Immunized
Epilepsy	844	46	1–0	0	**3**	0	1	0	2	0	2
			1–10	.....	**1**	0	0	.....	0	.....	1
			1–100	.....	**1**	0	0	.....	0	.....	0
	1075	16	1–0	0	**3**	0	1	0	2	0	1
			1–10	.....	**2**	0	1	.....	1	.....	1
			1–100	.....	**2**	0	0	.....	0	.....	0
	144	12	1–0	0	**3**	0	2	0	1	0	1
			1–10	.....	**2**	0	0	.....	0	.....	0
			1–100	.....	**1**	0	0	.....	0	.....	0
Schizophrenia	845	28	1–0	0	1	0	**4**	0	**3**	0	1
			1–10	.....	0	0	**2**	.....	**2**	.....	0
			1–100	.....	0	0	**1**	.....	**1**	.....	0
	896	11	1–0	0	2	0	**4**	0	**4**	0	2
			1–10	.....	1	0	**1**	.....	**3**	.....	0
			1–100	.....	0	0	**1**	.....	**2**	.....	0
	146	12	1–0	0	2	0	**4**	0	**4**	0	1
			1–10	.....	1	0	**2**	.....	**2**	.....	1
			1–100	.....	0	0	**1**	.....	**2**	.....	0
Arthritis	864	31	1–0	0	1	0	2	0	2	0	**3**
			1–10	.....	1	0	1	.....	1	.....	**2**
			1–100	.....	0	0	0	.....	0	.....	**1**
	862	1	1–0	0	1	0	2	0	2	0	**4**
			1–10	.....	1	0	2	.....	2	.....	**3**
			1–100	.....	0	0	0	.....	0	.....	**2**
	5592	12	1–0	0	1	0	2	0	1	0	**3**
			1–10	.....	1	0	0	.....	1	.....	**2**
			1–100	.....	0	0	0	.....	0	.....	**1**
Control NaCl solution			1–0	0	0	0	0	0	0	0	0

*Figures indicate laboratory number of respective pools.

beings is attributable to the serial dilution methods used in the primary isolations. The maintenance of respective original specificity for agglutination and precipitation studies, for the preparation of specific antiserums, and also for production of thermal antibodies with heat alone, or with hydrogen peroxide and much less heat, is attributable to the preservation of specific properties of freshly isolated streptococci in dehydrated form in the dense suspension of glycerine, 2 parts and saturated sodium chloride solution, 1 part. Viability and specific virulence were often maintained for as long as two years in this menstruum, and antigenic specificity was maintained apparently indefinitely. In extended studies of alpha streptococci as isolated in studies of various diseases we have not been able to find a medium which will maintain viability, elective localizing power, or virulence, and specific serologic properties over prolonged periods. From these studies it became apparent that dormancy of cultures kept under proper conditions is especially favorable for maintaining viability and specific properties. Keeping cultures frozen in dry ice or in dehydrated form in vacuo on glass beads, although a usually satisfactory method, is cumbersome and not suited for routine studies. Viability of cultures of the respective streptococci was maintained for several years at room temperature without transfer in autoclaved chick embryo medium in tall columns layered with liquid petrolatum; however, changes in virulence and antigenicity occurred seasonally in accord with epidemics of respiratory and other injections.

Viability and antigenic specificity but usually not virulence were maintained in cultures of streptococci and pneumococci on blood agar slants sealed with sterilized paraffined corks or screw caps when stored at room temperature in the dark for years without transfer. Here also dormancy and reduced oxygen tension are

TABLE 12

PRECIPITATION REACTION BETWEEN THE SERUMS OF HORSES IMMUNIZED WITH STREPTOCOCCI ISOLATED IN STUDIES OF EPILEPSY, SCHIZOPHRENIA AND ARTHRITIS, THE SERUM OF PERSONS SUFFERING FROM THESE DISEASES AND THE POLYSACCHARIDE OF THE RESPECTIVE STREPTOCOCCI.

SOURCE OF STREPTOCOCCI	SOLUTION OF POLYSACCHARIDE FROM 50 BILLION STREPTOCOCCI PER ml. OF FOLLOWING POOLS OF STREPTOCOCCI			PRECIPITATION (0-4) AT THE INTERFACE BETWEEN SOLUTIONS OF POLYSACCHARIDE AND THE SERUM OF					
				HORSES IMMUNIZED WITH STREPTOCOCCI ISOLATED IN STUDIES OF			PERSONS SUFFERING FROM		
	Pools*	Strains	Dilution	Epilepsy	Schizophrenia	Arthritis	Epilepsy	Schizophrenia	Arthritis
EPILEPSY	844	46	1–10	**3**	2	2	**2**	0	0
			1–100	**2**	1	1	**1**	0	0
	1075	16	1–10	**3**	1	2	**1**	0	0
			1–100	**2**	0	1	**1**	0	0
	144	12	1–10	**4**	1	2	**2**	0	0
			1–100	**2**	0	1	**2**	0	0
SCHIZOPHRENIA	845	28	1–10	2	**2**	1	0	**1**	0
			1–100	0	**1**	0	0	**1**	0
	896	11	1–10	2	**2**	1	0	**2**	0
			1–100	0	**1**	0	0	**0**	0
	146	12	1–10	1	**3**	1	1	**1**	0
			1–100	0	**2**	1	0	**1**	0
ARTHRITIS	846	31	1–10	2	1	**4**	0	1	**0**
			1–100	1	0	**2**	0	0	**1**
	862	1	1–10	2	1	**3**	0	0	**0**
			1–100	1	0	**2**	0	0	**1**
	5592	12	1–10	2	2	**4**	0	0	**1**
			1–100	1	0	**2**	0	0	**1**
CONTROL NaCl SOLUTION			1–10	0	0	0	0	0	0

*Figures indicate laboratory number of respective pools.

important. The overlying gas in sealed blood agar slant cultures became rich in carbon dioxide. When carbon dioxide was displaced with atmospheric oxygen on breaking the seal while making subcultures, death of the surviving organisms usually occurred promptly.

The data obtained in this study and those obtained in the experiments on animals with the freshly isolated strains and those of cutaneous tests made with natural and thermal streptococcal antibodies and antigen (to be reported elsewhere) indicate that (1) persons suffering from epilepsy and schizophrenia, despite the usual absence or slight symptoms of an active infection, harbor in their nasopharynx, apexes of pulpless teeth, and sometimes in their blood specific types of alpha streptococci which are not harmless or casual invaders but which are specifically antigenic; (2) the streptococci and the "neurotoxins" which they produce have predilection for certain structures in the brain, and (3) they may play an important role in the pathogenesis of epilepsy and schizophrenia. Studies on the nature of the respective neurotoxins produced by the streptococci and the use of streptococcal vaccines and thermal antibodies in prevention and treatment are in progress at the present time.

TABLE 13

PRECIPITATION REACTION BETWEEN SOLUTIONS OF THERMAL ANTIBODY AND POLYSACCHARIDE PREPARED RESPECTIVELY FROM STREPTOCOCCI ISOLATED IN STUDIES OF EPILEPSY, SCHIZOPHRENIA AND ARTHRITIS.

Filtrates of solutions of polysaccharide prepared from streptococci (50 billion per ml.) isolated in studies of	Streptococcal pools*	Streptococcal strains	Dilutions of polysaccharide	Precipitation at interface between polysaccharide of streptococci and filtrates of thermal antibody prepared from streptococci isolated in studies of epilepsy, schizophrenia and arthritis by autoclaving the respective NaCl solution suspensions for ninety-six hours (20 billion per ml.)			one hour + 1.5% H_2O_2 (10 billion per ml.)		
				Epilepsy	Schizophrenia	Arthritis	Epilepsy	Schizophrenia	Arthritis
Epilepsy	844	46	1–0	**1**	0	0	**2**	1	0
			1–10	**1**	2	1	**2**	1	0
			1–100	**2**	1	1	**3**	1	0
	1075	16	1–0	**1**	1	0	**3**	2	0
			1–10	**2**	1	1	**2**	1	0
			1–100	**1**	0	0	**1**	0	0
	144	12	1–0	**2**	1	0	**3**	2	0
			1–10	**2**	1	1	**2**	1	1
			1–100	**1**	0	0	**1**	0	0
Schizophrenia	845	28	1–0	1	**2**	0	3	**3**	1
			1–10	1	**3**	1	1	**2**	0
			1–100	0	**2**	0	1	**2**	0
	896	11	1–0	1	**2**	0	3	**4**	1
			1–10	2	**3**	1	1	**2**	0
			1–100	1	**2**	0	1	**2**	0
	146	12	1–0	1	**3**	0	3	**4**	1
			1–10	1	**2**	1	1	**2**	1
			1–100	0	**1**	0	1	**2**	0
Arthritis	864	31	1–0	1	1	**2**	2	2	**2**
			1–10	1	2	**2**	0	0	**0**
			1–100	1	1	**2**	0	0	**0**
	862	1	1–0	1	2	**3**	3	3	**2**
			1–10	1	1	**1**	0	0	**1**
			1–100	0	0	**1**	0	0	**1**
	5592	12	1–0	1	1	**1**	3	3	**1**
			1–10	1	1	**2**	1	1	**1**
			1–100	0	0	**1**	0	0	**1**

*Figures indicate laboratory numbers of respective pools.

TABLE 14

PRECIPITATION AT THE INTERFACE BETWEEN NaCl SOLUTION WASHINGS OF NASOPHARYNGEAL SWABBINGS OF PERSONS SUFFERING FROM EPILEPSY, SCHIZOPHRENIA AND ARTHRITIS AND THE SERUMS OF RESPECTIVE PATIENTS AND THE SERUMS OF HORSES AND RABBITS THAT HAD BEEN IMMUNIZED WITH THE RESPECTIVE STREPTOCOCCI.

SOURCE OF ANTIGEN: NASOPHARYNGEAL SWABBINGS	PERCENTAGE OF PRECIPITATION AT INTERFACE BETWEEN CLEARED NaCl SOLUTION WASHINGS OF NASOPHARYNGEAL SWABBINGS AND THE SERUM OF											
	HORSES IMMUNIZED WITH STREPTOCOCCI ISOLATED IN STUDIES OF				RABBITS IMMUNIZED WITH STREPTOCOCCI ISOLATED IN STUDIES OF				PERSONS SUFFERING FROM			
	Cases	Epilepsy	Schizophrenia	Arthritis	Cases	Epilepsy	Schizophrenia	Arthritis	Cases	Epilepsy	Schizophrenia	Arthritis
EPILEPSY	20	**65**	35	10	20	**85**	25	0	20	**70**	0	0
	21	**67**	38	14	.....	.....	.....	.....	.....	.....	.....	.....
	.....	.....	.....	.....	10	**80**	70	10	10	**40**	0	10
	17	**65**	29	0	.....	.....	.....	.....	17	**59**	.....	.....
	10	**70**	0	0	10	**50**	50	10	10	**70**	20	0
	68	**68**	32	7	40	**75**	38	5	57	**61**	4	2
SCHIZOPHRENIA	26	0	**58**	4	26	23	**69**	4	26	8	**62**	.....
	46	7	**72**	0	.....	.....	.....	.....	.....	.....	.....	.....
	10	0	**70**	0	.....	.....	.....	.....	10	0	**80**	.....
	20	10	**70**	5	.....	.....	.....	.....	20	10	**60**	.....
	102	5	**68**	2	26	23	**69**	4	56	7	**64**	2
ARTHRITIS	27	0	7	**41**	.....	.....	.....	.....	.....	.....	.....	.....
WELL CONTROLS	14	7	0	0	14	7	0	0	14	0	7	0
	34	3	0	0	.....	.....	.....	.....	.....	.....	.....	.....
	17	0	6	6	.....	.....	.....	.....	17	0	0	0
	20	0	0	0	20	15	10	5	20	10	0	0
	85	2	1	1	34	12	6	3	51	4	2	0

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