A DIAGNOSTIC CUTANEOUS REACTION IN ACUTE POLIOMYELITIS

E. C. Rosenow, M. D., Division of Experimental Bacteriology, Mayo Foundation: In studies on the effect of intradermal injection of the whole or euglobulin fraction of the serum of horses hyperimmunized with streptococci, it was found that an immediate (five to ten minutes) erythematous and edematous reaction occurred at the site of the injection among persons who had diseases caused by, or associated with, streptococci that were similar antigenically to the streptococci used in the production of the reacting antiserum[1-4]. This specific streptococcal antibody antigen reaction is similar to the antibody antigen reaction reported by Foshay in cases of tularemia[5,6] and by Tamura in cases of undulant fever[7] after injection of the respective antiserums for these diseases.

1. Heilman, F. R. and Rosenow, E. C.: Newer methods of study and treatment of chronic streptococcal disease. Proc. Staff Meet., Mayo Clin. 12: 252-256 (Apr. 21) 1937.
2. Rosenow, E. C.: Precipitin and cutaneous streptococcal antibody-antigen reactions in poliomyelitis. Proc. Staff Meet., Mayo Clin. 12: 531-535 (Aug. 25) 1937.
3. Rosenow, E. C.: Experimental and clinical studies on the relation of streptococci to various diseases. Illinois M. J. 75: 28-38 (Jan.) 1939.
4. Rosenow, E. C.: The early diagnosis and treatment of poliomyelitis with poliomyelitis antistreptococcic serum. Illinois M. J. 76: 144-149 (Aug.) 1939.
5. Foshay, Lee: Intradermal antiserum tests; a bacterial-specific response not dependent upon serum sensitization but often confused with it. J. Allergy 6: 360-364 (May) 1935.
6. Foshay, Lee: The nature of the bacterial-specific intradermal antiserum reaction. J. Infect. Dis. 59: 330-339, 1936.
7. Tamura, J. T.: Rapid presumptive diagnosis of lymphogranuloma inguinale; a specific intradermal test with antilymphogranuloma inguinale goat serum. J. Lab. & Clin. Med. 21: 842-844 (May) 1936.

The findings on examination of cerebrospinal fluid, while of great value in the diagnosis of poliomyelitis, are not always sufficiently characteristic to distinguish poliomyelitis from certain forms of encephalitis and tuberculous meningitis. Moreover, changes from the normal in cerebrospinal fluid do not occur until after penetration of the virus or streptococci into the central nervous system. The need, was, therefore, great for a means of determining the type of infection at the onset of suggestive symptoms, before penetration by the virus or streptococcus of the nerve centers in the spinal cord. Thus it should be possible by therapeutic injections of a curative serum at the onset of symptoms to prevent paralysis and death. Fortunately my studies yielded a simple method which makes practical this possibility.

Through the co-operation of physicians, superintendents of hospitals and health departments I have had the opportunity to apply this test to patients having sporadic or epidemic poliomyelitis in twelve widely separated cities in nineteen epidemics or seasonal outbreaks. The results in this report were obtained from 1938 to 1941. Nearly all of the patients were in hospitals and the diagnosis of poliomyelitis was established by examination of cerebrospinal fluid and other tests by the physicians in charge. The distribution of cases according to age and sex and type of disease was typical. Well children and adults and persons who had acute or chronic diseases other than those of the nervous system, at nurseries, dispensaries, orphanages, hospitals, nurses' homes, schools and in family groups, within and outside of epidemic zones of poliomyelitis, were tested as controls.*

The horses whose serums were used were immunized with the respective freshly isolated streptococci, antigenic specificity of which was preserved in dense suspensions in glycerol (2 parts) and saturated solution of sodium chloride (1 part).[8] Different batches of the poliomyelitis euglobulin were used with comparable results.

THE INTRADERMAL TEST

The test consists of the intradermal injection of 0.03 c.c. of a 10 per cent solution of the euglobulin fraction of the serum of horses that had been immunized to the streptococcus of poliomyelitis and as controls the euglobulin fraction of the serum of horses immunized with streptococci from diseases other than poliomyelitis, and normal horse serum diluted 1:10. A blanched, sharply demarcated, white

*I wish to express my gratitude to the many physicians and nurses and others in charge who so generously co-operated, and to Eli Lilly and Company for immunizing horses with suspensions of streptococci which I furnished and for refining and making available for study the antiserum used in treatment.

8. Rosenow, E. C.: Serologic specificity of streptococci having elective localizing power as isolated in various diseases of man. J. Infect. Dis. 45: 331-359, 1929.

bleb about 5 mm. in diameter indicates that the correct amount has been injected into the proper layer of the skin. A diffusely raised region indicates that the injection has been made too deeply and that the injection should be repeated.

A positive reaction consists of erythema and edema which begin almost immediately after injection and reach a maximum in five to ten minutes; they then begin to disappear at the periphery and usually disappear entirely in from thirty minutes to one hour. The reaction occurs without itching and is almost never associated with the formation of pseudopodia. The erythema usually is pronounced and diffuse during the early stages of poliomyelitis and the edema which develops more slowly is almost always proportional to the erythema and in the Negro race is often the only sign of a positive reaction. Reactions of persons retested at different times of the day or several days apart vary only slightly. The reaction must be distinguished from a transient blush which sometimes occurs immediately after test and control injections in the case of persons who have low vasomotor tone and also from the reaction caused by sensitiveness to horse serum. The former disappears within two to three minutes. The latter almost never develops within fifteen minutes, almost always is accompanied by itching and by formation of pseudopodia and lasts for hours. After some experience, the early five to ten minute diagnostic reaction nearly always can be distinguished from the later reaction attributable to sensitiveness to horse serum.

The reaction is considered negative when little or no erythema and no edema appear. The maximal area of erythema at the site of injection of the different euglobulins was outlined with pen and ink (figs. 1 and 2) and was reproduced on tissue paper; from the average diameter the area in square centimeters was calculated. About two-thirds of the injections and tracings of erythema were made by me; the other third, by various assisting physicians. Two groups of patients were tested as unknowns. Two or more observers agreed as to the size of the reactions.

The euglobulin, in the amounts injected, did not sensitize persons to horse serum. As a result of local sensitization slight late (three to seven days) reactions of equal intensity were common at the site of injection of the different euglobulins and of normal horse serum but these were mild and transient. Only two out of more than 5,000 persons tested in this and other studies had constitutional reactions and these two persons proved exceedingly sensitive to horse serum.

RESULTS OF THE CUTANEOUS TEST

The average area of erythema and the percentage incidence of reactions of 5 sq. cm. or greater after intradermal injections of the

poliomyelitis and control euglobulins into persons in different stages of poliomyelitis, into contacts and into large numbers of controls, are summarized in table 1. The average reaction and incidence of reactions 5 sq. cm. or more were greatest in the early stages of acute and abortive poliomyelitis, and far greater than reactions following control injections. Interestingly, the cutaneous reaction to the euglobulin of encephalitis was significantly greater among persons who had the bulbar type of poliomyelitis than those who had the spinal type.

The cutaneous reaction during the first two weeks after onset

Table 1

A CUTANEOUS REACTION DIAGNOSTIC OF EPIDEMIC POLIOMYELITIS: RESULTS FROM 1938 TO 1941

Groups of persons tested	Cutaneous reactions* to the euglobulin of antiserums prepared with streptococci from: Poliomyelitis: Persons tested	Poliomyelitis: Per cent, 5 sq. cm. or more	Influenza: Persons tested	Influenza: Per cent, 5 sq. cm. or more	Encephalitis	Ulcerative colitis	Arthritis
Poliomyelitis							
Acute, 1 to 14 days	324† / 9.5	[92]	53† / 3.2	11	202† / 3.4	53 / 2.0	139 / 2.7
Acute (spinal type)	17 / 8.8	[100]			17 / 3.0		
Acute (bulbar type)	11 / 8.2	[82]			11 / 7.0		
Abortive, 1 to 14 days	46 / 7.8	[85]			28 / 4.0		
Convalescent, 15 to 42 days	83 / 7.4	[42]			75 / 2.3		61 / 2.0
Recovered	17 / 2.6	[18]					17 / 2.8
Contacts	283 / 6.8	[54]	81 / 1.6	9	90 / 1.8	27 / 1.5	283 / 1.5
Noncontacts, well persons and those sick with other diseases within epidemic zone of poliomyelitis	408 / 5.2	[51]	209 / 2.1	6	110 / 1.3	12 / 1.8	86 / 3.7
Remote from epidemic zone of poliomyelitis and influenza	429 / 1.8	13	191 / 2.8	13	124 / 1.0	94 / 1.3	87 / 2.0
Epidemic colds and influenza	46 / 1.8	9	62 / 9.2	[79]	37 / 2.2	13 / 2.0	45 / 2.3

*Reactions to the control normal horse serum averaged less than 1 sq. cm. and in nonsensitive persons were never 5 sq. cm.

†The figures above the line in each instance indicate the number of persons tested; the figures below the line indicate the average reaction in square centimeters.

of the disease was about the same, whether the patient had no, slight or severe paralysis, whereas later the incidence and degree of reaction were greater among persons severely paralyzed, especially those who had little, if any, restoration of muscle function. Positive reactions in such cases often were obtained even six weeks after onset, whereas in mild or severe attacks in which improvement was satisfactory, reactions became negative in the second or third week.

The presence of enlarged tonsils, containing much infected material, in cases in which convalescence was not satisfactory, was found almost constantly throughout these studies. Abscesses, containing large numbers of pleomorphic streptococci, were found in

tonsils after death in all of seventeen cases in which tonsils were studied and in surgically removed tonsils from each of eleven patients who were not making satisfactory recoveries.[9]

The incidence and degree of reaction were highest among persons ill with poliomyelitis, next highest among contacts, less high among well persons and persons ill with diseases other than poliomyelitis residing within epidemic zones of poliomyelitis, and least among well and ill persons remote from epidemics of poliomyelitis and persons remote from epidemics who had recovered from poliomyelitis. During epidemic waves of poliomyelitis persons who had previously had poliomyelitis have been found to become carriers of the poliomyelitic type of streptococcus and to give a heightened incidence of cutaneous reactions similar to that of persons who have not had poliomyelitis.

In agreement with the relatively high incidence of reactors among well contacts and noncontacts in epidemic zones of poliomyelitis, it should be stated that six days after slight exposure to one sporadic case of poliomyelitis, I reacted positively to intradermal injection of the poliomyelitis euglobulin. The extent of my exposure consisted of swabbing the patient's nasopharynx, making the skin test and exposing culture media to the air in the room while I was wearing a face mask and sterile gown. Nine of ten nurses coming in contact with this patient also reacted positively to the cutaneous test, whereas all of nine control persons not exposed reacted negatively. Similar but greater exposures in five additional instances, in four of which I obtained negative cutaneous reactions at the time of exposure, were followed in from four to seven days by a specifically positive cutaneous reaction to the euglobulin of poliomyelitis.

Four patients who had facial paralysis, three patients whose condition was considered clinically as lymphocytic choriomeningitis and one patient who had residual paralysis from a previous attack of poliomyelitis and who was in a second attack reacted maximally to the euglobulin of poliomyelitis. The fact that well contacts and noncontacts often react, especially during the latter part of epidemics, has led to misinterpretation, in a few instances, by some who have used the test. Fifteen physicians who have used the test independently, however, have obtained results similar to the ones I am reporting. Some of these also have been reported.[10]

9. Rosenow, E. C. and Wheeler, G. W.: The etiology of epidemic poliomyelitis. J. Infect. Dis. 22: 281-312, 1918.

10. Kennon, C. L. and Mayer, R. A.: The epidemic of poliomyelitis in Miami and vicinity, Dade County, Florida, from September, 1940 to September, 1941. Jackson Memorial Hosp. Bull. 4: 49-57 (Jan.) 1942.

In September, 1940, during an epidemic of poliomyelitis at an orphanage the poliomyelitis euglobulin gave a high incidence and degree of reactions among well and ill persons whereas in April, 1941, during an epidemic of respiratory infection the influenza euglobulin gave a high incidence and degree of reactions among well and ill persons. The incidence and degree of reactions to each euglobulin was directly proportional to illness in the epidemic at the time, to the degree of contact and to the age and sex incidence or susceptibility. Therefore, the cutaneous reactions to the euglobulins of poliomyelitis and influenza appear to have seasonal and epidemiologic significance (table 2).

Table 2

CUTANEOUS REACTION TO THE POLIOMYELITIS AND INFLUENZA EUGLOBULINS IN RELATION TO EPIDEMIC POLIOMYELITIS AND INFLUENZA IN AN ORPHANAGE

Time of study and groups	Cutaneous reaction to the euglobulin of antiserums prepared with streptococci from: Poliomyelitis			Influenza		
		Reactions			Reactions	
	Persons tested	Average, sq.cm.	%, 5 sq.cm. or more	Persons tested	Average, sq.cm.	%, 5 sq.cm. or more
During epidemic of poliomyelitis of September, 1940						
Persons (4 boys, 1 girl) ill: symptoms resembling abortive poliomyelitis	5	10.5	80			
Contacts (boys) from same cottage	27	5.92	74			
Well persons						
2 to 10 yrs. of age	103	4.8	66	11	1.3	0
11 yrs. of age or more	87	3.9	45	15	2.3	0
Boys	102	4.5	59			
Girls	88	4.1	49			
Total	190	4.33	56	26	1.7	0
During epidemic of respiratory infections, April, 1941						
Persons ill with fever and sore throat				18	9.2	89
Well persons	29	2.9	24	155	5.6	66

Heating the euglobulin solution as used at 63° C. for thirty minutes did not destroy the reacting substance, whereas boiling for fifteen minutes or autoclaving for ten minutes destroyed it completely. The size of the reactions, on injection of the euglobulin after one filtration through Seitz or Berkefeld "N" filters, was reduced about a fourth. The reacting substance was absorbed specifically by the antigen used in its preparation (streptococci from poliomyelitis) and only to a slight degree by streptococci obtained from cases of arthritis, and completely by finely ground infusorial earth and by blood charcoal.

The euglobulin fraction is highly stable in the whole antiserum, if kept at about 10° C. in the refrigerator. Specific but often not as marked reactions occurred following cutaneous injection of the euglobulin solution freshly obtained from antiserums prepared from two to fourteen years previously in seven horses with streptococci isolated

during different epidemics of poliomyelitis, with streptococci from different strains of the virus of poliomyelitis and with mixtures of these. No apparent diminution occurred in the active principle in the 10 per cent solutions of euglobulin when kept at 10° C. for two years.

The reacting substance was found chiefly in the euglobulin (bacterial antibody) fraction. By injecting mixtures of the euglobulin and pseudoglobulin (antitoxic antibody) fractions, it was found that the pseudoglobulin reduced or prevented the reaction due to euglobulin (fig. 1). In agreement with this observation, it was discovered

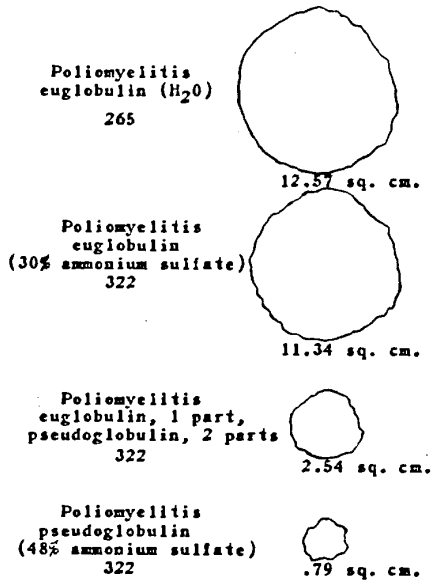

Fig. 1. Tracing of the cutaneous reactions to the euglobulin of poliomyelitis in a case of acute poliomyelitis, no reaction to the pseudoglobulin and interference with the reaction to the euglobulin by the pseudoglobulin fraction when a mixture of both was injected (x 2/5).

that when the refined antistreptococcic serum, consisting of a solution of the pseudoglobulin obtained by precipitation with 48 per cent ammonium sulfate (two parts) and the euglobulin obtained by precipitation with 30 per cent ammonium sulfate (one part) in a concentration approximating that in the whole serum, was injected intramuscularly in therapeutic amounts (5 c.c. for children one year of age or less and 1 c.c. more for each additional year of age up to twenty years, once or twice daily), a prompt and marked reduction in the cutaneous reaction occurred on reinjection of the euglobulin (fig. 2). Similar experimental and therapeutic results have been

obtained after therapeutic injections of only the pseudoglobulin fraction. Hence, this test appears to be a measure of specific antigen in the skin or blood, a Francis test in reverse.[11]

One or two therapeutic injections of the poliomyelitis antistreptococcic serum at the onset of the disease, especially in the prepoliomyelitic stage, sufficed to bring the temperature and reactivity of the skin to normal or nearly so and resulted in recovery without paralysis. In well established cases also, the therapeutic injection of the antiserum brought the temperature down and caused the cutaneous reactivity to disappear, but the latter tended to return in twenty-four

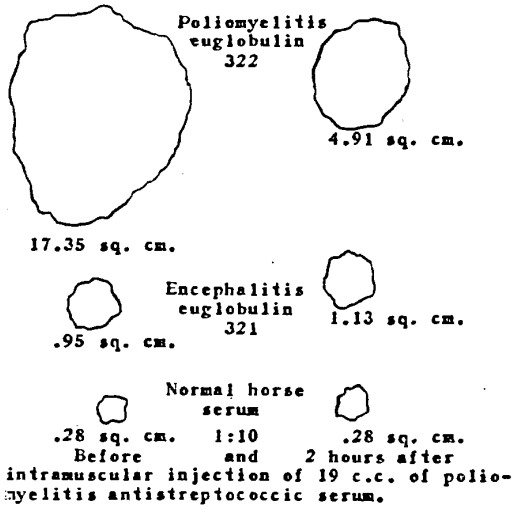

Fig. 2. Tracing of cutaneous reactions to the euglobulin of poliomyelitis and marked reduction in reaction of skin to reinjection of the euglobulin following intramuscular injection in therapeutic amounts of the poliomyelitis antistreptococcic serum in a case of acute poliomyelitis, sixth day (x 2/5).

to thirty-six hours due to absorption of more streptococcal antigen or toxin from the nasopharynx, tonsils, stool or elsewhere. By repeating the therapeutic injections twice daily for three, four or five days the cutaneous reaction was reduced more than it was in three, four or five weeks among patients who did not receive serum treatment. Similar cutaneous and serum reactions[12] and favorable clinical responses followed therapeutic injection of the antiserum[13] into Macacus

11. Francis, Thomas, Jr.: The value of the skin test with type-specific capsular polysaccharide in the serum treatment of type I pneumococcus pneumonia. J. Exper. Med. 57: 617-631 (Apr. 1) 1933.
12. Rosenow, E. C.: Specific streptococcal antibody-antigen reactions of the skin and serum of monkeys during attacks of experimental poliomyelitis. Proc. Staff Meet., Mayo Clin. 15: 382-384 (June 12) 1940.
13. Rosenow, E. C.: Protection of monkeys (Maracus rhesus) against experimental poliomyelitis with vaccine and antiserum prepared with the streptococcus from poliomyelitis: preliminary report. Proc. Staff Meet., Mayo Clin. 13: 328-330 (May 25) 1938.

rhesus monkeys during attacks of experimental poliomyelitis induced with filtered and unfiltered virus.

In addition to these definite effects of the antiserum, it was found in other published[4,13] and as yet unpublished experiments that the poliomyelitis antistreptococcic serum neutralized the virus of poliomyelitis and protected monkeys against intranasal inoculation of highly virulent virus.

Therapeutic injections of the poliomyelitis antistreptococcic serum should not be given to well persons who react to the euglobulin

Table 8

PRECIPITATION REACTION WITH SERUMS AND SALINE EXTRACTS OF NASOPHARYNGEAL SWABBINGS AND POLIOMYELITIS ANTISTREPTOCOCCIC SERUM

Material used as antigen	Cases	% of positive reactions* with antiserums prepared with streptococci from:			
		Poliomyelitis	Encephalitis	Influenza	Ulcerative colitis
Cases of poliomyelitis					
Saline extracts of nasopharyngeal swabbings: 1 to 21 days after onset	183	89	39	29	16
Serum: 1 to 21 days after onset	122	69	23	6	10
22 to 42 days after onset	22	5	5	0	0
Contacts					
Saline extracts of nasopharyngeal swabbings	30	70	21	29	18
Serum	10	30†	0	0	0
Controls within epidemic zones					
Saline extracts of nasopharyngeal swabbings	91	53	16	18	24
Controls outside epidemic zones					
Saline extracts of nasopharyngeal swabbings	80	16	3	16	9
Serum	52	32†	4	0	0

*No reaction with normal horse serum.
†Serums from samples of blood drawn from persons who, at the time, gave a positive cutaneous reaction to the poliomyelitis euglobulin.

but only to persons at the onset of symptoms resembling those of poliomyelitis, to persons throughout the active stage of the disease and during convalescence, even long after the temperature has become normal provided the cutaneous test gives a positive result.

SIGNIFICANCE OF THE CUTANEOUS REACTION

In order to determine the significance and nature of the cutaneous reaction, I obtained nasopharyngeal swabbings for cultures and precipitation tests. Animals were inoculated and agglutination tests were made with the streptococci isolated. Ether anesthesia was employed for inoculations into animals. Samples of blood were obtained from patients in various stages of poliomyelitis, from contacts and controls who reacted positively to cutaneous tests and from controls who reacted negatively to cutaneous tests. The serums were used as antigen in precipitation tests with the poliomyelitis and other

antistreptococcic serums. The methods employed in these experiments were similar to those used previously.[1,14,15]

Results of cultures and of inoculation of animals.—Of fifty-six rabbits that were inoculated intracerebrally with highly diluted dextrose-brain broth cultures of the streptococci from the nasopharynges of twenty-four persons who reacted strongly to the cutaneous test and who had acute poliomyelitis, flaccid paralysis was the outstanding manifestation in thirty-eight rabbits (67.8 per cent). Of fourteen rabbits that received streptococci derived from the nasopharynges of eleven well contacts who resided in epidemic zones and who reacted strongly to the cutaneous test, nine (64.3 per cent) became paralyzed. In sharp contrast, none of the twelve rabbits receiving

Table 4

AGGLUTINATION OF STREPTOCOCCI FROM THE NASOPHARYNX IN RELATION TO THE CUTANEOUS REACTION IN POLIOMYELITIS

Streptococci isolated from persons who had:	Strains or cases	Pools or single strains tested	Specific agglutination by serum: Of horses immunized with streptococci from poliomyelitis		Of human beings convalescing from poliomyelitis (15 to 21 days)	
			Number	Per cent	Number	Per cent
Positive cutaneous reactions						
Cases of poliomyelitis						
Streptococci as isolated	63	12	10	83	9	75
Streptococci after animal passage	45	7	6	86	4	59
Contacts	12	12	11	92	8	67
Controls outside of epidemic zones	21	9	7	77	7	77
Negative cutaneous reactions						
Controls outside of epidemic zones	24	7	0	0	0	0

streptococci obtained from the nasopharynges of eleven well persons who were remote from the epidemic zone of poliomyelitis and who reacted negatively to the cutaneous test, became paralyzed.

Results of precipitation tests.—The results of precipitation tests are summarized in table 3. The incidence of specific reactions was highest when swabbings from the nasopharynges of patients having acute poliomyelitis (89 per cent) were used as the antigen; next highest when those of contacts (70 per cent) and controls within the epidemic zones (53 per cent) were used and lowest when those of controls outside of epidemic zones (16 per cent) were used.

The control antiserums and the poliomyelitis antistreptococcic serum after absorption of antibody with the poliomyelitis streptococcus gave only a low incidence of precipitation regardless of the source of the antigen.

14. Rosenow, E. C.: Further studies of the poliomyelitis precipitin reaction. J. Infect. Dis. 38: 532-540, 1926.
15. Rosenow, E. C.: Recurring encephalomeningoradiculitis with fibromyositis following poliomyelitis. A bacteriologic study of sixty-four cases. Arch. Int. Med. 64: 1197-1221 (Dec.) 1939.

Sixty-five to 100 per cent of persons in the different groups whose nasopharyngeal swabbings or serums gave positive precipitation reactions with the antiserum of poliomyelitis gave a positive cutaneous reaction to the poliomyelitis euglobulin, whereas only 11 to 44 per cent of persons whose precipitation tests gave negative results were found to have positive cutaneous reactions.

The incidence of specific agglutination of the streptococcus in relation to the cutaneous reaction is shown in table 4. In agreement with the results of animal experiments, precipitation and cutaneous tests, there was a high incidence of specific agglutination of the streptococcus of poliomyelitis, both by the poliomyelitis antistreptococcic serum (77 to 92 per cent) and by convalescent serum (57 to 77 per cent), and no specific agglutination of any of the strains of streptococci isolated from control persons remote from poliomyelitis who did not react to the cutaneous test. In accord with the fact that serums of adults more often neutralized the virus of poliomyelitis than the serums of children who had not had poliomyelitis, it was found that agglutination of the streptococcus of poliomyelitis was greater and occurred in higher dilution by the serums from adults than by the serums from children. This was so definite as to suggest that the parallelism of higher agglutinating and antiviral titers of serums of adults than the serums of children may be due to a common cause, such as unapparent immunizing infection by the streptococcus or virus of poliomyelitis.

SUMMARY AND CONCLUSIONS

A cutaneous test has been developed that is diagnostic of poliomyelitis and which also serves as a control for the amount and number of therapeutic injections of the poliomyelitis antistreptococcic serum necessary for best results. The cutaneous reaction appears to be a measure of specific streptococcal antigen in the skin and blood serum and of clinical or subclinical immunizing infection by the poliomyelitic type of streptococcus.

During attacks of poliomyelitis the reactivity of the skin and blood serum of persons and monkeys to the poliomyelitic streptococcal antibody disappeared gradually as recovery ensued in untreated cases, and abruptly—often associated with corresponding improvement—following therapeutic injection of the poliomyelitis antistreptococcic serum.

It is concluded that the antigen or toxin is neutralized by the antistreptococcic serum and that the streptococcus is not a passive invader but a part of the infectious process in poliomyelitis now generally attributed to virus.