THE NEUTRALIZATION TEST IN POLIOMYELITIS

COMPARATIVE RESULTS WITH FOUR STRAINS OF THE VIRUS*

BY JOHN R. PAUL, M.D., AND JAMES D. TRASK, M.D.

(From the Departments of Medicine and Pediatrics, Yale University School of Medicine, New Haven)

(Received for publication, December 26, 1934)

During the last two decades various methods of determining the power of human sera to neutralize poliomyelitis virus have been employed, and from them various clinical and epidemiologic conclusions have been drawn. Few will deny that these studies have added to knowledge with regard to poliomyelitis but at the same time there is a growing belief that from some of them, perhaps, attempts have been made to obtain more clinical and epidemiologic information than the methods afford. For instance, although it is open to doubt whether the methods used to make these determinations in clinical poliomyelitis are very accurate, one fact is becoming increasingly apparent; namely, that in neutralization tests with poliomyelitis virus, different results may be obtained with some human sera when tested with different strains of virus. Thus certain convalescent sera tested with a strain which has been recently isolated from a human case will act differently than when tested with a strain which has been subjected to a long series of monkey passages. It is our present purpose to report similar comparative tests on sera obtained from normal human adults who did not give a history of having sustained a recognizable attack of poliomyelitis, and to discuss them in the light of previous results with sera from convalescent and normal persons representing younger age groups. The purpose of such a discussion mainly concerns the specificity of the antigen used in such neutralization tests on human sera. In experimental work in this disease anti-

* The expenses of this investigation have been defrayed by a grant from the National Research Council.

viral properties appear in the blood of the convalescent or immunized monkey, and, more or less coincidently, the animal may be found to be immune to a subsequent intracerebral inoculation of the virus. In human sera (both convalescent and normal) the presence of substance which neutralizes poliomyelitis virus has, by analogy therefore, been taken as a measure of "immunity," although it is recognized, of course, that the extent to which the mere presence of such neutralizing antibodies represents immunity is a matter of some speculation. But the difference between the situations in the monkey and in man is that in the monkey neutralization tests have usually been made with the actual strain which induced the experimental disease, whereas most human sera have been tested with strains of virus which may be quite different from those which induce the human disease. As a result it now seems questionable whether human immunity to poliomyelitis has been very accurately determined by the methods employed in the past and it will be with this fact that this paper will be concerned.

The early methods of performing virus neutralization tests in poliomyelitis, the results, and their interpretations will not be reviewed because the reader may be referred to a recent article by Wells (1) for a summary of these subjects. In the majority of these and subsequent studies it was assumed that the presence in human blood of a given amount of substance which neutralized poliomyelitis virus was a reliable test for immunity in this disease. On this basis comparisons have been made between tests performed on sera from patients who had sustained frank attacks of poliomyelitis, from contacts, and from individuals who were not known to have been contacts or to have sustained a recognized attack of the disease. Interpretations have also been drawn from comparisons made between tests on the sera of normal individuals from this country as opposed to those from regions where clinical poliomyelitis is thought to be rare, such as: Porto Rico (2), Liberia (3), Greenland (4), and certain parts of China (4, 5). Similar interpretations have been drawn from the results of tests on normal individuals from the northern as opposed to the southern part of this country (6); and on normal children and adults from rural districts as opposed to normal urban individuals of the same age groups (7, 8). All of these tests were performed with strains of the virus which had been passed through monkeys many times.

Almost from the beginning of this work it became apparent that the average neutralizing power of sera from normal, adult individuals did not differ much from that of the sera of convalescents, most of those tested also being adults. In com-

paring the two groups, Shaughnessy, Harmon, and Gordon (8) found that antiviral titres of individual samples of normal sera appeared to be more potent in their action against the virus than were those of the sera of convalescents. Brodie (9) found that in 70 per cent of his normal adult sera the neutralizing power was about four-fifths that of pooled convalescent sera, and in 30 per cent it was equal in titre. Subsequently Howitt (10) pointed out that the average power of individual samples of convalescent sera to neutralize poliomyelitis virus was perhaps less than had been generally supposed. This fact becomes even more evident if one compiles from the literature the available series of tests on individual samples of serum in which virus: serum mixtures have been set up in a dilution of 1:1, 1:2, or 1:3, and compares the normal with the convalescent results according to age groups. In fact, with the exception of Aycock's convalescent findings (7) (which are not included because they were based on pooled lots of sera), the average neutralizing power of individual samples of convalescent sera reported in the literature seems to be uniformly less in all age groups than that of normal sera. The many observations on this point will not be transcribed in detail but general trends of the results by different investigators are shown by the graphs in Fig. 1. In this figure we have included only those series of tests which have been reported since 1929. Prior to that date the number of reported tests in different age groups was small and certain variations in technique render a comparison with the recent work somewhat difficult. There are still technical differences which involve some of the results shown in Fig. 1, but only those tests have been included in which virus: undiluted serum mixtures have been set up in a proportion of 1:1, 1:2, or 1:3.

With the results expressed as they are in Fig. 1, and in so far as the data go, it will be seen that individuals from rural districts have less than the normal, average amount of antiviral substance, and that contacts appear to have a little above the average, as do also individuals from regions where poliomyelitis is thought to be rare. There is no evidence that the acquisition of the clinical disease in the remote or recent past, gives rise to an increase in antiviral substance when tested with passage strains, although it is unfortunate that so few tests are reported in the literature which have been done on convalescent sera from children.

We shall review next the question of variation in the virus and the comparisons between neutralization tests performed with different strains. That old passage strains of poliomyelitis virus may exhibit slight immunological differences, one from another, was described in 1929 by Stewart and Rhoads (21). That more easily demonstrable differences existed between a strain recently isolated from a human case, and a strain which had been passed through monkeys many times was observed in 1931 by Burnet and Macnamara (22). The principles of their observation have subsequently been confirmed (23, 24, 20, 25). There is no evidence to show that all such recently isolated strains are alike, and the actual number of series of neutralization tests on human sera in which results with several strains have been compared is few. Nevertheless, evidence has appeared in the literature

to suggest that the situation shown in Fig. 1 would be altered if the tests had been done with so called "human" strains.[1]

For instance, in comparing the neutralizing action of different human sera on their Australian human strain and on a New York passage (M V) strain, Burnet and Macnamara (22) found that although pooled, convalescent sera would neutralize both strains, a few tests with individual samples of convalescent sera failed to show this parallelism, in that only the human strain was neutralized.

Later Howitt (20) made similar, comparative observations upon seven samples of adult, convalescent sera. All of these convalescent sera neutralized her 1931 human strain, whereas less than half of these sera neutralized her passage (M V) strain. Howitt concluded that in judging the neutralizing ability of human sera it would seem preferable to employ a virus that had not undergone too great a modification by repeated transfer through monkeys. It is worth noting that the conclusion was based on work with a human strain which was not derived from the epidemic in which Howitt's patients had sustained their attacks of poliomyelitis.

We (25) have also performed this type of experiment upon sera obtained from children in which tests with our human (W—7th passage) strain were compared to those of a passage (M) strain. The children tested were either normal, contacts, or in early or late convalescent stages of an abortive or frank attack of poliomyelitis. Their exposure to, or acquisition of the disease occurred in the same epidemic from which the human strain was isolated. From the results, and in spite of the present difficulties inherent in the technique, it has seemed that those tests performed with the human strain gave a better concept of antiviral properties induced by the human disease than did similar tests performed with the passage strain.

It has been our object in the present study to amplify the previously reported results by performing a comparative series of tests on samples of sera from normal adults using two human and two passage

[1] Hereafter the term human strain will be used in this article to designate a strain recently isolated from a case of human poliomyelitis. The terms human and passage in referring to strains of poliomyelitis virus have not been defined. They are employed in this article because of priority of usage (23, 24). Criticism might be raised against our use of the term human, in referring later to seventh and tenth passage strains. As it is almost impossible at present to perform neutralization tests with strains in their first few passages because of their low virulence for the monkey, the term human strain will be defined in this article as referring to a strain which has not been passed many times beyond the point at which it is suitable for use in neutralization tests. Passage strains represent those strains passed many times beyond this point. Although both our human strains (W and F) came from the same epidemic, the W strain should perhaps be qualified by the terms,—1931, New Haven (seventh passage); and the F strain as,—1931, New York (tenth passage).

strains of the virus. The experiments have also been devised to throw some light on the following questions: (*a*) To what extent do human strains act like one another when tested by this method? (*b*) To what extent do passage strains act like one another in this respect? (*c*) What is the power of normal adult sera to neutralize human poliomyelitis virus? (*d*) To what extent do the findings in these human strain experiments alter certain concepts with regard to clinical aspects, and to the epidemiology of poliomyelitis based on passage strain neutralization experiments?

Methods

Strains Employed.—Four strains of poliomyelitis virus were used. They included two human strains, designated as W and F; and two passage strains, designated as Aycock and M.

Human Strains

W Strain, New Haven, 1931.—Our W strain was in its seventh passage. Obtained in 1931 from the throat of a child in the 1st day of an abortive attack of poliomyelitis, its isolation (26) and its first seven monkey passages (25) have been described. Material employed for all neutralization tests came from a single animal (Monkey B-6); a procedure which was followed in previous neutralization tests described with this strain.[2] The supernatant fluid (after centrifugalization) from a 10 per cent suspension of ground cord, when mixed with an equal part of normal monkey serum and injected intracerebrally in 0.5 cc. amounts, has infected nine fresh monkeys thus tested. The experimental disease so produced is clear-cut but usually not fatal. In nine monkeys so infected, the incubation period to the onset of fever averaged about 6 days; the febrile period prior to the onset of paralysis about 4 days; and the mortality in this series of monkeys was about 12 per cent.

F Strain, New York, 1931.—This was obtained through the kindness of Dr. Simon Flexner of The Rockefeller Institute for Medical Research. It had been isolated during the summer of 1931 from the medulla and spinal cord of a fatal case of poliomyelitis and the sample we received represented the eighth passage. A virulence titration at this time showed that 0.5 cc. of a 1 per cent suspension of this material would infect a monkey but the experimental disease so produced was mild, whereas when a similar amount of a 5 or 10 per cent suspension was injected it was severe.

Material from a single animal (No. 1-97) representing the tenth passage was

[2] Monkey B-6 had contracted a severe form of the experimental disease. For passing human strains it is our practice to select those animals which are most extensively paralyzed.

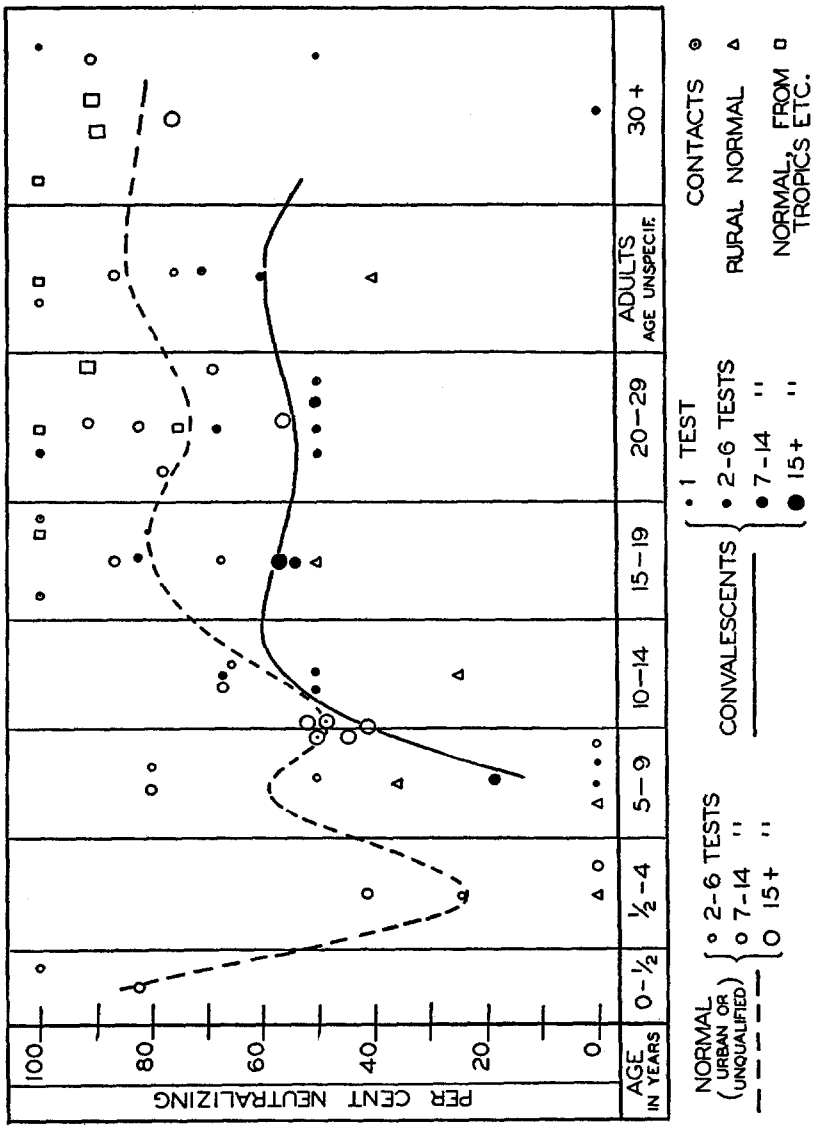

FIG. 1
Figure legend on facing page.

eventually employed for all neutralization tests, using the same dosage as that of the W strain, *i.e.*, a 10 per cent cord suspension mixed with one part of undiluted serum. The F strain was much more virulent for the monkey than our W strain. When mixed with normal monkey serum and inoculated in 0.5 cc. amounts into two monkeys the average incubation period was 3½ days, the febrile period of 2½ days, and the mortality 100 per cent. All of the monkeys which were infected during the course of the neutralization experiments were extensively paralyzed.

Passage Strains

Aycock Strain.—This was obtained through the kindness of Dr. W. L. Aycock of the Department of Preventive Medicine and Hygiene, The Harvard Medical School. It is a highly virulent strain for the monkey. It had been used in several of the investigations (4, 6, 7, 8, 11, 12, 14, 15, 19) referred to in an earlier section of this paper. The number of monkey passages to which it has been sub-

---

FIG. 1. The per cent of normal, convalescent, and exposed individuals whose sera neutralized passage strains of poliomyelitis virus, arranged according to age groups. Data included in this figure have been obtained from reports (2–20). In several of them (see Bibliography) the published data have been supplemented by a personal letter from one of the authors in which the ages of the individuals tested and other details, not appearing in the text, have been kindly supplied.

Included are the results of tests performed on individuals from: (*a*) rural districts in this country (shown as small triangles) and from: (*b*) regions where clinical poliomyelitis is thought to be rare (shown as small squares). Normal represents those individuals from this country, England, and France in whom the environment was not specified as rural. Convalescents represent individuals who in the great majority of instances sustained attacks of poliomyelitis accompanied by residual paralysis. No attempt has been made to separate the convalescent cases into groups depending upon the interval from the time at which they sustained an attack of paralysis and the time when the blood sample was obtained. In those series in which this interval was stated it averaged about 3 or 4 years. Only those individuals have been designated as contacts who had recently (within a few months) been exposed to the disease. The lines which have been drawn through the chart to represent the trends in which positive neutralization tests occurred in normal and convalescent persons, do not include Groups *a* and *b*.

Most of the points on the chart have been derived from groups of from 5 to 10 tests performed by a given investigator. A few (three convalescents) represent the results of single tests. Obviously, the result of a single test should not be charted on a percentage basis. They have only been included to aid in the construction of the graph in those age groups in which data on convalescents are scanty.

jected is unknown. After one passage just prior to its use in our experiments (Nov., 1933), a titration of the material showed that 0.5 cc. of a 0.01 per cent suspension would infect a monkey (a smaller dose was not tried). Material from a single animal (No. 1-74) was used for all neutralization tests. The dosage employed was a 1 per cent cord suspension mixed with one part of undiluted serum. When this dose was mixed with an equal part of normal monkey serum and inoculated intracerebrally in 0.5 cc. amounts into three monkeys the average incubation period was 4 days, the febrile period 1½ days, and all three of the monkeys died, as did all other monkeys which contracted the experimental disease in the course of the neutralization tests with this strain.

M Strain.—This highly virulent strain was obtained through the kindness of Drs. W. H. Park and E. R. Weyer from the Bureau of Laboratories, Department of Health, City of New York. It is a mixed strain and so its origin, or the number of monkey passages to which it had been subjected, is unknown. Since we received it in September, 1931, it has had two further passages. Material for all neutralization tests came from a single animal (No. 1-49) and was prepared as a 0.2 per cent suspension, which was mixed with one part of normal monkey serum and injected intracerebrally in 0.5 cc. amounts. The experimental disease in nine monkeys showed an average incubation period of 5 days, a febrile period of 2 days, and a mortality of 100 per cent.

Technique of Neutralization Tests.—This will not be described in detail because the methods employed have been given in a previous publication (25). It may suffice to say that the four strains were employed in the dosages mentioned above, and were mixed with an equal part of undiluted serum in all of our experiments reported in this paper. This mixture was placed in the incubator for 2 hours and monkeys were inoculated intracerebrally in 0.5 cc. amounts. Daily temperature readings were taken over a period of 4 weeks, on all animals thus inoculated. If any animal died within this period from some cause other than experimental poliomyelitis the test was repeated. In the course of the 126 neutralization tests performed in this series of experiments, three such animals died during the 4 week period of observation; two from dysentery, and one from a brain abscess. Individual monkeys were used one to eight times in the course of these experiments which were carried out over a period of a year.

In all experiments in which the neutralizing power of individual samples of human sera were tested, two control tests were employed; a protected control,—in which pooled convalescent serum was mixed with the virus,— and an unprotected control,—in which normal monkey serum was mixed with the virus. If unexplained results occurred in the controls the experiment was discarded. A result of this type occurred once in fourteen experiments. It was an experiment in which the W strain was used, and the protected control developed the experimental disease after a long incubation period. In another experiment, in which the M strain was used, the unprotected control failed to develop the experimental

disease but this monkey, which was used through an error, was subsequently shown to be resistant to a larger dose of this virus.[3]

The results of these tests have been expressed only in terms of a 1:1 serum: virus mixture. A plus sign signifies that neutralizing properties were present in the serum, in that the animal did not develop the experimental disease within the 4 week period of observation; a minus sign signifies that neutralizing properties were absent, in that the animal developed the experimental disease within a few days of the incubation period (to the onset of fever) shown by the unprotected control; and a plus-minus sign signifies so called partial neutralization, in that the incubation period was prolonged for a period of 6 or more days beyond that exhibited by the unprotected control, the longest incubation period noted in all of the experiments being 28 days. A questionable plus sign indicates that three tests were performed and that plus represents the majority result, but that discrepancies occurred in one of the three tests. Protocols of our experiments appear at the end of the paper (Table II). Here it will be seen that thirteen of the passage strain tests were repeated in satisfactory experiments either one or two times. In sixteen repetitive tests discrepancies were encountered twice (± as opposed to + once, and − as opposed to + once).

By comparing the results of repetitive tests with those previously reported (25) we find that discrepancies have been noted with the W strain in about 11 per cent of eighteen tests, and with passage strains in about 8 per cent of twenty-five tests. These experiences are mentioned to emphasize that in our hands unknown factors of error in the use of this test are not uncommon, and they reflect to some extent the experience of others (7).

One criticism of the technique of our comparative tests is that different dosages were used for the human strains than were used for the passage strain experiments. Owing to differences in virulence for the monkey exhibited by these strains this has proved a variable which we could not overcome. However, in comparing the human and passage strain tests described in this paper with those

---

[3] In order to conserve monkeys in the passage strain experiments we frequently used as the unprotected control a monkey which had recovered from a W strain infection. In a previous communication (25) we found that, provided 10 weeks or more had elapsed between the two inoculations, an antecedent infection by the W strain did not protect against an intracerebral inoculation of the M strain in the dose used for the neutralization tests. Reinfection occurred in the six instances in which the experiment was tried. A smaller number of experiments has also shown that the Aycock strain will also reinfect. Previously infected monkeys of this type were therefore used not only as unprotected controls, but also as checks on some of the passage strain neutralization tests (see protocols in Table II). The results of these check tests were only accepted if the monkey subsequently proved to be susceptible.

which we have previously reported (25) we can find no evidence to show that this variable influences the results in any particular direction.

Sera Employed.—Fourteen samples of normal adult sera obtained from individuals ranging from 21 to 45 years of age, who did not give a history of having had a recognized attack of poliomyelitis, were used in the course of these experiments. These samples were collected in New Haven during the summer of 1931 when poliomyelitis was epidemic in that city.[4] One specimen was obtained from a physician who had been exposed to poliomyelitis in the course of his hospital duties; the others were from individuals who offered their blood for therapeutic purposes to a member of their family ill with the disease. Two samples of convalescent sera obtained from adults who had sustained attacks of poliomyelitis in childhood, were included as controls in this series. Serum or blood from all of these individuals had been used for therapeutic purposes in 1931, and, as some of the patients so treated had apparently done well and others had done badly it was one of our original objects to attempt to correlate the supposed therapeutic efficacy of the serum or blood with the results of the neutralization tests. The outcome of the tests was such that no such correlation could be made.

Although collected during the summer of 1931 and subsequently kept in the ice box, these sera were not tested until 20 to 32 months had elapsed. It is possible that the neutralizing power of some might have deteriorated between the 20th and 32nd month but no evidence of this supposition was apparent in the course of the experiments. The majority of tests with the passage strains were performed between the 28th and 32nd month. Inasmuch as none of the sera failed to neutralize completely the passage strains of virus, which were the last tests performed, there was no evidence that this particular neutralizing property had been lost by deterioration through aging.

RESULTS

The results of our series of neutralization tests made with four different strains of poliomyelitis virus on fourteen samples of normal (or contact) adult sera and two adult convalescent sera appear in Table I. Comparisons between the different strains may be expressed on a percentage basis if we assume that when partial neutralization is observed with one strain, and either no neutralization or complete neutralization with another, a 50 per cent agreement exists; or, that when questionably positive neutralization is observed with one strain

[4] Criticism may be applied to the term normal in referring to these individuals. Some of them were intimate contacts. The difficulty of defining the degree of contact in this group was such, however, that it has seemed more practical to consider them all as normal, particularly as we do not believe that the differentiation is pertinent to the problem under investigation.

and positive with another, a 75 per cent agreement exists. On this basis the agreement between the tests with the two human as opposed to the two passage strains might be roughly placed at 75 per cent. On the same basis there is an 86 per cent agreement between the two human strains, and a 92 per cent agreement between the two passage

TABLE I

Neutralization of Normal Adult Sera by Two Human and Two Passage Strains of Poliomyelitis Virus

Subject	Type of individual	Age	Human strains		Passage strains	
			W	F	Aycock	M
		yrs.				
R. Hn.	Convalescent	25	+	+	+	+
D. Be.	"	27	+	0	+	+
Epn.	Normal	21	+	+	±	+
D. Bls.	"	26	−	−	0	±
Chn.	"	29	+	+	+	+*
J. Dn.	"	30	+	+	+	+? (*a*)
Sdb.	"	30	+	+	+	+
Ptg.	"	30	−	+	+*	+*
Klk.	"	34	−	−	+*	+*
Msn.	"	35	+	+	+*	+*
Cls.	"	38	+	+	+	+*
Wnr.	"	38	+	+	+	+
Brn.	"	40	+	−	+*	+*
Dvl.	"	43	+	+	+	+
Ttr.	"	45	−	−	+	+? (*b*)
Cpn.	"	45	+	+	+	+*

+, neutralization; ±, partial neutralization; −, no neutralization; 0, test not done (insufficient serum).

+? (*a*), three tests performed; one −, two +.

+? (*b*), three tests performed; one ±, two +.

* Test repeated, result confirmed.

strains. The last difference does not appear to be significant if one takes into account the experimental error of the method which we would place at about 10 per cent. It will be seen, however, that in general these normal adult sera showed less power to neutralize human than passage strains. Thus the percentage of positive neutralization

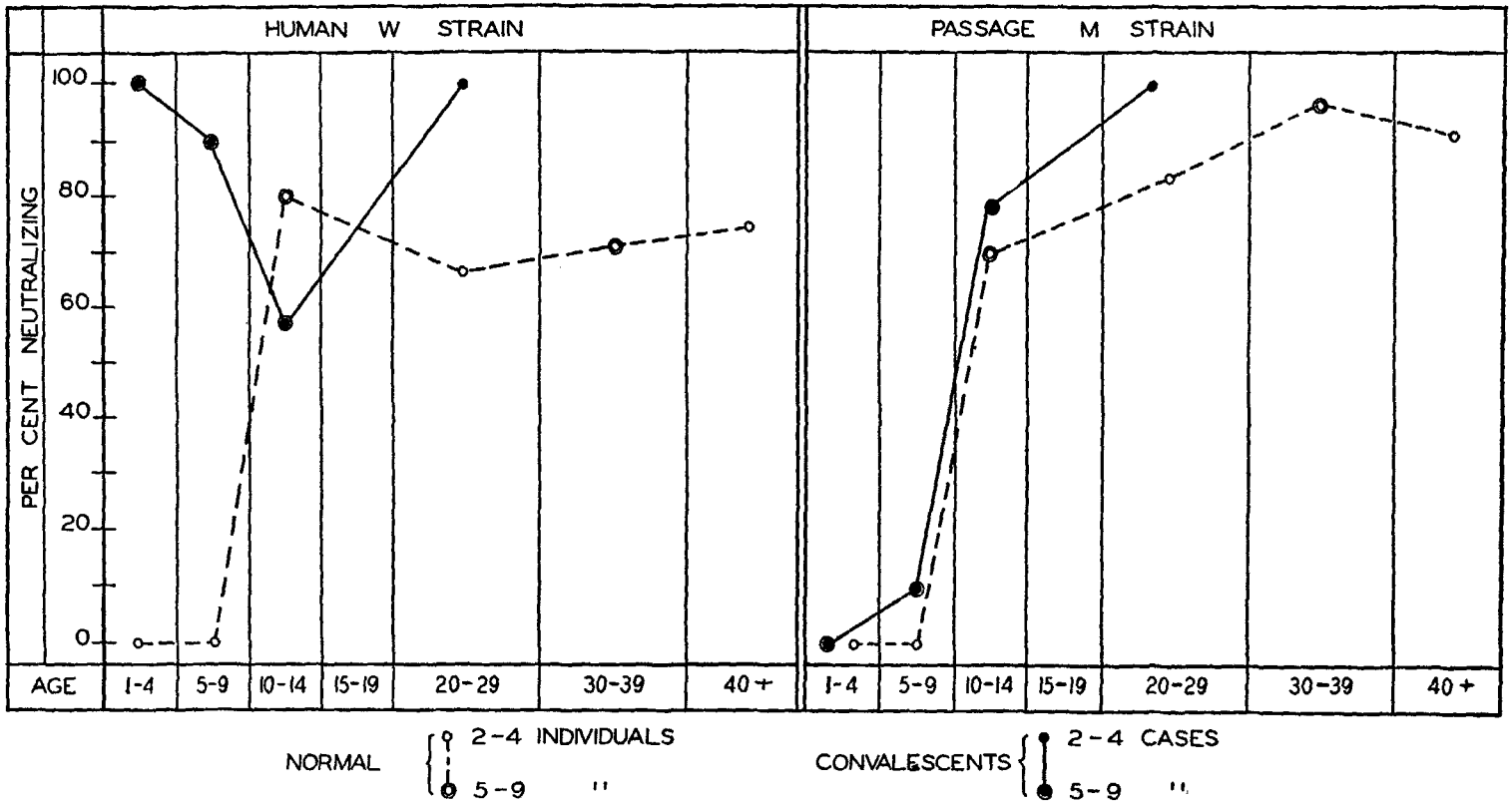

FIG. 2. The results of comparative tests performed on normal persons and patients convalescent from paralytic and abortive poliomyelitis when tested by a human (W) and a passage (M) strain. All of the convalescent sera were obtained within 14 months of the onset of illness.

The points in each half of the chart are based on 44 tests, representing 23 normal individuals and 21 convalescent cases.

tests noted with the W strain was 71; with the F strain 71, with the Aycock strain 95, and with the M strain 92.

Our previously reported comparative results were performed on sera from normal children, or children who were in early, late, or convalescent stages of abortive or frank attacks of poliomyelitis. In this younger group, which was largely composed of recent convalescents, not only was there less agreement in the results obtained with the two strains than in the normal adults, but the character of disagreement was generally in the opposite direction; namely, a higher percentage of positive neutralization tests was obtained with the human strain.

The sum total of our results on different types of sera can perhaps be best expressed in terms of graphs which appear in Fig. 2. These have been devised along the same lines as those in Fig. 1. Here it becomes apparent that in our passage strain experiments there is little evidence that the acquisition of either frank or abortive poliomyelitis has any influence upon the results of the tests, and by comparing the right half of Fig. 2 with Fig. 1, it will be noted that our experience with this passage strain has, in general, been similar to those of others with passage strains. With the human strain there is closer agreement with events which one might predict on the basis of analogy with other virus diseases; or in other words, in childhood at least, the acquisition of the clinical disease tends to elevate the "anti-human" viral properties in the blood, although this elevation may not be permanent.[5]

COMMENT

In reviewing our results it should again be pointed out, as has been done in a previous article (25) that many factors are unknown regarding the neutralization test in poliomyelitis. These include: (*a*) the extent to which a positive test signifies immunity; (*b*) the time of

[5] We found in previous work (25) that in testing convalescent sera from mild abortive attacks of poliomyelitis the elevation of "anti-human" viral substance in the blood was occasionally transient. Thus an increase in antiviral substance, demonstrated in two patients in the (3 week) convalescent sample, was not demonstrated in a later (14 month) sample. This transient increase was not noted in several paralytic convalescents similarly tested.

		Human strains																	
Strain		W—1931, New Haven									F—1931, New York								
Date of experiment		May 17, 1933			Sept. 21, 1933			Jan. 30, 1934			Feb. 27, 1934			Apr. 23, 1934			Nov. 28, 1933		
		Monkey No.	Incubation*	Result	Monkey No.	Incubation*	Result	Monkey No.	Incubation*	Result	Monkey No.	Incubation*	Result	Monkey No.	Incubation*	Result	Monkey No.	Incubation*	Result
			days			*days*			*days*			*days*			*days*			*days*	
Control sera	Human convalescent	1-39		+	1-66	12	±	1-94		+	1-83		+	1-88		+	1-71		+
	Normal monkey	1-52	5	−	1-75	5	−	1-90	7	−	1-84	5	−	1-87	5	−	1-62†	3	−
	" "																		
	" "																		
Normal human sera	Brn.	B-19		+										1-86	8	−	1-40†		+
	Epn.	1-60		+										2-11		+	1-44†	16	±
	Klk.	1-44	6	−										2-03	10	−	1-45†		+
	D. Bls.	1-45	5	−										2-04	4	−			
	Msn.	1-48		+										1-94		+	1-67§		+
	Ptg.	1-47	7	−										1-83		+	1-61†		+
	Cpn.				1-71		+	1-83		+	1-86		+						
	Chn.				1-72	9	−	1-84		+	1-85		+						
	Cls.				1-73		+	1-85		+	1-88		+						
	Dvl.				1-74		+	1-86		+	1-87		+						
	J. Dn.				1-76	10	−	1-87		+	1-91		+						
	Sdb.				1-77		+	1-88		+	1-92		+						
	Ttr.				1-78	5	−	1-89	6	−	1-93	6	−						
	Wnr.							1-93		+	1-94		+						
Convalescent sera	R. Hn.				1-48		+	1-91		+				1-91		+			
	D. Be.				B-19		+	1-92		+				2-02	Died from abscess				
					Experiment discarded because of result in protected control												Preliminary experiment on previously infected monkeys		

+, protection; ±, partial protection; −, no protection. * Incubation period in days to the onset

† Monkey previously infected with W strain, but susceptible to passage strain. ‡ Monkey previ

§ Monkey previously infected with W strain, subsequently resistant to passage strain.

E II

ization Experiments

Passage strains																									
Aycock						M																			
Mar. 25, 1934			May 12, 1934			Oct. 25, 1933			Jan. 9, 1934			Feb. 6, 1934			Mar. 6, 1934			Apr. 5, 1934			May 23, 1934		
Monkey No.	Incubation*	Result	Monkey No.	Incubation*	Result	Monkey No.	Incubation*	Result	Monkey No.	Incubation*	Result	Monkey No.	Incubation*	Result	Monkey No.	Incubation*	Result	Monkey No.	Incubation*	Result	Monkey No.	Incubation*	Result
	days			*days*			*days*			*days*			*days*			*days*			*days*			*days*	
2-02		+	2-10		+	1-71		+	1-71		+	2-00		+	1-71		+	1-96		+	2-11		+
1-40†	4	−	2-00	5	−	1-73	5	−	B-11	4	−	1-59‡		+	1-61†	6	−	2-09	2	−	1-71	4	−
			1-59‡		+													1-45†	6	−	1-76	5	−
			1-67§		+													1-59‡		+	1-89	5	−
			2-08		+	1-40†		+													1-94		+
						1-44†		+															
			2-07		+	1-45†		+													1-83		+
			Insufficient serum			1-59†	20	±															
			2-05		+	1-62†		+													1-88		+
			2-06		+	1-61†		+													1-91		+
2-03		+							1-40†		+	1-71		+				2-00		+			
2-04		+							1-76†		+	1-95		+	1-96		+						
1-83		+							1-45†		+	1-96		+	1-95		+						
1-94		+																2-05		+			
1-86		+										1-98	8	−	2-00		+	2-06		+			
1-88		+																2-07		+			
1-85	Died, dysentery		1-95		+				1-61†		+	1-99	28	±				2-08		+			
1-92	" "		1-96		+													1-71		+			
1-87		+																1-95		+			
1-91		+																2-10		+			
						Preliminary experiments on previously infected monkeys						Immune monkey used (through error) as unprotected control												

of fever.

ously infected with W strain, later infected with M strain, subsequently resistant to passage strains.

appearance and duration of neutralizing antibodies in the blood after an individual has sustained an attack of the disease; and (*c*) the specificity of antiviral substance detected by this method. Furthermore, methods of performing neutralization tests in poliomyelitis are not very accurate, and perhaps this criticism can be more justly applied to human than to passage strain experiments. The expense involved, which precludes the performance of large numbers of repetitive tests, and the differences in resistance to infection exhibited by different monkeys, tend to vitiate the results obtained. From the character of the methods employed, therefore, all one can perhaps learn are certain trends or directions in which the results seem to fall. The interpretation of these trends or the clinical or epidemiologic significance of an experiment of this type is dependent, of course, upon the nature of the differences between different types of poliomyelitis virus. This unfortunately is unknown. The implication is, perhaps, that the difference between human and passage strains is comparable to the difference between the street virus as opposed to the fixed virus of rabies; or between yellow fever virus recently isolated from a human case by monkey inoculation as opposed to yellow fever virus which has been adapted to infect mice. However, as far as poliomyelitis virus is concerned, sufficient data do not exist on which to base such an analogy. There is no evidence that all human strains of poliomyelitis virus are alike. There is no evidence that the strains which we and others have designated as human would necessarily acquire the properties of passage strains if they were carried further through five, ten, or twenty monkeys. These and other features go to show that the significance of human *versus* passage strain experiments in poliomyelitis is unknown and that such experiments should be taken at face value and do not warrant wide interpretations.

SUMMARY

In experiments devised to compare the neutralizing action of normal adult human sera on different strains of poliomyelitis virus, and to fill in certain gaps in our series of neutralization tests with different strains of virus on different types of cases in different age groups, we have made the following observations.

1. The difference between two human and two passage strains of

the virus when tested by this method amounted to about 25 per cent, and there was less power in normal adult sera to neutralize human than passage strains of virus.

2. The differences between the two human strains amounted to 15 per cent, and between the two passage strains to 8 per cent, the last figure falling within the limits of the experimental error of the method.

The extent to which these findings affect certain concepts with regard to the epidemiology of poliomyelitis based on passage strain neutralization experiments cannot be determined from the data presented in this paper, except that they more or less confirm the view previously derived from passage strain experiments, that 70 to 95 per cent of normal urban adults possess in their blood, substance which neutralizes poliomyelitis virus in a given amount. However, certain other indications appear when the present results are supplemented by those we have previously obtained (25). Primarily, we have found no relation between the clinical acquisition of poliomyelitis and the presence of substance in the serum which neutralizes a passage strain of poliomyelitis virus. With a passage strain the results seem rather to bear a closer relationship to age than to illness. With a human strain we have obtained results in which there is some evidence, shown only in the juvenile group, that acquisition of the clinical disease is accompanied by the appearance of antiviral properties in the blood.

BIBLIOGRAPHY

1. Wells, M. W., in Poliomyelitis, International Committee for the Study of Infantile Paralysis, Baltimore, The Williams & Wilkins Co., 1932, Chapter VII, 432–441.
2. Soule, M. H., and McKinley, E. B., *Proc. Soc. Exp. Biol. and Med.*, 1931, **29**, 168.
3. Hudson, N. P., and Lennette, E. H., *Am. J. Hyg.*, 1933, **17**, 581.
4. Jungeblut, C. W., *J. Immunol.*, 1933, **24**, 157.
5. Lennette E. H., and Hudson, N. P., *Proc. Soc. Exp. Biol. and Med.*, 1933, **30**, 449.
6. Aycock, W. L., and Kramer, S. D., *J. Prevent. Med.*, 1930, **4**, 201.
7. Aycock, W. L., and Kramer, S. D., *J. Prevent. Med.*, 1930, **4**, 189. Data supplemented by personal communication from Aycock.
8. Shaughnessy, H. J., Harmon, P. H., and Gordon, F. B., *J. Prevent. Med.*, 1930, **4**, 463.

9. Brodie, M., *J. Bact.*, 1932, **23,** 102. Data supplemented by personal communication from author.
10. Howitt, B. F., *J. Infect. Dis.*, 1932, **51,** 565. Data supplemented by personal communication from author.
11. Jungeblut, C. W., *J. Immunol.*, 1932, **23,** 35.
12. Kramer, S. D., and Aycock, W. L., *Proc. Soc. Exp. Biol. and Med.*, 1931, **29,** 98.
13. Hudson, N. P., and Litterer, W., *J. Infect. Dis.*, 1933, **53,** 304.
14. Schultz, E. W., and Gebhardt, L. P., *Proc. Soc. Exp. Biol. and Med.*, 1931, **28,** 409.
15. Aycock, W. L., and Kramer, S. D., *J. Exp. Med.*, 1930, **52,** 457.
16. Fairbrother, R. W., and Brown, W. G. S., *Lancet*, 1930, **2,** 895.
17. Netter, A., Levaditi, C., and Hornus, G., *Bull. Acad. méd.*, Paris, 1933, **109,** series 3, 529.
18. Weyer, E. R., Park, W. H., and Banzhaf, E. J., *J. Exp. Med.*, 1931, **53,** 553.
19. Kramer, S. D., *J. Am. Med. Assn.*, 1932, **99,** 1048.
20. Howitt, B. F., *J. Infect. Dis.*, 1933, **53,** 145.
21. Stewart, F. W., and Rhoads, C. P., *J. Exp. Med.*, 1929, **49,** 959.
22. Burnet, F. M., and Macnamara, J., *Brit. J. Exp. Path.*, 1931, **12,** 57.
23. Weyer, E. R., *Proc. Soc. Exp. Biol. and Med.*, 1931, **29,** 289.
24. Flexner, S., *J. Am. Med. Assn.*, 1932, **99,** 1244.
25. Paul, J. R., and Trask, J. D., *J. Exp. Med.*, 1933, **58,** 513.
26. Paul, J. R., and Trask, J. D., *J. Exp. Med.*, 1932, **56,** 319.