A COMPARATIVE STUDY OF RECENTLY ISOLATED HUMAN STRAINS AND A PASSAGE STRAIN OF POLIOMYELITIS 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)

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Since the early days of the discovery of the virus of poliomyelitis it has been known that quantitative differences in virulence for the monkey exist between recently isolated strains, and strains which have passed through successive monkeys. Only within the last few years has it been clearly demonstrated by the work of Burnet and Macnamara (1) in Australia, that immunologic differences exist between such strains, which might be termed qualitative differences, in that they do not seem to be confined to those of virulence alone.

These investigators made a comparative study of two strains; one a local virus recently isolated from a fatal human case, and the other the Rockefeller Institute strain of so called mixed virus (M. V.). They described three instances in which monkeys contracted a fatal attack of poliomyelitis following the intracerebral inoculation of the M. V. strain despite the fact that some weeks previously the monkeys had sustained a typical attack of poliomyelitis produced by the local strain. The reverse of this experiment was also demonstrated in a single instance, in which a recovered monkey, partially paralyzed by the M. V. strain, was subsequently brought down with complete paralysis by the local virus. Furthermore, they found in neutralization tests that, although pooled convalescent serum would neutralize both strains, a few tests with individual samples of convalescent sera failed to show this parallelism, in that only the local virus was neutralized.

In corroboration of this work Weyer (2) later described differences in the neutralizing values of human convalescent and antiviral horse serum for recently isolated strains and for a monkey passage strain. Flexner (3) has also pointed out that immune sera prepared from "human" and old passage strains of virus

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exhibit differences in cross-neutralization tests, although old passage strains of the virus preserve their immunizing power in part, at least, against recent strains.

The observations to be reported in this paper are essentially a repetition of the experiments of Burnet and Macnamara, and our results are in accord with their findings. They show that: (*a*) the experimental disease in the monkey induced by our two human strains failed to immunize monkeys against a subsequent reinfection by an old passage strain; and (*b*) that the neutralizing power of human sera for a recently isolated human[1] strain differs from the neutralizing power for an old passage strain.

Methods

Macacus rhesus monkeys, most of them small (weighing 6 pounds or under), were employed in all our experiments. All inoculations were done under ether anesthesia by the intracerebral route.

Strains of Virus Employed.—The old passage strain which we have termed the M 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 the date of its first isolation or the actual number of monkey passages to which it had been subjected is unknown.

The usual incubation period of the experimental disease produced by this strain was from 3 to 5 days. This disease was heralded by a sharp elevation of temperature to 105° or 106° to be followed within 24 to 36 hours by a sharp drop to subnormal values, widespread paralysis, and usually the death of the animal. A virulence titration done in October, 1931, on material from the cord of a single passage monkey (No. 34) revealed the minimal infecting dose of this material to be greater than 0.5 cc. of a 0.01 per cent suspension of ground monkey cord, and less than 0.5 cc. of a 0.05 per cent suspension. The dose subsequently employed for all neutralization tests with this strain was 0.5 cc. of a 0.1 per cent cord suspension. This technique gave uniform results from October, 1931, to January, 1932. The strain was not used for neutralization tests during the next 14 months, but when later tested (March, 1933), the virulence of material from glycerinated cord No. 34 was found to have fallen. A single passage (Monkey 149) at this time seemed to restore the virulence and, although a titration was not done, the dosage which had been employed with material from Cord 34 was employed with Cord 149.

The recently isolated or human strain which we have termed our W strain was isolated in September, 1931, from the oral washings of a child of 5 years of age on the 1st day of a mild abortive attack of poliomyelitis. The circumstances under

[1] The term human strain to designate a recently isolated strain has been employed because of its usage in previous articles (2, 3) on this subject.

which the strain was isolated, the technique employed, and the results of the first two passages have been described in a previous publication (4). The results of subsequent passages will be described in a later section of this paper. Material from a single (sixth passage) monkey (No. B-6) was employed for all of our reinoculation and neutralization tests. The usual incubation period of the experimental disease induced by the seventh passage of this strain was 6 days; the febrile period was generally longer than that of the old passage strain, and the development of paralysis only occasionally as severe. The mortality might be roughly placed at 15 per cent, in that out of twenty-seven positive inoculations but one monkey died within 3 days of the onset of the disease and three other monkeys, which developed severe and extensive paralysis and probably would have died, were sacrificed on the 3rd or 4th days of the disease. The histological lesions from this and our other recently isolated human strains failed to show any differences from those noted with the passage strain. The virulence of the sixth passage of the W strain was far lower than that of the M strain. The dose employed in all reinoculation and neutralization tests was 0.5 cc. of a 5 per cent cord suspension, and, as will be subsequently shown in Text-fig. 1, this dose infected fresh monkeys consistently.[2]

A third strain (F) 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 a fatal case of poliomyelitis and the sample we received represented the eighth passage. A titration of virulence at this time led us to employ the same dosage as that used in our W strain for all neutralization tests. The experimental disease induced by the F strain was quite similar to that produced by the seventh passage of our W strain.

Technique of Neutralization Tests.—In all of the neutralization tests with the M strain the virus-containing material was derived from two monkeys, Nos. 34 and 149. A virulence titration had been done with the former lot of material. In all tests with the W strain the virus was derived from a single monkey, No. B-6, and a series of monkeys had been brought down consistently by the dose employed. With the F strain pooled material from three monkeys was used representing the eighth and ninth passages.

In performing neutralization tests the following technique was employed: A small portion (0.1 to 1.0 gm.) of glycerinated spinal cord was weighed and ground for 6 or 8 minutes in a mortar with sterile sand and 2 to 3 cc. of saline solution. Enough saline was then added to make the percentage of the virus suspension equal to twice that of the dose to be inoculated. This material was centrifuged at moderate speed for 8 to 10 minutes. The opalescent supernatant fluid was then removed with care to avoid the presence of macroscopic particles. Individual tests were set up by mixing equal volumes of the virus suspension and the

[2] To our knowledge this is the first strain, isolated from the throat, which has been studied intensively through a series of monkey passages.

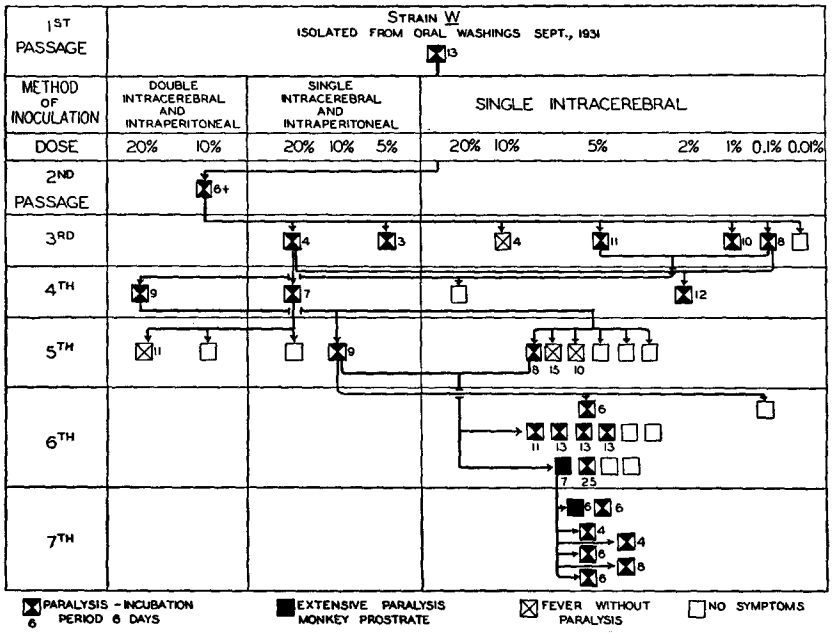
serum to be tested, using from 0.3 to 0.5 cc. of each. Thus the total volumes varied between 0.6 and 1.0 cc. From one to twenty samples of sera were employed in each experiment but our usual experiment consisted of six or eight samples. Included in each experiment were two control samples of sera: (*a*) the protected control, consisting of a pooled sample of sera from adults recently convalescent from a frank case of poliomyelitis; and (*b*) the unprotected control, which consisted of a sample of normal monkey serum. The serum-virus suspensions were mixed and incubated at 37°C., for 2 hours. A single monkey was inoculated intracerebrally with 0.5 cc. of each serum-virus mixture. Temperatures were taken daily for a period of 4 weeks on all monkeys inoculated with our human (W) strain, on most of the monkeys inoculated with the passage (M) strain, and on a few with the (F) strain. The results of individual tests were expressed by three terms which are, of course, only relative in their meaning: (1) no neutralization (or no protection); (2) partial neutralization; (3) complete neutralization. Sera were judged as having no neutralization when inoculated monkeys came down with the experimental disease within 5 days of the time when the unprotected control came down; partial neutralization when the incubation period proved to be 6 or more days longer than that of the unprotected control; and complete neutralization when the animals failed to come down during the period of observation, which in all instances was 4 weeks. Untoward results were encountered in three out of twenty-one experiments of this type, in all three of which the protected controls developed the experimental disease after a prolonged incubation period. This occurred once with each of our three strains of virus. The results of all tests in these three experiments were discarded and the experiments repeated. It may be worth mentioning, however, that the majority of results of individual tests in the unsatisfactory experiments were in agreement with the results from satisfactory experiments.

In the interpretation of the results of this test it should again be emphasized that in our hands, it has proved to be a rather crude, qualitative test. We seldom attempted to quantitate our results because, owing to the expense involved, the use of several monkeys for each determination was impossible. The degree of accuracy of the test has been difficult to determine. We have had occasion to repeat eighteen individual tests, representing ten different samples of sera, with the W strain and discrepant results were encountered twice, or in about 11 per cent of these repetitions When discrepant results were encountered at least three tests on the sample of serum were always done and the majority result accepted. Nine tests with the M strain were repeated with a reduplication of results in all but one in which the difference was slight (partial protection with an incubation period of 24 days, as opposed to complete protection).

EXPERIMENTAL

It was our aim in these experiments to employ a strain of poliomyelitis virus as little influenced as possible by monkey passages. This is a

difficult ideal to attain; for, if a strain of virus is to be adequate for use in a series of neutralization tests, it should be of sufficient virulence so that a single inoculation of at least a 5 per cent cord suspension should consistently give rise to the experimental disease in the monkey. In neither of two freshly isolated strains, W and Rn (4), with which we have worked was this the case in the first few passages, and considerable difficulty was encountered in rendering one of them ade-



TEXT-FIG. 1. Diagram of the first seven monkey passages to which our human W strain was subjected.

quate for this purpose. As the manner in which a strain of virus, recently isolated from a human case, becomes established in the monkey is pertinent to the problem under discussion, the steps of this process will be given in some detail.

Enhancement of Virulence in the W Strain.—As previously mentioned the circumstances under which this strain was obtained from oral washings, and the results of the early monkey passages have been described in another publication (4). The results of subsequent pas-

sages in which the character of the experimental disease which appeared in the various passages and the incubation period, are shown in Text-fig. 1. The latter has been measured from the time of inoculation to the onset of fever.

In the second passage, using the method of Flexner (5), large double doses inoculated both intracerebrally and intraperitoneally, were employed. In the third passage a titration was attempted with somewhat irregular results for, although the experimental disease was produced with the surprisingly small dose of 0.5 cc. of 0.1 per cent suspension, one of the monkeys which received a much larger dose failed to develop the typical experimental disease. On the fifth passage the virulence had fallen and, on a first attempt, two out of three monkeys, which received what might be called massive doses (one of them a double inoculation) failed to develop any symptoms; a third monkey developed fever without paralysis. When, on a second attempt the fifth passage was successful, an estimate of the virulence was tried and six monkeys were inoculated with a single intracerebral dose of 0.5 cc. of a 5 per cent suspension; but two developed fever and a third paralysis. On the sixth passage another titration was done which still showed that the virus was apparently weaker than it had been in the third passage. Two lots of monkeys were inoculated with a 5 per cent suspension. Of the first lot of six monkeys but four developed the experimental disease, and of the second lot of four but two. However, one of the latter monkeys (No. B-6) developed a severe form of the disease with widespread paralysis of all of the limbs, similar to the usual picture produced by the M virus. In the seventh passage samples of cord from this monkey were used, and out of seven monkeys all, inoculated at different times with different samples of this cord (No. B-6), developed satisfactory examples of the experimental disease.[3]

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[3] There were certain points exhibited by Monkey B-6 which seem to be of sufficient significance to warrant recording. On the day following inoculation it was noted that the animal was apathetic, and showed a tendency to move slowly in a circular direction to the left. These symptoms continued for the next 4 days during which time the temperature remained at 101°; on the 6th day after inoculation the temperature rose to 103°, and on the 8th to 105°. On the 9th day the animal was prostrate with widespread paralysis. It was sacrificed the next day. The autopsy besides revealing extensive lesions of poliomyelitis, showed a large cerebral hemorrhage in the right hemisphere at the site of the inoculation, probably due to a traumatic injury of a vessel. Inasmuch as this was the first monkey out of thirty-four previously inoculated with material supposedly containing this virus, which succumbed with such widespread paralysis, it has seemed to us that possibly the injury to the brain may have contributed either to the establishment of, or the dissemination of the infection.

A consideration of our efforts (shown by the data in Text-fig. 1) to establish in the monkey this rather refractory strain of the virus brings out a few important points, many of which have been noted before, but they now assume a new significance. It is to be noted that apart from some of the monkeys which received massive doses, the incubation period, as measured from the time of intracerebral inoculation to the onset of fever, was as a rule longer in the earlier passages than it was in the seventh. Furthermore, with the exception of the two monkeys shown in Text-fig. 1 as having developed extensive paralysis and having been prostrated by the experimental disease, in none have we reason to believe that the experimental disease would have been fatal, although as previously mentioned subsequent fatalities were recorded. It is probable that a more rapid establishment of virulence might result in a repetition of this work, for this strain seemed to require more monkey passages than have been reported in other detailed descriptions of the establishment of human strains, but whether our difficulties were due to differences in susceptibility of the individual monkeys used or to the character of the virus, or both, has not been determined. It seems pertinent to recall in this connection, however, the experiments of Stuart and Krikorian (6), which illustrate the differences in the number of passages required to establish different strains of rabies virus in the rabbit.

Multiple Cross-Inoculations with the M, F, and W Strains.—An intensive study of the immunologic differences in these three strains has not been done. The degree of protection against infection by a heterologous strain has, however, been observed although by one method alone; namely, that of intracerebral reinoculation.

The value of this method, which is perhaps not an ideal one for the purpose, rests upon the fact that it has been the previous experience of most investigators that if the experimental disease is successfully produced in the monkey by intracerebral inoculation and the animal survives, it is usually refractory to a subsequent reinoculation. Thus, Flexner and Lewis (7) reinoculated ten monkeys at periods varying from 8 to 60 days after the first paralysis appeared and failed to detect evidences of the experimental disease resulting from the second inoculation. Similar results have been recorded by Schultz, Gebhardt, and Bullock (8) in eight reinoculation experiments at intervals varying from 2 to 67 days. Leiner and von Wiesner (9) have reported thirteen reinoculation experiments in eight paralyzed monkeys, given from 2 to 99 days after the onset of the first paralysis. The sec-

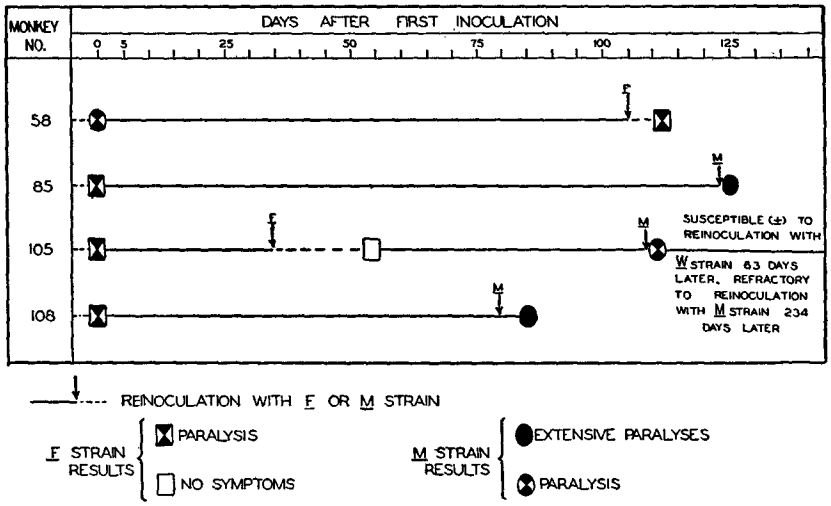
ond inoculation failed to give rise to symptoms except in one instance. This was a monkey reinoculated 17 days after the first onset of paralysis; 18 days later the animal again became ill and new paralyses developed. Krauspe (10) reports a similar isolated example of reinfection. This was described as a severe infection following a third reinoculation, administered 4½ months after the first. Presumably the same virus was used for each inoculation but no mention of this fact is made. In any event, although the number of intracerebral reinoculation experiments which appear in the literature are few and briefly described, it would seem as if the successful reinfection of the monkey by an homologous strain was distinctly uncommon.

We have performed thirteen reinoculation experiments, in which homologous strains of the virus were used and daily temperature records were kept over a period of 4 weeks from the second inoculation. With the passage (M) strain opportunities for experiments of this type do not often arise because monkeys infected with the M strain nearly always die. In all of our homologous reinoculation experiments with the M strain the monkeys employed represented examples of the experimental disease which had been modified by methods we shall presently describe. In these reinoculations the same dose was employed as that which had previously infected the monkey, and the intervals between the onset of paralysis and the second inoculation were 20, 20, 52, 60, and 230 days respectively. None of these reinoculated monkeys showed any symptoms such as fever or signs of illness as a result of the second inoculation. With the recently isolated strain (F), two homologous reinoculation experiments were done at intervals of 34 and 146 days respectively, and, with our other recently isolated strain (W), six such experiments were done at intervals of 17, 20, 21, 56, 101, and 111 days respectively. In none of these were any symptoms produced as a result of the second inoculation. This is a small series and certainly does not exclude the possibility that monkeys may occasionally be reinfected with homologous strains during the intervals studied but we believe, as others have often shown, that such instances of reinfection must be uncommon.

Results of our first group of heterologous experiments appear in Text-fig. 2. The first observation recorded in this group is of particular value not only because the outcome was illuminating to us at the time it was performed, but because it represents the only monkey (No. 58) which survived in our series of more than 50 fresh monkeys

infected with the M strain. Several other survivors, which will be subsequently described, represent monkeys which had previously been partially immunized.

Monkey 58 was inoculated with a human serum-virus mixture on Dec. 1, 1931. The usual infecting dose for this strain (0.5 cc. of a 0.1 per cent virus suspension in serum) was employed. The human serum in this test possessed some neutralizing properties for the M strain but failed to neutralize it completely. After a prolonged incubation period the animal became ill on Dec. 25, paralysis of both hind legs was noted on Dec. 27. It eventually recovered and there was a slight return of function to the legs. On Apr. 7, 1932, the monkey was reinoculated with 0.5 cc. of a 5 per cent suspension of the F strain. It became ill on Apr. 15 with paraly-



TEXT-FIG. 2. Four reinoculation experiments with the human F strain and the passage M strain.

sis of the arms, tremor, and increased weakness of the legs. The animal was sacrificed on Apr. 18 and an examination of the tissues from the central nervous system revealed the presence of both old and fresh lesions of poliomyelitis in the brain and cord.

The reverse of this experiment is recorded in three instances which also appear in Text-fig. 2, representing Monkeys 85, 105, and 108. These experiments were done early in our work and may not be as satisfactory as some of the others, in that a larger dose of the M strain (ten times the usual infecting dose) was employed to reinfect the mon-

keys. Two of these monkeys rapidy developed an extensive fatal disease as a result of the second inoculation. A third (No. 105), which had proved refractory to a second inoculation of the F strain, was reinfected with the M strain but survived.

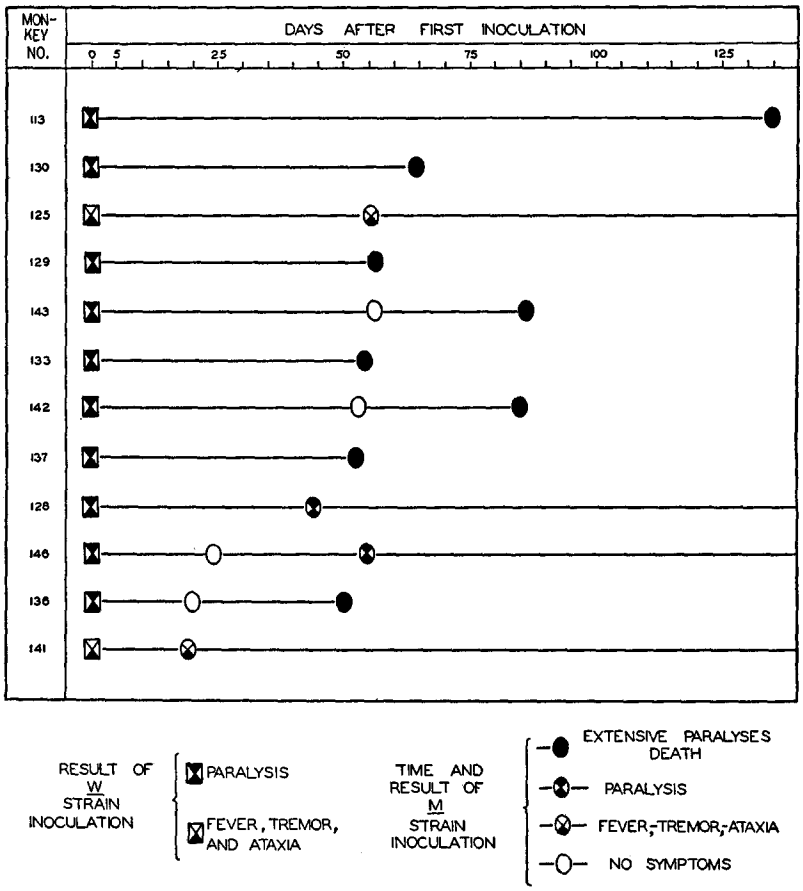
A second series of heterologous reinoculation experiments was then tried using our W and M strains. The results, which appear in Fig. 3, again show the ease with which monkeys which have survived an infection by a human strain may be subsequently infected with a passage strain. The usual infecting dose for each strain was employed throughout, although in several instances the virus was mixed with "impotent" human sera; that is, human sera which on subsequent or previous tests failed to reveal the presence of neutralizing antibodies. The reasons for this were that many of these monkeys were used as checks in our neutralizing tests on human sera to confirm or establish the absence of neutralizing antibodies in the serum.

It is unfortunate that the opportunity seldom arose to test the reverse of this experiment; namely, the degree of protection against the W strain, conferred by an infection with the M strain. The reason for this was that but one animal from our entire series of fresh monkeys infected by the M strain survived. A single experiment of this type was, however, tried on a partially immunized monkey, as follows:

Monkey 105 (see Text-fig. 2) was inoculated first with the F strain and survived with paralysis. It proved refractory to a second inoculation with the F strain but was reinfected by the M strain and, as a result, developed more extensive paralyses involving both hind legs and the left fore leg. 63 days after the infection with the M strain this monkey was inoculated with the W strain in its third passage. After an incubation period of 16 days the animal became ill, weak, very clumsy, and developed tremors of the head and strabismus. A lumbar puncture performed on the 9th day of fever showed 300 red blood cells and 70 white cells per c.mm. Fever persisted somewhat irregularly for about 2 weeks, the animal eventually recovered and did not sustain any new permanent paralyses as a result of this inoculation. Subsequently it was found to be refractory to a second inoculation of the M strain.

We believe that this animal was infected by three different strains, and that the experiment suggests, as does that in Monkey 58 (see Text-fig. 2), that an infection of the passage or M strain does not wholly protect the animal against an infection by the human strain. The result is important in suggesting that the difference between the

human and passage strains is not merely a quantitative one. It is also remarkable that the human F strain in its eighth and ninth passage did not wholly protect against the human W strain in its third passage. This point will be referred to later.



TEXT-FIG. 3. Twelve reinoculation experiments with the human W strain and the passage M strain.

Early in our work it became apparent that the length of the time interval between inoculations was a significant factor and for that reason the results shown in Text-fig. 3 have been arranged in series according to diminishing intervals between inoculations. The pres-

ence of this and other variables probably influenced this experiment. Included among them is perhaps that of the susceptibility of individual monkeys which we believe is an appreciable factor more readily manifest in experiments which concern the relatively weak human strain than the passage strain but it is also reflected in the latter. Thus it will be seen in Text-fig. 3 that of the series of twelve monkeys recorded in this group which were initially infected with the W strain, two (Nos. 125 and 141) developed relatively mild forms of the experimental disease. These mild forms were characterized by the picture already described in Monkey 105. Generally there was a high and rather prolonged temperature reaction which was not followed by a sharp fall to subnormal values. The animal usually developed tremor and ataxia during the febrile period but made a good recovery and was left perhaps with slight weakness of one or more limbs, which after 3 to 6 weeks was often difficult to detect. The only two mild examples of the experimental disease induced by reinoculation with the M strain occurred in these same two monkeys (Nos. 125 and 141) and we have been inclined to attribute this phenomenon to the presence of a hypothetical resistance to the acquired experimental disease in these two monkeys.

Another variable which we have attempted to control and to which reference has already been made is that of the time interval between inoculations. These intervals have been recorded in Text-fig. 3 in a little different manner from that which is usually employed in order that those monkeys which developed mild forms of the disease might be uniformly included in this chart. The interval between inoculations has been numbered from the day of the first onset of fever to the day on which the next inoculation was done. The effect of this interval on the reinoculation result may be best summarized by the statement that all of the failures to reinfect monkeys by a heterologous strain were encountered when the reinoculations were done less than 60 days from the onset of the previous experimental disease. An analysis of the effect of this time interval is also shown in Table I. The figures speak for themselves.

Comparison of Different Human Strains by Reinoculation Experiments.—The attempt has not been made to make a comparative study by methods just outlined, of our two human strains, F and W; or of

the early (third to fifth) passages of the W strain as contrasted with the later (sixth) passage of this strain. A few cross-inoculation experiments were done but the results are insignificant. It may be worth recording, however, that the experimental disease induced by a third passage W strain, gave complete protection to reinoculation by the F (eighth and ninth passage) strain in but one of three experiments; partial protection was developed in the other two.

Comparative Results of Serum-Virus Neutralization Tests with the W and M Strains.—As a further means of comparing the properties of human and passage strains, their individual susceptibility to neutralization by different samples of human sera was tested. In spite of the

TABLE I

Character of Symptoms Induced by the Inoculation of a Passage Strain in Relation to the Interval from the Onset of a Previous Infection Induced by a Human Strain

	Time interval between inoculation of passage strain (M) and the onset of a previous human strain infection*	No. of monkeys employed	Per cent which developed			
			Extensive paralysis—animal prostrate	Paralysis of one or more limbs	Fever, tremor, ataxia, and slight weakness	No symptoms
	days					
A	More than 70	6	83%	17%	0	0
B	35–70	10	50%	20%	10%	20%
C	20–35	3	0	0	33%	66%

* All of the human strain infections were induced by the W strain with the exception of three in Group A which were induced by the F strain.

occasional irregularities encountered with this test (see under Methods) the results, which appear in Table II, show that it is not too crude for demonstrating differences in these two strains. Here are listed a series of comparative tests with the W and M strains on blood samples representing fifteen individuals which include seven frank cases with paralysis, one frank case without paralysis, five abortive cases, and two contacts. The cases have been listed in series according to age and it appears that, regardless of the age of the patient, all but one of the recent convalescent samples from frank and abortive cases showed either partial or complete neutralization with the W strain of virus. The exception, J. D., a child 11 years of age, who had sustained

paralysis of both legs, failed to show any neutralization in a sample of blood obtained 18 days from the onset of his disease and again 11

TABLE II

Comparative Neutralization Tests with a Recently Isolated Human Strain and a Passage Strain of Virus

Patient	Character of illness or contact	Age	Recently isolated W strain			Passage M strain		
			Before or during attack or contact	1½ to 10 wks. from onset of attack or contact	11 to 14 mos. later	Before or during attack or contact	1½ to 10 wks. from onset of attack or contact	11 to 14 mos. later
		yrs.						
R. O.	Intimate contact	1	−	−		−	−	
G. M.	Frank case with paralysis	2		+			−	
D. D.	Frank case with paralysis	2½		+			−	
E. D.	Abortive case	4	−	+		−	−*	
F. O.	Mild frank case	4½		+	+		−	−*
A. H.	Frank case with paralysis	4½		+			−	
R. W.	Abortive case	5	−	±		−	−	
A. P.	Frank case with paralysis	6		+	+		−	−
I. O.	Frank case without paralysis	6½	−*	+	+	−*	±	−
M. D.	Abortive case	10	+†	+*		−	±	
B. E.	Frank case with paralysis	11		+			−	
Ev. O.	Abortive case	11	−*	+*	−	+*	+	+
J. D.	Frank case with paralysis	11		−	−		+*	+
Ed. O.	Intimate contact	12	+*	+*	+	+*	+	+
C. O.	Abortive case	13	+†	+*	−	±†	+	+

−, no neutralization. ±, partial neutralization. +, complete neutralization.

* Test repeated, similar result obtained.

† Test repeated, discrepant results obtained; majority or average result accepted.

months later. An interpretation of the results of the neutralization tests with the W strain on the mild abortive cases and the contacts will be discussed in the following paper (11).

Quite a different pattern of results is obtained with the M strain. Of the thirty-one tests recorded in this series there is practical agreement with those in the W series in about one-third. With the M strain the presence of neutralization seems to be somewhat an expression of the age of the child. With the older group ($6\frac{1}{2}$ to 13 years of age), there is some correlation between the increase of neutralizing power and the presence of either a frank or an abortive attack as shown by the result obtained with I. O., a frank case; and M. D. and C. O., abortive cases. Of the thirteen instances in which complete or partial neutralization was present for the M strain it was present for the W strain in eight; there are five instances in which neutralization was present for the M strain and absent for the W strain; *i.e.*, the first and third samples from Ev. O., both samples from J. D., and the third from C. O. Although the character of the differences between these two strains is unknown, it is also on the basis of these results that we believe that the use of the term qualitative differences is justifiable in a comparative description of the properties of the M and W strains.

DISCUSSION

Experiments in this report, together with previous observations in the literature (1–3), seem to leave little doubt that by the methods we and others have employed, differences can be demonstrated between recently isolated and passage strains of poliomyelitis virus, although there are common immunologic factors between these two strains. The situation recalls those differences which exist between the street virus and the fixed virus of rabies, or perhaps between vaccine virus and smallpox virus. Our own observations may not afford much opportunity for generalizing upon the subject, based as they are on studies of but two so called human strains, and a single passage strain of poliomyelitis virus, and we recognize that there is no exact knowledge as to the stability or the nature of the differences described between these strains. Nevertheless, we are inclined to consider them as qualitative. Furthermore, it should be emphasized that there are no data which define the limits of a human strain. In fact, those strains which we have designated as human do not perhaps really justify this term, for, during the few passages to which they have been exposed, the properties of one of them, W at least, have changed as has been shown in Text-fig. 1. In spite of these limitations, how-

ever, the observations do afford some information for which a practical application may be found. Thus it is our belief that in spite of the present difficulties inherent in the technique of the serum neutralization test, such tests with human serum with a recently isolated human strain of the virus, give a better concept of the antiviral properties induced by the human disease than do similar tests performed with passage strains of the virus. With the passage strain some correlation with clinical events was, however, noted in children who were older than 6 years. The correct interpretation of our neutralization tests performed with this passage virus is still a question and it seems to us that much of the data now in the literature concerning the neutralization of poliomyelitis virus by human sera, founded as it has been, largely upon passage strain experiments, is also open to some question in so far as giving complete or accurate information which has to do with the anti-human poliomyelitis virus properties of the blood.

SUMMARY

1. Confirmation of the qualitative differences which exist between so called human and passage strains of poliomyelitis virus has been established by the following observations.

(*a*) The experimental disease induced by two human strains usually failed to protect monkeys against a subsequent infection by a passage strain, and in the few instances in which the reverse experiment could be tried a similar lack of protection was observed.

(*b*) In some human sera the neutralizing power for a human strain differed qualitatively from the neutralizing power for a passage strain.

2. The time interval between the intracerebral inoculation of heterologous strains has been found to be an important factor bearing upon the results of the reinoculation experiments reported. Within the intervals used, the greater the period between the original infection and the reinoculation with a heterologous strain, the less was the degree of cross-immunity observed.

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