A STUDY ON SEROTHERAPY OF POLIOMYELITIS IN RHESUS MONKEYS

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Received for publication September 26, 1958

In work previously reported (Liu, Carter, DeSanctis, Geating and Hampil, 1958) a severe infection in the central nervous system (CNS) of monkeys was induced by the intraspinal inoculation of a small amount of the Mahoney strain of Type I *poliovirus hominis*. Paralysis of the infected animals could be prevented or limited by the administration of rabbit antiserum. In the majority of the experiments the serum was administered when the infected monkeys showed no apparent clinical symptoms; such management, strictly speaking, should be considered as prophylaxis. However, encouraging results were obtained with small groups of animals that received delayed treatment. Although only 8 monkeys were employed in the experiment in which the serum was injected at 48 hr. post-infection, the authors concluded tentatively that the therapy was effective in preventing further spread of the infection in the CNS.

In view of these favorable results, further work was carried out. In the present study, emphasis was placed on the therapeutic aspect of poliomyelitis, dealing mainly with the following questions: What happens when delayed treatment is applied to more animals? What are the clinical signs of the infected monkeys at the time of therapy? What is the effect of serotherapy if infection by another route is employed? What is the effect of antibody obtained from human sources in treating infected monkeys?

MATERIALS AND METHODS

Virus used for infection of monkeys

The Mahoney strain of Type I *poliovirus hominis* was employed throughout the experiments for infection of the monkeys. The procedures for preparation of the seed virus, production of the monovalent vaccine and titration of the virus intraspinally in rhesus monkeys have been described (Liu *et al.*, 1958). In addition to the intraspinal injection of virus, in some experiments intracerebral inoculation was used. The procedures of inoculation *via* this route were described in detail by Bodian, Morgan and Schwerdt (1950). All animals were anesthetized by injection of 30 mg./kg. of sodium pentobarbital intravenously. Under deep anesthesia, each animal was infected with 500,000 50 per cent tissue culture infective doses ($TCID_{50}$) of Mahoney virus in 1 ml. of medium 199 (Salk, Youngner and Ward, 1954) by injecting one-half the volume into each of both thalami through a hole in the skull made by a small " Moto tool " over the central parietal region, about one-half inch lateral to the sagittal suture.

Hyperimmune rabbit serum

The method for preparing the Type I poliovirus hyperimmune serum in albino rabbits has been described in the previous report (Liu *et al.*, 1958).

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Hyperimmune human γ-globulin

A pool of plasma was obtained from 39 donors as described in a later section. The plasma was fractionated according to a combination of methods: No. 6 (Cohn, Strong, Hughes, Mulford, Ashworth, Melin and Taylor, 1946) and No. 9 (Oncley, Melin, Richert, Cameron and Gross, 1949). The distribution of plasma proteins by electrophoresis has been described by Cohn *et al.* (1946) and Oncley *et al.* (1949).

Titrations of $TCID_{50}$ and neutralizing Antibodies

The colorimetric method of Salk, Youngner and Ward (1954) was adopted, with a minor modification that 2 per cent calf serum was substituted for the horse serum in mixture 199. Virus dilutions were set up at 0·5 and 1·0 log steps and antibody dilutions in 2-fold steps. The 50 per cent end point of these titrations was calculated according to the method of Reed and Muench (1938).

The serum was tested for neutralizing antibodies against not only Type I *poliovirus hominis* but also the MEF_1 (Type II) and Saukett (Type III) strains originally obtained from Dr. J. E. Salk and designated M-80 and M-83 respectively. The stock cultures were used at their third passage in monkey kidney cells. The $TCID_{50}$ lay between $10^{-5.3}$ and $10^{-7.3}$ ml.

RESULTS

Studies with hyperimmune rabbit serum

Delayed treatment after intraspinal inoculation. Two experiments were carried out in order to ascertain the efficacy of the delayed therapy with the hyperimmune serum prepared from the rabbits and to determine more fully the clinical signs at the time when therapy was given. Each experiment included 12 rhesus monkeys weighing 2–3 kg. each. In the first trial, 8 animals received an intravenous dose of 20 ml./kg. of the standard hyperimmune rabbit serum intravenously as described in the previous report (Liu *et al.*, 1958), and 4, which received no serum, were controls. In the second trial, 7 were treated and 5 used as controls. All monkeys were infected intraspinally with 10–16 $TCID_{50}$ of Mahoney virus 48 hr. prior to therapy. Thereafter, these animals were under careful observation for a period of 4–5 weeks.

Two objective criteria were applied to establish the clinical diagnosis of poliomyelitis in the infected monkeys when therapy was administered: elevation of body temperature and presence of muscular paralysis. The clinical signs of each monkey in these 2 experiments at 48 hr. post-infection are shown in Fig. 1. It is known that the rectal temperature of normal monkeys varies a great deal. Nonetheless, it is reasonably certain that a temperature above 39·5° may be considered abnormal. As is shown in the figure, approximately half the animals had developed pyrexia at the time therapy was started. Also, as indicated by the blackened areas in the monkeys, all manifested a certain degree of paralysis, involving from one of the lower legs to both of them. In our experience, any grade of paralysis beyond paresis of one of the lower legs is unequivocally due to the viral infection.

The fate of these animals is summarized in Fig. 2. As can be observed, 3 of 4 and 4 of 5 in the control groups of the first and second experiments, respectively, died from severe paralysis caused by the viral infection. Thus the total mortality was 7 of 9 or 78 per cent. The muscular paralyses of the monkeys that succumbed to the infection extended to the entire body and 4 limbs, except for 2 animals (16 in exp. 10, and 16 in exp. 33); these animals died too soon to permit full development of paralysis. Such a phenomenon has been described previously (Liu *et al.*, 1958) and may be ascribed either to an extensive encephalitis or to rapid involvement of the majority of the respiratory muscles. Two monkeys which survived for the entire period of observation displayed paralysis of muscles below the waistline. In addition to the paralysis of the lower body and limbs, one of the animals (15 in exp. 33) showed paralysis of the left facial muscles, a condition that occurred rarely after an intraspinal inoculation.

In the treated groups, only one monkey (12 in exp. 33) succumbed to the infection with complete paralysis on the 5–6th day post-infection. The reason for this one failure remains obscure, because no necropsy could be performed. Two additional animals (17 and 22) in exp. 10) expired in the 4th week post-infection from uncontrollable diarrhoea, but their paralyses were definitely less extensive than those of controls. Therefore, the serum treatment

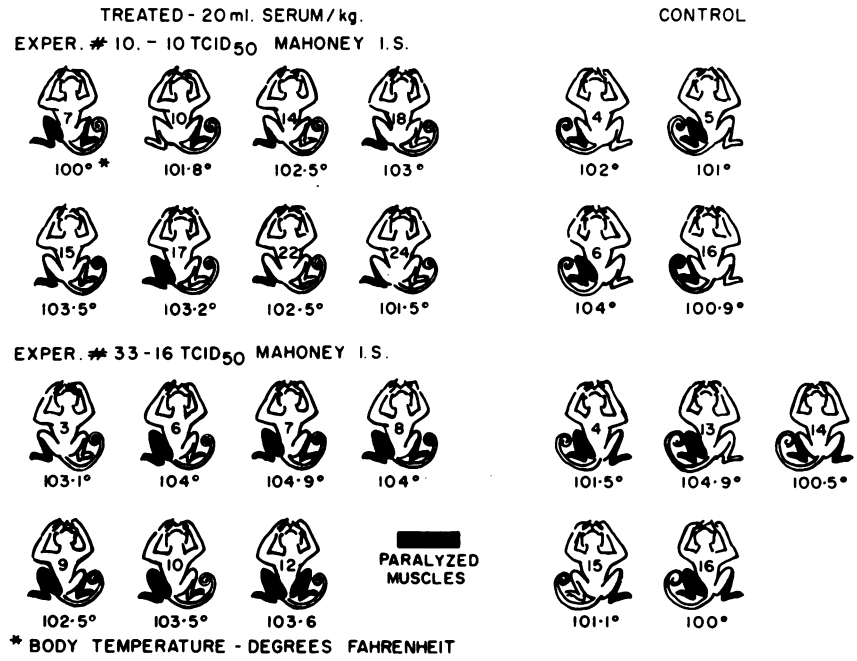

FIG. 1.—Clinical signs in monkeys at 48 hr. after intraspinal inoculation of 10–16 $TCID_{50}$ of Mahoney strain of poliovirus.

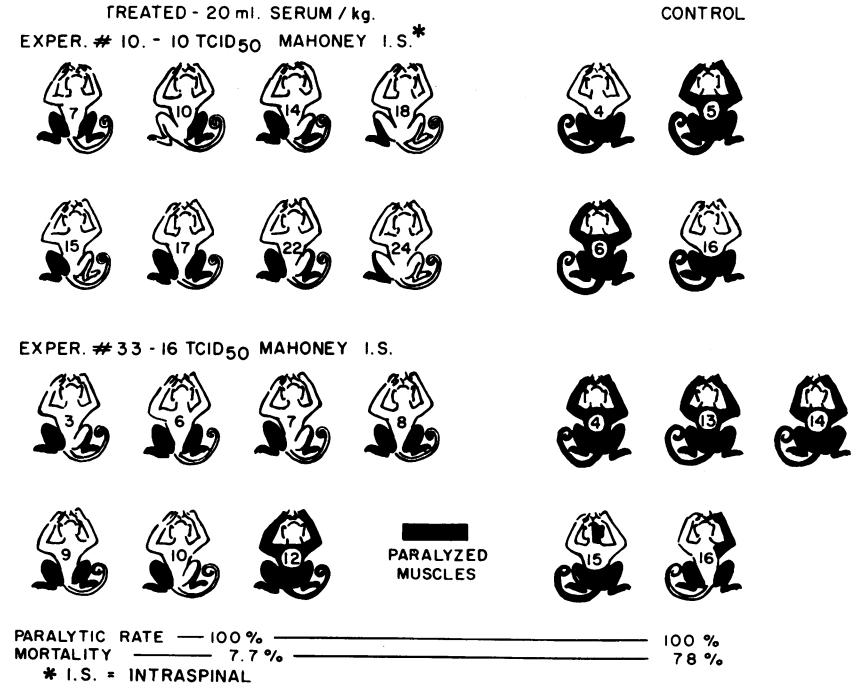

FIG. 2.—The fate of the monkeys shown in Fig. 1 ($P < 0{\cdot}01$; 95 per cent confident limits of mortality ; treated 0·2–32 per cent and controls 40–97 per cent).

for these 2 monkeys should be considered successful. The remaining monkeys survived with limited paralysis for the entire follow-up period. The mortality of the treated groups thus averaged 7 per cent (1 of 15). Even if the other 2 animals that died from diarrhoea were assumed to have succumbed to poliomyelitis, the total mortality would then be only 20 per cent which still would be substantially lower than that found in the control groups (78 per cent). With regard to the degree of muscular paralysis of the treated animals, it should be emphasized that at the termination of observation no monkey acquired a paralysis involving more than the 2 lower limbs, except 12 of exp. 33, listed above. Hence, there appeared no doubt that the extent of muscular disability in the treated was much less than that in the controls.

Comparison of Figs. 1 and 2 shows that the progression of muscular paralysis in the treated animals was completely arrested within a period of 1–3 days after administration of the serum. The length of time required varied in individual monkeys. The possible reasons for such a delay in stopping the progress of the disease are discussed in a later section.

Serotherapy of poliomyelitis in monkeys infected intracerebrally. Only intraspinal infection was used throughout the prior study. One may wonder what would be the efficacy of the treatment if infection through another route were employed. Among the possible routes for infection, intrathalamic inoculation was selected for further study.

The first experiment included a total of 11 monkeys. Each animal was infected intracerebrally with 500,000 $TCID_{50}$ of Mahoney virus in 1 ml. of medium 199. The animals were examined daily for rectal temperature, muscular weakness and other neurologic signs. On the basis of clinical observation, 2 animals which appeared conspicuously sick were selected for treatment on each of the following days: 3rd, 5th, and 7th day post-infection. The treatment consisted of an intravenous injection of 15 ml./kg. of the hyperimmune rabbit serum. The remaining 5 monkeys, untreated, served as controls. Among these 5, one monkey was sacrificed on the 3rd and another on the 7th day post-infection. The viral contents of various levels of the CNS were examined according to the method described in the previous report (Liu *et al.*, 1958). It was found that as early as the 3rd day after inoculation the virus was disseminated throughout the CNS except in the occipital lobe, and significant quantities, $10^{3.6}$ to $10^{6.0}$ $TCID_{50}$ per g. of tissue, were found at all levels in the spinal cords of these 2 sacrificed animals.

In the second experiment, 6 monkeys were employed. Two groups of 2 monkeys received 5 ml./kg. and 10 ml./kg. of the same serum, respectively on the 6th day after infection with the identical amount of virus. The remaining 2 animals without treatment were controls.

All animals in these experiments were observed for 5 weeks after the infection. The results revealed that all the 6 monkeys treated with 15 ml./kg. were free of any detectable muscular paralysis at the end of the observation period, whereas the 5 controls and the 4 monkeys receiving less than 15 ml./kg. of serum showed varying degrees of paralysis involving 2–4 limbs.

The clinical course of poliomyelitis in monkeys following intracerebral inoculation

The clinical course of the disease in monkeys after intrathalamic infection differed considerably from that of intraspinal inoculation. Typical clinical records of an untreated monkey and monkeys that received the serum 3, 5, and 7 days post-infection, respectively, are shown in Fig. 3. In general, on the first day after inoculation, the untreated animals invariably showed elevated rectal temperatures which lasted for only 1–2 days. The fever began to fall by lysis on the following day and the temperature stayed within normal range for the next 1–4 days. During this period, the animals appeared alert as usual. Thereafter, the second spike of temperature developed, and the animals manifested anorexia, sluggishness roughened coats and other neurologic signs *i.e.*, tremor, unsteady gestures, irritability. As the lysis of the second spike began, weakness of one of the limbs, ptosis of the eyelid, or deviated neck became detectable. The paralysis progressed for a period of 1–2 weeks. Finally, a wide involvement of muscles of limbs, face and neck resulted. In general, the paralysis was not severe enough to cause death and the majority of the animals lived until the end of the usual observation period.

As shown in the top chart of Fig. 3, a typical picture of an untreated control (12) is illustrated. Although the monkey showed involvement of 4 limbs at the end of 2 weeks, sufficient movements of its neck and hands persisted permitting the animal to live until the end of the experiment. In the second chart, monkey 6 was treated on the 3rd day post-

infection when the second elevation of temperature was apparent. Two days after treatment, the monkey appeared sick. This lasted for only one day, and throughout the rest of the time the animal was in good health. The third chart shows monkey 1 that received the serum on the 5th day following infection. At that time, pyrexia and shaking of the head were observed. The latter sign persisted for 6 days. In addition, paresis of the left lower leg was noticed between the 7th and 9th day. However, the animal recovered completely by the end of the second week and stayed well for the rest of the period. In the last chart, monkey 7 was treated on the 7th day post-infection when fever had been present for 3 days and at the time of treatment neurologic signs were evident. Despite these, the monkey did not develop permanent paralysis and appeared normal again on the 3rd day post-treatment.

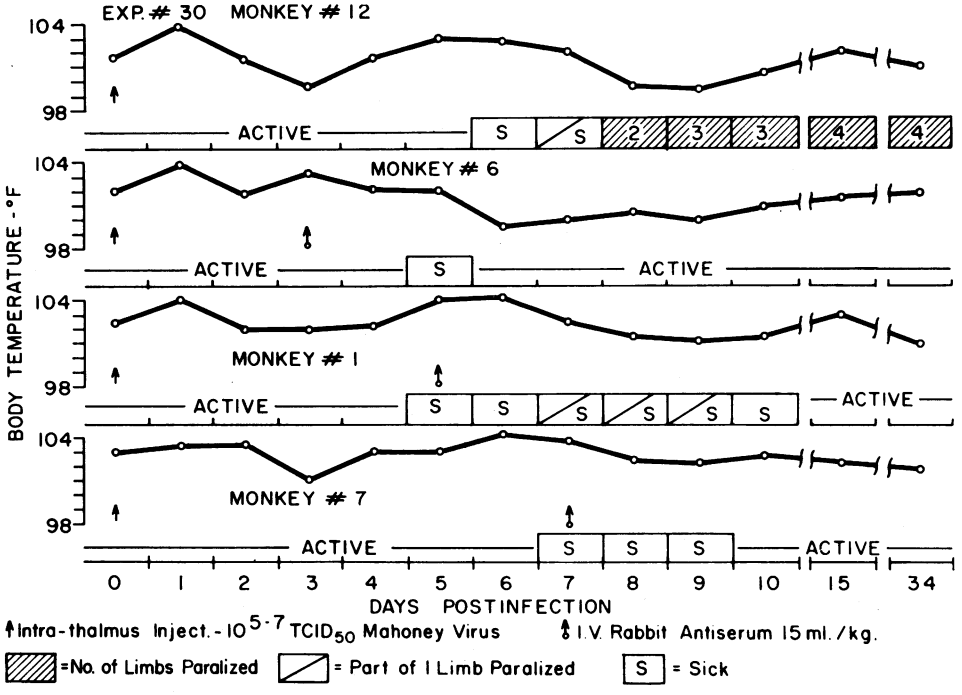

FIG. 3.—The effect of serotherapy on polio infection in Rhesus monkeys after intracerebral inoculation of 500,000 $TCID_{50}$ Mahoney strain of poliovirus.

Antibody levels in the blood of monkeys before and after treatment

In all experiments which were carried out, including those in the previous paper (Liu *et al.*, 1958), serum specimens were taken from the monkeys routinely before and after the infection and their neutralizing antibodies against Mahoney virus were titrated. Since many reports have appeared in the literature concerning this subject, these data have not been presented. However, in order to illustrate the antibody content in the blood of animals before and after treatment with the immune serum, the results of the titrations of the sera obtained from 4 animals in the first experiment using intracerebral challenge are summarized in Table I.

As can be seen, no animal had demonstrable antibody in the serum before treatment. At 10 min. after the treatment was given, the neutralizing antibody against the Mahoney virus in the serum increased to an enormous level. The arithmetic mean titre of the 4 specimens was 17,700 units per 0·5 ml. In the experiment, the animals received 15 ml./kg. of rabbit serum with 75,000 units of antibody per 0·5 ml. Therefore, a total of 2,250,000 (75,000 × 15 × 2) units of antibody should be present in every kg. of body weight of these animals. If approximately 10 per cent of the body were whole blood, the volume of plasma would be

TABLE I.—*Neutralizing Antibody Titres against Mahoney Strain of Poliovirus in the Blood of Monkeys Before and After Treatment with Hyperimmune Rabbit Serum*

Monkey No.	Serum taken: 10 min. before treatment	Serum taken: 10 min. after treatment
1	<5*	15,600
6	<5	16,900
8	<5	17,000
11	<5	21,400
Average	<5	17,725

* Reciprocal of the 50 per cent neutralizing end point.

about 50 ml. per kg. of body weight. Theoretically, the total amount of antibody should be distributed evenly into 65 ml. of the intravascular fluid (50 ml. of plasma and 15 ml. of the injected serum), hence the titre of the serum of the animals immediately after treatment should be 1 : 17,300 per 0·5 ml. The average titre of the neutralizing antibody found in the animal serum, 1 : 17,700, is indeed quite close to the theoretical calculated value recorded above.

Preparation of hyperimmune anti-polio γ-globulin from human plasma

Seventy-five donors were selected from the Philadelphia Donor Center of Merck Sharp and Dohme Division, Merck and Co., Inc. Each was immunized twice with 1 ml. of Salk vaccine intramuscularly at an interval of 2 months. Samples of serum were procured from all donors prior to immunization. Titres of neutralizing antibodies against the 3 types of *poliovirus hominis* were determined by Microbiological Associates, Inc. It was found that all pre-immunization sera of the 39 donors who had completed the course of 2 injections contained antibodies against at least one type of virus ; 33·4 per cent had antibodies against any combination of 2 types and 61·5 per cent, antibodies against all 3 types. The titres varied from 1 : 4 to greater than 1 : 1024.

One pint of blood was collected from each of these 39 donors between 1 and 2 weeks after the second injection. The plasma samples from these bleedings were pooled and fractionated according to the methods indicated previously. The pool of plasma and the 16 per cent solution of γ-globulin prepared from it were tested repeatedly for neutralizing antibodies against 3 types of poliovirus. The average titres from 4 different assays for the plasma and γ-globulin and the arithmetic mean titres of the 39 sera obtained before immunization are shown in Table II. It is evident that the levels of antibody in the blood of these donors increased from 77- to 154-fold after a course of 2 injections of vaccine. An approximate 8-fold concentration was achieved in the 16 per cent γ-globulin as compared with the original plasma pool.

TABLE II.—*Titres of Neutralizing Antibodies against 3 types of Poliovirus of the Sera from 39 donors before immunization, of the hyperimmune plasma pool and of γ-Globulin prepared from the pool*

	Reciprocal of the 50 per cent neutralizing end point: Type I (Mahoney)	Type II (MEF_1)	Type III (Saukett)
Prevaccination serum	126	87	76
Post vaccination plasma	9,700	13,400	6,000
Folds increased (compared with prevaccination)	77	154	80
γ-globulin 16 per cent solution	75,050	86,280	52,130
Concentration factor (compared with plasma)	7·7×	6·4×	8·7×

Samples were removed from each step during the process of fractionation. Levels of antibody against 3 types of poliovirus were determined for all the samples, and the final

preparation, 4·7 per cent γ-globulin. The total units of antibody in each fraction were computed by multiplying the titre by its corresponding volume in litres. The percentage of the antibody content in every preparation was calculated on the basis that the number of units in the pool of plasma is considered as 100 per cent of the source of the antibody. These results are summarized in Table III.

TABLE III.—*Neutralizing Antibodies against 3 types of Poliovirus in Various Fractions of Human Hyperimmune Plasma*

Fraction (per cent)	Amount in litres	Type I antibody			Type II antibody			Type III antibody		
			Total units			Total units			Total units	
		Titre	(×10³)	per cent	Titre	(×10³)	per cent	Titre	(×10³)	per cent
Plasma (5·2)	14·2	8,050*	114·31	100·0	12,800	181·76	100·0	9,000	127·80	100·0
Fraction I (3)	1·7	154	0·26	0·23	600	1·02	0·39	400	0·68	0·53
Sup I (2·35)	31·8	4,160	132·20	115·65		N.D.		4,460	141·85	110·99
Sup. II & III (4·26)	12·0	524	6·29	5·50	1,048	13·58	7·42	1,230	14·76	11·55
Sup. II & IIIw (0·46)	10·0	1,200	12·00	10·50	800	8·00	4·40	800	8·00	6·03
Sup. II	25·0	63	1.58	1·39	100	2·50	1·38	143	3·58	2·72
Ppt. III (2·5)	2·5	2,240	5·60	4·90	2,240	5·60	3·08	2,240	5·60	4·38
γ-globulin (4·7)	1·5	30,800	46·20	40·42	38,400	57·60	31·69	30,800	46·20	36·15

* Reciprocal of highest dilution neutralizing 10–50 $TCID_{50}$ of 3 types of *poliovirus hominis* (50 per cent end point).
N.D. = Not done.

As is revealed, a considerable quantity of neutralizing antibody was lost in various fractions and in various steps during processing. In the final product, γ-globulin contained only 31–40 per cent of the total antibody present in the plasma. Fraction I (fibrinogen, antihaemophilic globulin, etc.) contained 0·23–0·53 per cent of the entire antibody; supernatant II + III (albumin, α-lipoprotein, metal binding globulin, etc.) 5·5–11·5 per cent; supernatant II + III-W (lipoprotein and a small quantity of γ-globulin), 6·0–10·5 per cent; supernatant II (residual γ-globulin), 1·4–2·7 per cent and precipitate III (iso-agglutinin, prothrombin, plasminogen, fibrinogen, γ-globulin, etc.) greater than 3·1–4·9 per cent. Presumably, the latter fraction should contain considerably more antibody than was shown (Sanders, unpublished). Unfortunately, this particular test cannot be repeated because of a limited supply of certain samples. Also, supernatant III containing the γ-globulin fraction should have been assayed prior to fractionation and precipitation. At any rate, there was approximately 30 per cent of antibody which cannot be accounted for. This loss may be ascribed to the filtration and other manipulations during the process. However, there is no way to ascertain whether or not inactivation or denaturation of the antibodies had occurred.

Treatment of poliomyelitis in monkeys with human γ-globulin

Three experiments were carried out to test the efficacy of the prepared γ-globulin in treating poliomyelitis in monkeys. Six to 8 rhesus monkeys weighing 2–3 kg. were used for each experiment. Immediately after intraspinal infection with 100 $TCID_{50}$ of Mahoney virus, half the animals were treated with the 10–16 per cent aqueous solution of human γ-globulin intravenously and the remainder used as controls. The amounts of γ-globulin employed in these experiments were 0·3, 0·6 and 1·5 g/kg., respectively. All animals were observed for a period of not less than 4 weeks following infection and therapy. Special attention was paid to their muscular activities for the entire period. The outcome of these experiments are illustrated in Fig. 4. As is shown in the figure, almost half the monkeys died too rapidly to develop an extensive paralysis, probably for the reasons which were referred to previously, *i.e.*, disseminated damage in brain stem or paralysis of the respiratory muscles. The number of animals developing such fulminating disease increased as a larger infectious dose was employed.

In the groups treated with 0·3 and 0·6 g./kg. of γ-globulin, there was no difference in the course of illness and the extensiveness of muscular involvement as compared with the corresponding controls. In contrast, definite therapeutic effects were observed in the animals receiving 1·5 g./kg. of the human material. None of the treated group died while all the

controls succumbed to the infection. One monkey (2) among the treated died on the 17th day post-infection from apparent enteritis and malnutrition. This same animal showed a partial paralysis and the other 2 appeared to have no disability, whereas all the controls were completely paralyzed. From these data, it appeared that the antibodies derived from human sources were as effective in arresting polio infection in the CNS of monkeys as those of rabbit origin.

It has been reported (Stokes, 1944 and Janeway, personal communication) that shock can be elicited in humans by giving γ-globulin intravenously. Shock occurred in the above experiments when the hyperimmune human γ-globulin was injected into monkeys. Generally, administration of the total volume of the globulin took 10–15 min. for each animal. About

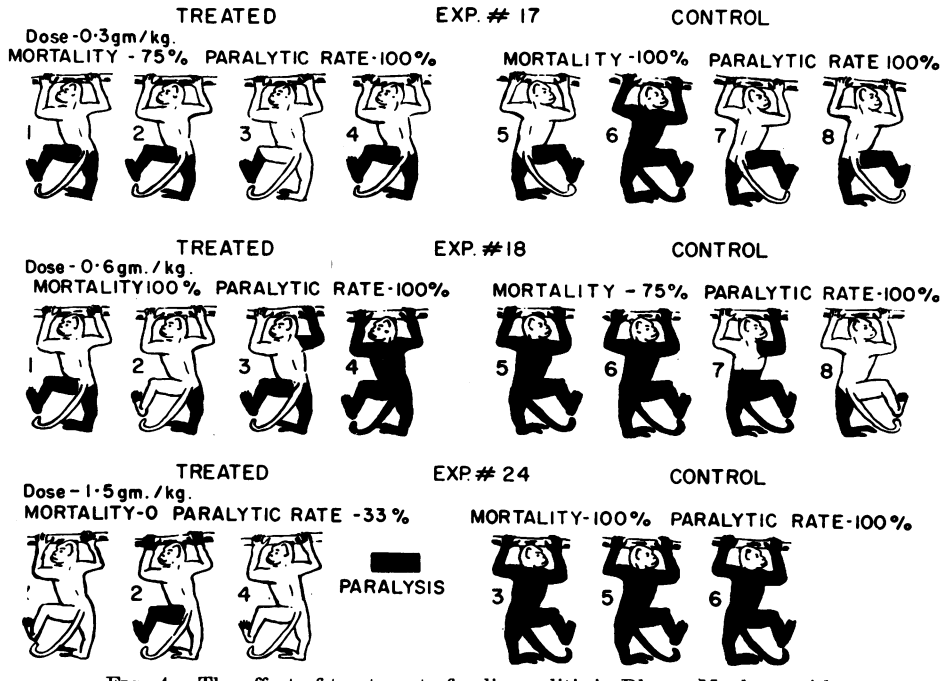

FIG. 4.—The effect of treatment of poliomyelitis in Rhesus Monkeys with varying amounts of hyperimmune Human γ-globulin.

25 per cent of the treated animals developed shock-like symptoms at the early stage of injection, within 1–2 min. after beginning the injection. It seemed that this adverse reaction did not appear to be related to the volume and concentration of the preparation used, but rather that certain animals were apparently sensitive to this material. Fortunately, no monkey succumbed from this reaction which was controlled by subcutaneous injection of 0·1–0·2 ml. of 1 : 1000 epinephrine either prior to administration of the γ-globulin or immediately following the appearance of early signs of this syndrome, *i.e.*, increased depth of respiration and pallor of face.

DISCUSSION

As early as 1944, Stokes analyzed the possibility of serotherapy of viral infections and indicated its future potential. In the following decade, various investigators attempted to treat a score of viral diseases with specific antiserum or its concentrates. Some encouraging information was obtained for measles (Stokes, Maris and Gellis, 1944); mumps (Derman and Lehew, 1944; Gellis,

McGuinness and Peters, 1945); rabies (Habel, 1945; Ghodssi, 1947, and Koprowski and Cox, 1951); equine encephalomyelitis (Zichis and Shaughnessy, 1945; and DeBoer, Cadilek and Walters, 1955) and so on. However, the clinical trials with the γ-globulin prepared from convalescent human plasma to treat poliomyelitis in the preparalytic stage did not appear promising (Bahlke and Perkins, 1945). Consequently, wide application of specific antiserum in this disease was deemed impractical.

So far as is known, the function of antiserum in the treatment of a viral infection is only to prevent further spread of the pathogens. The cells which have already been invaded by virus probably will be completely destroyed even under adequate therapy. Of greatest importance to the host is the number of susceptible cells which are protected from invasion by the virus. It is logical to assume that if a considerable number of the cells were kept intact by the serum, and particularly if the vital respiratory and circulatory centers were protected, it would be possible for the host to live. Factors which are essential for achieving protection of as many cells as possible by antiserum are: early diagnosis of the particular disease, and prompt administration of the largest feasible dose of the serum intravenously. These principles have been practised in humans for the past half century on bacterial diseases, viz. diphtheria, tetanus.

As knowledge in the field of virology advances, it has been accepted that each virus behaves characteristically in its mode of spread, propagation in certain tissues, production of definite sequelae and the ultimate effect on its host. Therefore, the therapy for any given viral disease should be considered as an individual problem. According to Horstmann (1949) and Smadel and Adair (1952), poliomyelitis in man can be differentiated into 2 phases, the minor and the major. A case of poliomyelitis is diagnosed only after the beginning of the major phase, especially during an epidemic. In childhood-type poliomyelitis, the paralysis usually sets in 2–3 days after the onset of the major phase; whereas in the adult-type disease, paralysis may appear as late as 6–8 days after the major phase has started. Since Salk vaccine has become widely available, with special emphasis on the vaccination of children, it is reasonable to expect that cases of the adult-type would occur relatively more often than the childhood type. Hence diagnosis can be established early enough before the onset of paralysis to allow for administration of the serum.

Both the present and the previous (Liu *et al.*, 1958) reports deal with different aspects of serotherapy of poliomyelitis. This experimentally produced condition in which the CNS has been seeded with active virus is probably analogous to the commencement of the major phase of the disease in man (Bodian, 1955). The experiments previously reported (Liu *et al.*, 1958) were based on the assumption that any measure which can prevent the muscular paralysis 1–2 days before its occurrence would be a valid treatment for this disease in its pre-paralytic stage. In the current study, as mentioned in the beginning, emphasis was placed on the therapeutic angle. The experiments were designed to show whether or not the serum can modify the progression of muscular paralysis after it has begun. The success of serotherapy for such a condition depends upon not only the general principles just stated, but also the amount of specific antibody which can cross the blood-brain barrier. As discussed previously (Liu *et al.*, 1958), there appears to be a definite threshold existing for the antibody between the general circulation and the brain-tissue proper. This concept is confirmed particularly by the experi-

ments carried out in the current study. In the experiments using intracerebral challenge, 15 ml./kg. of the hyperimmune rabbit serum were solidly curative whereas 10 ml./kg. of the same serum were completely devoid of effect.

When an intracerebral challenge was used, the course of the experimental illness resembled the natural disease even more closely. The data presented leave no doubt that the serum treatment is effective in preventing further paralysis at any stage of the disease. When an adequate quantity of serum was given, the non-infected neurons in the CNS were protected. As shown in Figs. 1 and 2, the ascending paralysis was arrested completely within a period of 1–3 days according to the individual animals. The reasons for the delayed effect of the treatment may be twofold: a certain length of time is required for the antibody to reach the site of infection. As illustrated by the experiment summarized in Table I the neutralizing antibodies were dissipated evenly into the general circulation within a few minutes after intravenous injection. This has been confirmed by using ^{131}I labelled γ-globulin. However, 24–48 hr. were needed for a significant amount of the iodinated globulin to enter the brain tissue. These results will be reported in detail elsewhere (Liu and Carter, unpublished). And the neurons which had already been invaded by the virus ultimately degenerated.

It has been pointed out in the previous report (Liu *et al.*, 1958), that 3 factors may be responsible for the negative results of the therapeutic trials in poliomyelitic patients (Bahlke and Perkins, 1945). These factors are: low potency of the antiserum used, incorrect route of injection of the serum and existing high viral content in the CNS at the time of treatment.

Colio, Criley and Coriell (1958) reported recently that they prepared a hyperimmune antipoliomyelitis γ-globulin with very high neutralizing titers against 3 types of Poliovirus hominis. However, when this was used for treating monkeys infected with Mahoney virus after the onset of paralysis, it failed to produce a clear-cut therapeutic effect. Their methods differed in several important respects from those used in this laboratory. The paralysis was induced by the intramuscular administration of undiluted poliovirus of high titre, so the 2 challenge methods are not comparable. More important, perhaps, are the 2 methods of administration of antibody, *i.e.*, intravenous against intramuscular. The intravenous route has 3 advantages over the intramuscular route: larger volumes can be given, higher antibody levels can be attained in the systemic circulation and therefore in the CNS and the time required to attain maximum levels in the CNS is much shorter since the intramuscularly administered antibody will require many hours to produce maximum serum levels in contrast to 10–15 min. for intravenously administered antibody. Other aspects of the distribution of antibody by various routes have been discussed by Stevens (unpublished). He concluded that only the intravenous and intracisternal routes held promise for the therapeutic use of antibody.

As indicated in the experiments in which intracerebral inoculation was employed, the infectious dose for each animal was approximately 50,000 times that used for the intraspinal infection in the majority of the experiments. As early as 3 days post-infection, abundant pathogens were found at all levels in the spinal cord of the monkey. In spite of the high content of virus present in the CNS, the disease was clearly milder than that produced by the intraspinal inoculation. This is shown by a longer incubation period, a prolonged course of illness, fewer paralyzed muscles, and lower mortality in the infected hosts. More-

over, in the disease thus produced, the serum treatment appeared much more efficacious. Therefore, it seems unlikely that the previous failure of sero-therapy resulted partially from the third factor, *i.e.* high content of virus in the CNS at the time of treatment. On the contrary, at least experimentally, the location in the CNS where the virus was first seeded plays an important role in the efficacy of therapy. There is as yet no satisfactory explanation for this phenomenon.

On the basis of the data presented in Fig. 4, the antibody derived from human sources appeared definitely effective in the treatment of monkeys infected intraspinally with poliovirus. However, difficulties may be anticipated if a large dose of γ-globulin is given by the intravenous route to man. For the purposes at the present time, e.g., study on the treatment of poliomyelitis in cynomologus monkeys infected orally or clinical trial in human cases, the hyperimmune human plasma (or serum) would be sufficiently adequate. The potency of these preparations can be improved by more intensive schedules of immunization with Salk vaccine for donors before bleedings and by careful selection of blood samples afterwards.

SUMMARY

A study was carried out on the effect of delayed treatment of poliomyelitis in rhesus monkeys. In two experiments, a total of 24 monkeys was infected intraspinally with 10–16 $TCID_{50}$ of Mahoney virus. At 48 hr. after infection, when all animals demonstrated various degrees of paralysis and half the number showed elevated rectal temperatures, 15 were treated intravenously with 20 ml./kg. of hyperimmune rabbit serum. At the end of 4 weeks, the mean mortality of the treated groups was 7 per cent as compared with 78 per cent of the 9 controls. Also, the degree of muscular paralysis in the treated animals was milder when compared with that of the controls.

Within a period of 3–7 days after intracerebral inoculation of the Mahoney virus (500,000 $TCID_{50}$ per animal), 10 monkeys were treated with varying amounts of the hyperimmune rabbit serum intravenously. The 6 animals which received 15 ml./kg. of the serum, even as late as 7 days post-infection, recovered completely, whereas the 4 monkeys treated with 5–10 ml./kg. of serum as well as the 5 controls developed extensive muscular paralysis.

Hyperimmune human γ-globulin was prepared from a pool of plasma obtained by bleeding 39 donors after 2 injections of Salk vaccine. Its efficacy was tested in monkeys infected with the Mahoney virus intraspinally. Its therapeutic effect was similar to that earlier observed with rabbit serum, suggesting that the quantity of antibodies, not the source, was essential in arrest of the polio infection in the CNS.

The authors wish to express their deep appreciation to Drs. Joseph Stokes, Jr., Werner Henle and Kingsley M. Stevens for their valuable suggestions throughout this work.

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