IMMUNIZATION AGAINST POLIOMYELITIS[1]

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TABLE OF CONTENTS

I. Introduction 339
II. Methods and criteria 340
A. Methods 340
B. Immunity following manifest poliomyelitis 342
1. Second attacks in man 342
2. Second attacks in laboratory animals 343
3. Development of neutralizing antibodies 344
C. Tissue immunity 346
1. Antiviral substances in tissues 346
2. Inoculation of specific tissues 348
D. Antibody protection against intracerebral challenge 348
III. Active immunization 349
A. With active, unmodified virus 349
1. Primates: intra- and subcutaneous, intraperitoneal, intramuscular 349
2. Use of adjuvants 357
3. Primates: intranasal and miscellaneous routes 359
4. Vaccination of rodents 364
5. Primates: Immunization through the alimentary tract 367
B. With inactivated or modified virus 370
1. Vaccines treated with physical agents 370
2. Vaccines treated with chemical agents 376
3. Live, rodent-adapted virus 401
IV. Passive immunization 404
A. Effects on antibody level 404
B. Passive protection in animals 405
1. Intracerebral challenge 405
2. Intranasal challenge 415
3. Challenge by other parenteral routes 419
4. Challenge by way of the alimentary tract 421
5. Combined active and passive immunization 423
C. Human trials 431
V. Conclusions 438
VI. Bibliography 438

I. INTRODUCTION

Experimental transmission of poliomyelitis to animals was first accomplished in 1908, and soon thereafter the etiologic agent was shown to be a filterable virus. Within two years it was known (a) that monkeys surviving one attack were resistant to infection; and (b) that the blood serum of such survivors acquired the property of neutralizing the virus *in vitro*. The association of (a) and (b) suggested that susceptible subjects might be passively protected against infection by blood from immune donors. Experience with other viral

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diseases also encouraged attempts at active immunization with vaccines. Although work toward both objectives has gone on since that time, only one decisive experiment on prophylaxis in man, by either method, has yet been carried out. That was a trial of passive immunization with gamma globulin, prepared from immune blood and containing the specific antibodies in concentrated form. From the outcome, published within the last year, we now know that passive immunization in man does confer temporary protection. The present and prospective supply of gamma globulin, however, is wholly inadequate for treatment of the entire susceptible population, and the protection it affords is limited to a few weeks.

The alternative procedure, active immunization, was long ago shown to be effective in some degree in animals. The methods of testing first employed failed to reveal its full potentialities. Recent advances of knowledge and techniques have made it possible to prepare vaccines that give to animals a high degree of protection over a substantial period of time, and to control the dosage through assay. Within the last two years three laboratories have published reports of active immunization against poliomyelitis in small groups of human subjects. More extensive investigations may obviously be anticipated.

The rationale of experiments on immunization against poliomyelitis has thus become a matter of interest to many persons outside the narrow group of those working directly with the virus in the laboratory. To review the literature critically at first hand is a formidable task because it is so voluminous and so complex. The following is a combination and revision of two papers originally written in 1951 and 1952, respectively. They were not at the time meant for publication but were distributed in mimeograph to a limited group of personnel concerned with specific projects. It was believed that such readers would not be particularly interested in a reviewer's personal interpretation of the literature, but might find useful a concise account of methods and results, sufficient to enable a reader to judge for himself the significance of the various individual contributions. The present version has been submitted for publication in response to several suggestions that it might be of interest to a larger audience. Literature appearing up to approximately the mid-year of 1953 has been included. The original method of treatment has been retained.

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II. METHODS AND CRITERIA

A. *Methods*

The known manifestations of immunity to poliomyelitis are (a) resistance to infection by natural or artificial means, in species naturally susceptible; (b) appearance of neutralizing antibody in the circulating blood; and (c) appearance of complement-fixing antibody. Of these, only the first two have been used in connection with the development or evaluation of immunizing procedures.

The complement-fixation test as applied to poliomyelitis has been rendered workable only recently.

Test inoculation of immunized animals was first made intracerebrally, and no other route of challenge has been so widely used. Since intracerebral inoculation induces paralytic poliomyelitis most consistently in all susceptible laboratory animals, it facilitates comparison with controls. No other method lends itself so well to quantitative control of virus dosage.

The first nontraumatic method found for experimental poliomyelitis infection was intranasal inoculation, applicable to primates but not to mice or cotton rats. It has been employed for the challenge of immunized animals, particularly during a period when the olfactory nerve was believed to be the pathway of natural human infection. In monkeys infected intranasally, anatomical evidence of virus invasion is usually demonstrable in the olfactory centers, but post mortem study of fatal human cases rarely shows involvement of those areas (1, 2). For this and other reasons human infection is now generally believed to occur by some other route.

Effective immunization of animals against paralytic poliomyelitis proved extraordinarily difficult to achieve so long as intracerebral or intranasal challenge was the test applied. It has long been recognized, however, that these artificial modes of challenge might be misleading with respect to the practical problems of human immunity and prophylaxis. Subclinical poliomyelitis infection in man, first inferred from epidemiologic observations, is now known to be much more common than the clinically recognizable disease. Even at the clinical level, human poliomyelitis runs a variable and usually a mild course compared to the fulminating paralysis so frequently observed in animals infected intracerebrally or intranasally.

Until the last decade, no method was known for producing in animals a pattern of infection corresponding to that observed in man. No method has yet been found for producing such a pattern in some of the animal species susceptible to poliomyelitis infection by artificial inoculation—(rhesus monkeys, cotton rats, mice). There is a striking parallel, however, between the infection produced by oral inoculation of chimpanzees or cynomolgus monkeys and that occurring naturally in man [for comparison see Bodian (3)].

The first published reports of oral challenge of animals immunized against poliomyelitis are those of Howe and Bodian (4) in 1945, and of Melnick and Horstmann (5) in 1947, dealing respectively with passive and active immunization of chimpanzees. The relative ease with which protection can be achieved against challenge by this route has been largely responsible for the revival of interest in the possibility of human prophylaxis through immunization. Meanwhile, the older method of intracerebral challenge has been recognized as inapplicable to this problem. It has continued to be useful in basic studies on immunity, such as the typing of virus strains.

Challenge by inoculation into subcutaneous or other peripheral tissues has occasionally been tried. In general, however, infection of control animals by these routes is infrequent and the percentage inconstant. Adequately controlled

experiments, utilizing such routes for challenge, have been reported only recently and have taken advantage of the relatively high infectivity of certain strains of virus in appropriate hosts (6, 7).

Development of quantitative methods of assay for virus and for antisera was slow. For some years only qualitative tests were employed, not always with adequate controls. In the literature of the first two decades virus dosages are generally given only as volume of inoculum and (irregularly) concentration of the infected tissue suspension. Titering methods were introduced about 1930, and the basic procedure has remained the same. A unit infecting dose of virus is first established by inoculation of animals with serial dilutions. Serial dilutions of serum are then tested against a constant dose of virus (occasionally *vice versa*), again by animal inoculation. The chief source of error in the early methods was failure to take into adequate account the biologic variation in response of test animals to identical inocula. Thus a relatively mild infection, or an incubation period longer than average in a single animal, was frequently considered as an index of borderline virus dosage or, in neutralization tests, of "partial protection". Such an interpretation seems unwarranted unless the number of animals involved is large enough to exclude the factor of chance variation. Similarly, it was considered possible to define the "minimal infective dose (MID)" or the "minimal completely paralyzing dose (MCP)" of virus by inoculation of 1 to 3 animals per dilution (8, 9). It now seems clear that these methods were incapable of defining infective units with any degree of precision, and that further error was introduced when serial dilutions of serum were tested, again in small numbers of animals, against such indeterminate dosages of virus. Limitations of the assay procedures employed up to 1940 are pointed out in the critical review of Schaeffer and Muckenfuss (10).

References below to the earlier literature include brief mention of the methods of titration where any were used. It would be tedious and probably futile, however, to describe in detail and attempt to evaluate the various techniques. Within the last decade quantitative methods have been put on a sounder statistical basis, employing animals in sufficient number to make possible a valid calculation of the minimal virus dosage required to infect, paralyze, or kill 50% of those inoculated (ID_{50}, PD_{50} or LD_{50}). Similarly, serum titer is expressed as the highest dilution adequate to protect 50% of animals against the same effects, using a known virus dosage. Since laboratories generally employ such methods at the present time, references to recent literature will omit descriptions of assay procedures.

Tissue culture techniques, recently introduced through the work of Enders and associates, have greatly facilitated qualitative and quantitative tests of poliomyelitis viruses and antisera. Nearly all the work here reviewed, however, was done before these methods were available.

B. *Immunity Resulting from Manifest Poliomyelitis*

1. *Second attacks in man.* Immunity to poliomyelitis appears first to have been inferred from the rarity of recorded second attacks in man. Actual data on their

frequency, however, are available only for limited population groups and periods of time. From such data it has not been possible to establish statistical proof that the incidence of poliomyelitis is any less in persons with a history of previous attacks than in the population at large (11). The existence of immunologically distinct types of virus provides a rational explanation of second attacks that is consistent with immunity, but only two such cases have been subjected to laboratory study of the infecting types (12). In both of these the virus responsible for the second attack was isolated and identified as Type 3. Serologic evidence of a previous infection with Type 1 was obtained in one patient, but no evidence could be obtained regarding the virus type responsible for the first attack in the other case.

2. *Second attacks in laboratory animals.* The subject of second paralytic attacks in monkeys has recently been reviewed in detail by Bodian (13) and will be passed over briefly here. Monkeys surviving an attack induced by one virus can become paralyzed again when inoculated with a heterotypic strain. The attack rate, however, is considerably lower than in previously uninfected animals. Bodian used 4 strains of virus, of which 3 were of Type 1 and the fourth of Type 2. Animals were inoculated with 100 to 10,000 PD_{50} of virus intracerebrally. The attack rate in control monkeys, with the 4 virus strains, varied from 18/18 (100%) to 20/22 (91%). Second attacks occurred in 36 of 72 monkeys (50%) on inoculation with virus of a type different from that responsible for the first. No evidence of reinfection was found in any of 77 monkeys that had survived one attack and were later reinoculated with homotypic virus. Kessel and Pait (14), using 65 monkeys that had survived attacks induced by intracerebral inoculation with BK strain of virus, reinoculated them by the same route, 2 to 3½ months later, with 100 or 1,000 PD_{50} of the same strain. Two showed symptoms of reinfection, and virus was recovered from the cord of one. Earlier, Howe and Bodian (15) had reported second paralytic attacks in two monkeys, the second attack in each being induced by intranasal inoculation, the first by a different route. In each of these two animals the first attack was induced by the Wfd strain and the second by MV, but subsequent work classified these as being of the same immunologic type (16). The same authors also report several instances of two successive infections by the same strain where the second inoculation route gave access to previously uninvaded parts of the CNS.

In the older literature there are several reports of relatively high second attack rates in monkeys. Brodie (17) reinoculated 11 convalescents, of which 4 had suffered severe and 7 mild first attacks. Second attacks induced in these were 2/4 and 4/7, respectively. The dosage of virus used for reinoculation was 1 to 3 MCP, but it is not clear whether the same strain of virus was used throughout or not.

Toomey (18), also Moore and Kessel (19), reported relatively high second attack rates in animals reinoculated by the same route and with the same virus strain used to induce the first. That experience has not been confirmed in later studies. Possible explanations of this discrepancy are discussed by Bodian (13). Second attacks as a rule are relatively mild and difficult to diagnose. Hence,

studies in which no histological examination is made have a large inherent error. It seems fair to conclude that one paralytic attack in a laboratory animal confers partial protection against reinfection by heterotypic strains of virus, and practically complete protection against homotypic strains.

Paralytic poliomyelitis is usually fatal in rodents, but cotton rats that survived one attack have been found resistant to reinfection with homologous virus (20).

3. *Development of neutralizing antibodies.* Neutralization of virus by the serum of convalescent monkeys was reported from four different laboratories in 1910 (21, 22, 23, 24). In the same year Netter and Levaditi (25, 26) examined the sera of human patients who had suffered attacks from 6 weeks to 3 years previously. In the light of subsequent developments, it is interesting that their first reported tests (on 4 patients) were arranged by adding 1 ml of each serum to a tube containing 0.7 ml of a virus suspension (concentration not stated) and 0.2 ml of fresh normal human serum. The latter was added "*afin de réactiver les sérums*". A control tube contained 0.7 ml of virus and 1.2 ml of normal human serum. From each mixture 0.25 ml was used to inoculate one monkey intracerebrally. The control monkey developed paralysis. The 4 inoculated with mixtures containing the patients' serums all remained well. In their second report Netter and Levaditi had changed the procedure, no longer mixing normal serum with serum under test. They continued, however, as did a number of later investigators, to use normal human serum in the control mixture.

It would thus appear to have been an accident that the first tests on the sera of human patients were in such perfect agreement with the data from monkey sera. Later studies on human sera revealed that neutralization of poliomyelitis virus was not limited to subjects with a clinical history of poliomyelitis, that it occurred with sera of a majority of adults in samples from the population at large, and that in younger groups the percentage of positive reactions increased with age. Moreover, serum tests at various stages of human poliomyelitis sometimes failed to show an increase of antibody titer between onset and convalescence, or even to show positive reactions at any stage. Instances were also found where paralysis developed although serum obtained during the preparalytic stage neutralized the virus (27, 28). It was, therefore, questioned whether the virucidal agent in human blood was actually an antibody produced in response to viral antigen or a nonspecific substance originating in some other way. Any protective action by this agent against poliomyelitis was also doubted.

Data from animal experiments had not, as late as 1941 (29), helped to resolve these questions but only added further difficulties of interpretation. One was that monkeys convalescent from paralytic attacks, induced by intracerebral or intranasal inoculation, were found resistant to reinfection whether serum antibody was demonstrable or not.

Jungeblut (30) reinoculated 11 convalescent monkeys and tested the serum of each from a bleeding just prior to challenge. Sera of 6 neutralized; sera of 5 did not. All resisted intracerebral challenge with 1.0 ml of a 10% virus suspension, whereas 12 controls, inoculated with 0.1 to 0.6 ml of the same suspension, all

became paralyzed. The route of inoculation used for the original infection and the virus strain were unspecified. Sabin and Olitsky (31) used 9 monkeys convalescent from paralytic attacks induced by the MV virus strain (inoculated intranasally in 5, intracerebrally in 4). All were challenged intranasally with homologous virus. Three died within 4 days of causes not determined but apparently not poliomyelitis. Six resisted, and in tests on sera obtained at the time of challenge 5 did not neutralize the virus. Sera from 3 other convalescent resistant monkeys were pooled, and the pool titered for neutralizing potency by mixing progressivly diminishing amounts of serum with a constant amount of virus. A parallel test was run on another pool of sera from 3 vaccinated monkeys, all of which had become paralyzed on challenge. The end point of both pools was found to be the same.

Both Jungeblut (30) and Sabin and Olitsky (31) tested the sera of monkeys at varying intervals during the incubation period, the acute illness, and convalescence. Jungeblut tested 9 sera obtained during the preparalytic stage, reporting 4 positive, 4 negative and 1± (test monkey paralyzed, incubation period beyond 14 days). Sabin and Olitsky found all of 6 preparalytic sera negative. Other tests resulted as follows:

	TIME AFTER ONSET	NUMBER OF SERA TESTED	RESULTS
1. Jungeblut	Acute stage	12	1+, 10−, 1±
	2 weeks	3	1+, 2−
	1 month	6	3+, 2−, 1±
	2 months	6	4+, 2−
	3 months	2	2+
	4 months	2	2+
2. Sabin & Olitsky	4 weeks	5	5−
	2 months	6	4+, 2−
	3 months	6	6+

There are discrepancies between these two sets of data in regard to the neutralization tests in the early stages, but it seems clear that the neutralizing agent appears in the serum of monkeys in response to the infection, even though its appearance is delayed. Uninoculated rhesus monkeys were found to remain seronegative regardless of age (32, 33), in contrast to the serologic pattern found in the general human population.

These puzzling inconsistencies have largely been resolved within the past few years. The frequency with which inapparent infection in man has been demonstrated, by actual virus recovery, seems to provide a satisfactory explanation for the occurrence of neutralizing antibody in persons with no clinical history of poliomyelitis. The existence of multiple immunogenic types of virus, indicated as early as 1931 (34), but definitely established much later (16, 35), offers a rational explanation for the instances in which serum from human convalescents failed to neutralize. It is now known that neutralizing antibody is generally present in the human disease by the time a clinical diagnosis can be established, provided the serum is tested against the virus isolated from the patient himself

(36, 37). Neutralizing antibody develops early in chimpanzees and cynomolgus monkeys infected by feeding virus (38, 39), in contrast to its delayed appearance following infection by intranasal or intracerebral inoculation. Where paralytic disease results from oral infection in these species, antibody is demonstrable at the onset of symptoms as in the naturally acquired human disease (3, 39, 40). Finally, in an experiment on oral inoculation of man with poliomyelitis virus (41), there was an early development of neutralizing antibody associated with the virus carrier state although with no clinical evidence of infection.

C. *"Tissue Immunity"*

The rôle of neutralizing antibody, in protection against the paralytic infection, has been difficult to elucidate. From the observations of Jungeblut (30) and of Sabin and Olitsky (31), already cited, it appears that convalescent monkeys are resistant to intranasal or intracerebral challenge in the absence of demonstrable serum antibody. This has been interpreted as evidence of a local cellular form of immunity in tissues that have survived one invasion by the virus.

1. *Antiviral substances in tissues from immune animals.* Attempts have been made to demonstrate neutralizing or inhibitory effects of immune or naturally refractory tissues upon the virus *in vitro.*

Jungeblut (42) used a Berkefeld filtrate from a 1% suspension of virus (strain not stated). Each tissue, in 0.4 g amount, was ground in 2 ml of virus suspension. The emulsion was allowed to stand for 3 hours, then centrifuged. Monkeys were inoculated with 1.0 ml of the supernate. Tissues so studied included (a) normal monkey brain; (b) normal monkey cord; (c) poliomyelitic (acute) monkey cord treated with heat to inactivate virus in it; (d) convalescent monkey cord; and (e) normal monkey testicle. Of these, the first three tissues listed had no demonstrable influence on the infectivity of the filtrate or on the course of the resulting infection. Of 3 monkeys inoculated with filtrate treated with convalescent cord tissue, two became paralyzed, one remained well. Of 4 monkeys used in the experiment with monkey testicular tissue, symptoms were mild and poliomyelitis questionable in two. The other two became paralyzed after relatively long incubation periods.

Sabin and Olitsky (43) made neutralization tests with 10% suspensions of CNS tissue from 3 convalescent monkeys. The animals were sacrificed two to three months after onset and the sera of all 3 at that time neutralized the infecting strain of virus (MV). Suspensions of spinal cord, medulla, and pons tissue were tested separately against 10 to 20 MID of the same strain. No antiviral activity was found in any of the tissues.

Morgan (44) made comparative studies on neutralizing antibody (or antiviral substance) in serum, CSF, and central nervous tissue of normal monkeys. Neutralization tests were made in mice with serial dilutions of blood, or CSF, mixed with equal volumes of a 1:160 dilution of Lansing virus. A 0.03 ml inoculum of this mixture was calculated to contain 10 PD_{50} of virus. Tissues were ground and saline suspensions made by adding 2.2 ml of saline to 1.0 g of tissue. Serial dilutions of the suspension were then tested as with serum.

In one control uninoculated monkey, no antibody was demonstrable in any of the CNS tissues tested, in blood, or in the CSF. Four groups of monkeys, exposed to the virus in one way or another, were used:

(1) A group of 4, each vaccinated intramuscularly with Lansing virus, 6 doses divided over 3 weeks, totalling 1.2 g of infected monkey cord. All had remained well and were sacrificed at 6 weeks. Serum antibody titers at that time varied from 1:60 to 1:2,000. Spinal fluid, cord gray matter, and visual cortex tissues failed to neutralize virus.

(2) Two monkeys that developed paralysis during a course of vaccination and were sacrificed one and two months after onset. One showed equal titers of antibody in serum and in gray matter of the cord (1:600). In the other gray matter titered 1:300, serum 1:30.

(3) (a) Eight monkeys convalescent from paralysis induced by intracerebral inoculation with Lansing virus and sacrificed 16 to 23 days after onset. (b) Three monkeys, similar history, sacrificed 4 to 5 months after onset.

Of the 8 in (a), one showed no neutralizing antibody in any of the fluids or tissues. The others all showed titers of 1:80 to 1:600 in the cord gray matter. The visual cortex failed to neutralize. Serum was positive in 4, and CSF in 5, titers ranging from 1:2 to 1:80. Of the 3 monkeys (b) killed after a longer convalescence, all showed highest titers of antibody in the gray matter of the cord, 1:600 to 1:2,000. Antibody also was present, in lower titer, in other parts of the CNS. Titers in CSF and serum varied from 1:10 to 1:300.

(4) Five monkeys that had been inoculated intracerebrally with a dose of Lansing virus that paralyzed the majority of animals, but had escaped without evidence of infection. They were sacrificed at 21 days to 5 months after inoculation. No appreciable titer of antibody was found in any fluids or tissues from these animals.

Studies of a similar kind have more recently been reported by Sabin and Steigman (45), who failed to find in the CNS tissues of convalescent monkeys such antiviral activity as Morgan described. Seven monkeys were used, all convalescent from attacks of paralytic poliomyelitis induced by intracerebral inoculation with strains of virus recently isolated from human patients. They were sacrificed respectively at 27, 28, 28, 35, 37, 39 and 82 days after onset. They were perfused with saline, and the lumbar and cervical enlargements of the cord excised and placed on solid CO_2. Gray matter of the anterior horns was dissected free from other tissues, and suspensions of it in saline prepared by a procedure much the same as that employed by Morgan.

Varying dilutions of the gray matter suspension were tested against 10 LD_{50} of Lansing virus in mice, with results interpreted bv the authors as negative. Tests were then made against the homologous virus in each instance. Virus pools were titered using 3 monkeys per dilution, and neutralization tests run against a calculated 20 PD_{50} of virus. No significant neutralization was found.

Thus the evidence on record, concerning the existence of poliomyelitis antibody in CNS tissues previously invaded by the virus, consists of conflicting data. The discrepancy remains to be explained.

2. *Inoculation of specific tissues from immune animals.* Rasmussen and Clark (46) report that in convalescent monkeys peripheral nerves are resistant to virus invasion. They inoculated 7 convalescent and 7 control monkeys by dipping the central stump of a sectioned sciatic nerve into a suspension (potency and concentration not stated) of MV virus. The convalescent animals had suffered paralytic attacks from the same strain, and 4 of them had resisted reinoculation by the intracerebral route (dosage not stated). The animals were sacrificed at 2 to 5 days after inoculation. Tissues were removed from (a) the sciatic nerve at the site of inoculation, near the knee; (b) the same nerve at the sciatic notch; (c) the lumbar cord; and (d) the cervical cord. Emulsions (10%) of these tissues were prepared and tested for the presence of virus by inoculation of 1.0 ml intracerebrally into one test monkey for each tissue sample. No virus was recovered from any tissues of the convalescent monkeys. In the controls it was found at progressively higher levels with increase of the time interval since inoculation. Lesions were present in the CNS in areas where the virus was found. Serum neutralization tests were not made.

It seems pertinent to compare these observations with those of Howe and Bodian (15) referred to above. The latter authors reported successive infections by the same virus strain in situations where the second pathway of infection led to parts of the CNS not previously invaded.

Cultures of testicular tissue from immunized monkeys have been found to support virus growth as readily as do similar cultures from tissues of nonimmune monkeys (47). No comparable studies on tissues from convalescent animals have been reported.

D. *Antibody Protection Against Intracerebral Challenge*

The earlier literature presents abundant evidence that the mere presence of serum antibody does not insure against paralytic infection by intracerebral or intranasal inoculation. Kramer (48), however, reported that mice could be protected against intracerebral inoculation with Lansing virus by large doses of immune serum of high titer. Unpublished data by the same author (49) show that monkeys can be similarly protected for a brief period by human gamma globulin. Rhodes and associates (50) have reported similar findings. Morgan (51) studied the outcome of intracerebral challenge in relation to antibody level in a group of 79 monkeys. These had all been vaccinated with varying dosages of Lansing virus, and none had suffered paralytic attacks from the vaccination process (p. 355). It is sufficient here to note that the animals all were challenged intracerebrally with a very large dose (10,000 PD_{50}) of the homologous virus; and that of 37 monkeys with a serum antibody titer of $10^{-3.0}$ or higher, all resisted. Since Morgan's report, the possibility of protecting monkeys by vaccination against intracerebral challenge has been amply confirmed. Other work reviewed below shows that protection against paralytic infection by some other routes can be achieved at antibody levels quite low in comparison with those required to protect against intracerebral inoculation.

To summarize, there are two possibly independent mechanisms by which the

CNS tissues can be protected against invasion by poliomyelitis virus. In previously uninfected animals, serum antibody can protect against challenge inoculation, the effective antibody level varying with the route of challenge. CNS tissues once invaded by the virus may be resistant to reinfection whether serum antibody is demonstrable or not.

III. ACTIVE IMMUNIZATION

A. *With Active, Unmodified Virus*

Exposure to live poliomyelitis virus may, under certain conditions, result in immunity in the absence of clinical evidence of infection. The probability of such an outcome has been found to depend on the strain of virus employed, the host species, and the route of inoculation. On the first of these variables, the available information is fragmentary and no attempt will be made here to review it. We may merely note that pathogenicity for a given host may be modified by repeated passage in another species (52); that the ratio of antigenic to infective titer, as measured in the same host, is not constant from strain to strain (53) or even from one line to another of the same strain (54); and that two strains of approximately equal infectivity by intracerebral titer may be quite unlike in their ability to infect by other routes (55, 56). While the hope is thus encouraged that nonpathogenic, immunizing strains may be found in nature or developed in the laboratory, no strain now available is definitely known to have these ideal qualities.

Many of the published reports deal with several variables. To avoid repeating references and details of procedure, the literature on subcutaneous, intracutaneous, and intramuscular vaccination of primates will be reviewed roughly in chronological order and in one section (i). Separate sections will be devoted to (ii) the use of adjuvants to enhance antibody response; (iii) intranasal and other miscellaneous routes of vaccination in primates; (iv) vaccination of rodents; and (v) immunization of primates by way of the alimentary tract. The extensive literature on production of antisera, by vaccination of animals naturally refractory to poliomyelitis, will be omitted.

1. *Primates: Subcutaneous, Intracutaneous, Intraperitoneal and Intramuscular Vaccination. Monkeys.* In 1910 Flexner and Lewis (21) and Römer and Joseph (57) each reported vaccination of one monkey with live virus subcutaneously and resistance of the animal to subsequent intracerebral challenge. Flexner gave 18 injections over a period of 70 days, with dosage increasing from 0.05 to 5.0 ml of a virus suspension of unspecified concentration. Römer and Joseph gave a single injection, 1.0 ml of 5% suspension. Control monkeys were paralyzed in each instance.

Zappert, von Wiesner and Leiner (58) briefly mention 4 monkeys given increasing doses of live virus subcutaneously, dosage not stated. No tests for immunity are mentioned, but one monkey was paralyzed after 4 injections. Another developed an illness diagnosed as a "marantic" form of poliomyelitis. This is apparently the first reported instance of infection by subcutaneous inoculation

with poliomyelitis virus. The remaining two monkeys died of intercurrent infection.

Thomsen (59) vaccinated monkeys subcutaneously with a filtrate from a suspension of infected monkey CNS tissue (1 part) in saline (5 parts). In his first series of 7 monkeys, 2 developed paralytic infection from the vaccine. One had received a single dose of 0.5 ml; the other, 3 doses of 0.02, 0.04 and 0.08 ml, respectively. One monkey was lost by intercurrent infection. The remaining 4, vaccinated with repeated doses gradually raised from 0.02 or 0.03 to 2.0 ml, all remained well. None became paralyzed on challenge although 3 showed mild symptoms of illness.

Thomsen vaccinated a second series of 7 monkeys by injections repeated weekly for 62 weeks. Dosage was started at 0.005 ml and gradually increased to 2.0 ml at the final injection. All remained well and were challenged two weeks after vaccination was complete. Five remained symptomless, one showed tremor only, one developed mild paralysis and survived.

Intracutaneous vaccination was apparently first tried by Aycock and Kagan (60). The strain of virus used, the concentration, and the challenge dosage are not stated. Virus was rubbed into the scarified skin of one monkey, the treatment being repeated 9 times over one month. On intracerebral challenge the animal became paralyzed. Twelve monkeys were vaccinated by multiple injections (20 or more at each time) into the skin. About 0.05 ml was given through each piqûre. The procedure was repeated daily over 6 to 45 days, the total dosage amounting to as much as 76 ml over the entire period for some of the animals. One developed paralysis from the vaccine. Of the others, 1/11 became paralyzed on first challenge. Six were challenged a second time with a paralytic rate of 2/6. Two were challenged a third time and both resisted. Blood serum from 8 of the vaccinated monkeys was tested for antibody. Serum was mixed with an equal volume of virus suspension (concentration not stated) and 1.0 ml of the mixture given intracerebrally to test monkeys. Six of the 8 sera neutralized. Controls (the number is not clear but apparently was 9) were all paralyzed.

Subsequent workers administering vaccines intracutaneously used the method of multiple injections into the skin to make up the indicated dosages.

Stewart and Rhoads (61) compared the immunizing efficiency of intracutaneous with that of subcutaneous vaccination. A 5% suspension of glycerolated virus was used for vaccination, for challenge, and for neutralization tests. Each monkey received biweekly doses of 1.5 to 2.0 ml, continued 3 to 5 months, total dosage 42 to 66 ml per animal. One group (I) of 7 monkeys was vaccinated intracutaneously. Another group (II) of 8 was vaccinated subcutaneously. Six of each group received the MA, two the Aycock strain of virus. Finally all were bled for neutralization tests and challenged intracerebrally with 0.5 ml of 5% virus, in some instances of the homologous strain, in others not. Neutralization tests were made with 0.5 ml of undiluted serum against an equal volume of virus. Whether tests were made against the homologous or heterologous strain is not stated. Sera from all monkeys of Group I neutralized. Of this group, one monkey developed a transient deltoid weakness on challenge; none of the others showed more than

transient minor symptoms. In Group II, subcutaneously vaccinated, the outcome was as follows:

MONKEY NUMBER	STRAIN USED FOR Vaccination	Challenge	OUTCOME OF CHALLENGE	SERUM NEUTRALIZATION
9	MA	MA	Resisted	Sera of 9, 10 and 11 pooled
10	MA	MA	Resisted	
11	MA	MA	Resisted	+
12	MA	Aycock	Paralyzed	+
13	MA	Aycock	Paralyzed	Not tested
14	MA	Aycock	Paralyzed	−
15	Aycock	MA	Resisted	Not tested
16	Aycock	MA	Paralyzed	+

Three control monkeys for each group were all paralyzed. No monkeys of these two groups suffered infection from the vaccine. An eighth monkey originally in Group I died of intercurrent infection (tuberculosis).

Rhoads (62) suggested that vaccination starting with minimal doses of virus might actually sensitize animals and induce paralytic infection by the vaccine. He therefore tried, with 4 monkeys, single massive intradermal doses of 15 ml of a 5% suspension of MV virus. No illness followed. The monkeys were bled, and challenged intracerebrally, 30 days later. The dosage of virus used for challenge tests was 0.01 ml of filtrate from a 5% suspension of homologous strain. Undiluted serum, 0.99 ml, was tested against 0.01 ml of virus filtrate. Serum from all 4 monkeys neutralized. One control was paralyzed. Two of the 4 immunized monkeys resisted intracerebral challenge, one became paralyzed, one died of intercurrent infection.

Jungeblut and Hazen (63) vaccinated 4 monkeys intradermally, giving to each 2 or 3 doses totalling 7 to 9 ml of 20% suspension of virus (strain unspecified). One developed paralysis from the vaccine. The others were challenged intracerebrally with 1.0 ml of 5% virus. Two of the 3 were paralyzed, 3/3 controls.

Rhoads (64) also used massive intracutaneous doses of live virus to raise the titer of neutralizing antibody in the serum of convalescent monkeys. Six monkeys, convalescent from attacks 15 to 19 months earlier, were bled, the serums pooled and stored. Each monkey then received a series of 10 doses of 5% MV virus intracutaneously at 3 day intervals. Each dose was 15 ml, the total per animal thus amounting to 150 ml. One month after vaccination was complete the monkeys were again bled and the serums pooled. The pre- and post-vaccination pools of serum were titered for neutralizing potency, varying volumes of each being tested against a constant dose of virus (0.12 ml of filtrate from the 5% suspension of MV). The minimal volume of pre-vaccination serum required to neutralize was 0.75 ml; of post-vaccination serum, 0.1 ml. Three comparative tests were made with identical results.

Flexner (65) briefly reports intracutaneous vaccination of 5 monkeys with infected human cord material from the 1931 epidemic. Repeated injections were

given, but the number and dosage are not stated. One monkey became paralyzed from the vaccine but survived; the other 4 remained symptomless. All 5 were bled and the serums tested for neutralization against the MV strain of virus (no protocols). None neutralized. Two of the vaccinated monkeys were challenged intranasally with MV. Both developed paralysis. Reference also is made to an unspecified number of monkeys vaccinated intracutaneously with first monkey passage material of the 1931 virus. Their serums likewise failed to neutralize the "monkey passage" (presumably MV) strain.

Brodie (66) studied the antigenic potency of monkey spinal cord tissue taken at varying intervals after onset of paralysis. Two monkeys were vaccinated intracutaneously with 2.0 and 1.5 g, respectively, of cord tissue obtained 23 and 35 days after onset. Other monkeys were similarly vaccinated but with cord tissue obtained earlier in the course of infection. Sera of the first two monkeys failed to neutralize 1 MCP of virus; sera of the latter group did neutralize.

Brodie (67) also studied the time course of the antibody response to intracutaneous vaccination. In neutralization tests, the virus, in volume of 0.1 ml or less, was mixed with undiluted serum to make up an inoculum of 1.0 ml. One rhesus monkey received 5 ml of a 10% suspension of virus intracutaneously at one time. Serum samples, obtained respectively before vaccination and at 2 and 6 days after, all failed to neutralize 1 MCP of virus. Subsequent samples all neutralized and were titered against varying dosages of virus. End points found were:

SERUM OBTAINED AT	NEUTRALIZED MCP	FAILED TO NEUTRALIZE MCP
10 days	6	10
16 days	35	45
20 days	60	70
30 days	50	60
6 months	40	50

Eleven controls all became paralyzed when normal serum or saline was used with more than 1 MCP of virus.

Thus the maximum antibody response to a single dose of vaccine intracutaneously was reached at about 20 days. Essentially similar results were obtained with another monkey vaccinated with a single dose of live virus.

The effect of repeated vaccination was studied on 4 monkeys. Each received two intracutaneous doses of 5 ml of 10% suspension, spaced at varying intervals apart (6, 10, 14 and 20 days, respectively). All were bled and the serums titered at intervals. No antibody response was found in any until about the sixth day following the first dose. A second injection had little effect if given at 6 or 20 days but enhanced the antibody response markedly if given at 10 or 14 days during the rise of titer evoked by the first dose. Serums of 3 monkeys, all vaccinated with two doses of 5 ml of 10% suspension, spaced at the optimum interval, each neutralized 75 to 85 MCP of virus at the height of the response. One still maintained this maximum level 6 months later. Two showed some reduction of potency but still neutralized 50 MCP.

Kolmer and Rule (68) vaccinated one monkey with MV virus intracutaneously. The dosage was 0.01 ml per kg of 2% suspension, repeated daily for 10 days. The animal resisted intracerebral challenge with 0.2 ml of 5% suspension of the same virus. Two controls were paralyzed.

Observations of Gordon, Hudson and Harrison (69) on subcutaneous vaccination with live virus are mentioned below (p. 380).

Stimpert and Kessel (70) attempted to compare the immunizing properties of 6 different strains of virus, all given by intracutaneous injection. Ten monkeys were vaccinated with each strain. Each animal received 3 doses, 1 ml each, of 5% virus suspension at 0, 3, and 9 days, respectively. The viruses were untitered. One control animal was inoculated intracerebrally with the suspension of each virus used for vaccination, and the 6 controls were all paralyzed. One monkey developed paralysis from the vaccination (with McS strain). The remaining 59 of the 6 groups were challenged intranasally on two successive days (at 70 days following the end of vaccination), and the challenge was repeated 34 days later on those resisting. Each group was challenged with the virus strain used to immunize, and two control monkeys were inoculated with each strain. Results shown in the table below are somewhat difficult to interpret because of irregular behavior of the controls. Figures for the first and second challenge are here combined.

VIRUS USED FOR IMMUNIZING	PARALYTIC RATE AT CHALLENGE	IMMUNE	PARALYTIC RATE CONTROLS
		Per cent	
McS	4/9	56	1/2
P	0/10	100	2/2
McK	2/10	80	2/2
ST	7/10	30	1/2
BK	5/10	50	1/2
BO	6/10	40	2/2

The resistant animals surviving 8 months after the second intranasal challenge were again challenged by the same route, two series of intranasal instillations on 5 and 4 successive days. Two controls were inoculated intranasally in the same way, and one intracerebrally (single inoculation), for each group. Three of the intranasally inoculated controls died of intercurrent infection, and the behavior of the others was again irregular. Paralytic rates were as follows:

VIRUS STRAIN	MONKEYS CHALLENGED INTRANASALLY Immunized and Previously Challenged	Controls	INTRACEREBRAL CONTROLS
McS	0/1	0/1	1/1
P	0/3	0/1	1/1
McK	0/3	0/2	1/1
ST	0/2	1/2	1/1
BK	0/3	0/2	1/1
BO	2/3	1/1	1/1

Morgan, Howe and Bodian (71) compared subcutaneous, intravenous and intramuscular vaccination in rhesus monkeys, using methods quantitatively more precise than those employed by earlier workers. In a preliminary experiment, 5 monkeys each received 7 doses subcutaneously, 2 ml at each injection, of 5% suspension of Riley virus. Vaccination extended over 6 weeks. One monkey became paralyzed from the vaccine but survived. One died of intercurrent infection. Serum from each of the surviving monkeys (including the one paralyzed by the vaccine) was tested at 5 months for neutralization of the homologous virus. Serum and 20% virus suspension were mixed in equal volumes, and 0.4 ml of the mixture containing 1,000 PD_{50} or more of the virus was inoculated into each of two test monkeys intracerebrally. None of the sera neutralized. The 3 vaccinated, nonparalyzed monkeys were challenged intranasally at 5 months, one instillation of 0.1 ml of homologous virus suspension into each nostril. All 3 developed paralysis.

Additional groups of monkeys were vaccinated subcutaneously, intravenously or intramuscularly with Lansing virus. Neutralization tests were made at varying intervals. Serial dilutions of serum were mixed with 1:160 dilution of Lansing virus, and 8 mice inoculated with each mixture. Virus dosage in the inoculum was calculated as 10 LD_{50}.

Most of the vaccinated animals were finally challenged intracerebrally with Lansing virus. Three groups of 5 monkeys each were vaccinated subcutaneously. The most intensively immunized of these groups received 12 injections per animal, totalling 2.0 g of infected cord tissue, over a period of 3 months. Two monkeys of this group failed to develop serum antibody. Two developed maximum titers of 300; the fifth, a maximum of 10 (all at 4 months). On challenge, 3 of the 5 were paralyzed. The other two groups both received vaccine in fewer and smaller doses. Only two animals from the two groups developed demonstrable antibody with maximum titers of 20 and 50, respectively. Three died of intercurrent infection, and on challenge all the rest (7) became paralyzed. In controls similarly challenged the paralytic rate was 16/17.

One group of 5 monkeys was vaccinated intravenously, total dosage 0.2 g of infected CNS tissue divided into 4 doses over 3 months. None developed detectable antibody. Two died, and the paralytic score on challenge of the 3 survivors was 2/3.

Three groups of 10, 4 and 14 monkeys, respectively, were vaccinated intramuscularly as follows:

GROUP	MONKEYS	DOSES PER MONKEY	VOLUME PER DOSE	CONCENTRATION	TOTAL G OF TISSUE	VACCINATION PERIOD
			ml	%		
IX	10	3	0.8	10	0.24	3 weeks
XII	4	6	1.0	20	1.20	3 weeks
XIII	14	15	1.0	20	3.0	4 months

All of these animals, excepting 4 in Group XIII that died of intercurrent infection, developed demonstrable antibody, though in two monkeys of Group IX

only undiluted serum neutralized. In Group IX serum titers were 1 to 1,000; in Group XIII, 60 to 2,000. In Group XIII the 10 surviving monkeys all developed serum titers of 1,000 to 8,000. On challenge, the paralytic rate of Group IX was 6/9, in Group XIII 0/9. Group XII was not challenged. Paralytic rate of controls was 16/16.

These experiments indicated, for the first time, that solid immunity against intracerebral challenge can be achieved without previous paralytic infection, provided the serum antibody level is sufficiently high. They also demonstrated that intramuscular is more effective than subcutaneous vaccination.

Morgan (51) made a more detailed study of the relation between serum antibody level and resistance to intracerebral challenge. Rhesus monkeys were vaccinated, all intramuscularly, with Lansing virus of known infectivity titer. Levels of serum antibody reached were measured by the quantitative mouse neutralization test referred to above. All monkeys remaining well through the vaccination period were challenged intracerebrally with a uniform dosage of 10,000 PD_{50} of Lansing virus. In a first series of experiments, 3 groups of 7 monkeys each were vaccinated with 20% virus suspension. The volume given at each injection was 4.0 ml for Group I, 2.0 and 1.0 ml, respectively, for Groups II and III. In other respects the procedure was uniform for all groups. Each monkey received 4 doses of vaccine at 0, 1, 3 and 5 weeks. Each was bled at 0, 3½ and 6 weeks and finally challenged at 6 weeks. Four monkeys of Group I developed paralytic infection from the vaccine. One of Group II was lost. The 16 remaining all resisted the final challenge. No significant difference of serum antibody level could be determined among the 3 groups. All serums were initially negative. All reached antibody titers of $10^{-3.0}$ or higher, 5 at 3½ weeks and the rest by 6 weeks.

Eight additional groups of monkeys were then vaccinated by a similar procedure. The dosage of vaccine was uniformly 1.0 ml at each injection, but the concentration of virus was varied from 20 down to 0.05%. The following table taken from Morgan's paper shows the relation of immunity achieved to the concentration of virus used for vaccination:

Suspension of virus, %	None	0.05	0.1	0.5	1.0	2.5	5.0	10.0	20.0
Paralytic rate	35/35	4/4	5/6	2/6	2/5	2/6	1/6	5/43	0/7
% immune	0	0	17	67	60	67	83	88	100

In additional experiments no appreciable enhancement of immunity was accomplished by increasing the volume per injection from 1.0 to 4.0 ml of 20% virus, nor by increasing the number of injections from 4 in 6 weeks to 15 spaced over a period of 3 months.

Results of challenge, in relation to serum antibody level, are presented for 79 monkeys. Of these, 37 had antibody titers of $10^{-3.0}$ or higher and all resisted challenge. Within the antibody range of 2.5 to 2.9 the paralytic rate was 5/16, and it rose with diminishing antibody levels.

Of the monkeys immunized in this study, 3 were subjected to repeated tests over a prolonged period to note changes in antibody titer (72). They had developed titers of $10^{-2.4}$ to $10^{-3.6}$ at the end of a 5 week course of vaccination in 1947; had resisted intracerebral challenge with homologous (Lansing) virus;

and had each received one booster dose of vaccine in November of 1947. A year later, their serum titers were $10^{-1.5}$ to $10^{-2.3}$, no known exposure to the virus having occurred in the meantime. A second booster injection was given in November of 1948. Five months later the serum titers were 2.4 to 2.7.

In brief, intramuscular vaccination is the only method so far found capable of inducing a degree of immunity sufficient to protect monkeys consistently against intracerebral challenge with known large doses of poliomyelitis virus. The intracutaneous route has been used only occasionally for more than a decade, and its effectiveness has never been studied in animals by the quantitative methods now available. The intramuscular route is more convenient than intracutaneous and more effective than subcutaneous. Currently, subcutaneous vaccination is used in the laboratory for one or two doses, preliminary to a series of intramuscular injections (73). Initial administration of live virus by the subcutaneous route is believed to reduce the hazard of paralytic infection from subsequent intramuscular injection.

Intramuscular vaccination has, since 1948, been extensively used in immunologic studies on poliomyelitis. Animals vaccinated with a prototype virus strain, and resistant to intracerebral challenge with it, have been challenged with strains of unknown type for purposes of typing (74). Specific antisera of high potency are prepared by vaccination of monkeys with appropriate types of virus. Reference will not be made to the numerous instances in which one or both of these procedures have been used.

Chimpanzees. Melnick and Horstmann (5), in reports chiefly concerned with oral immunization, list 3 chimpanzees initially exposed to poliomyelitis virus by combined intra- and subcutaneous injection (2 ml, 10 or 20% suspension of Y-SK strain). All 3 developed inapparent infections, manifest by appearance of neutralizing antibody and intestinal carriage of virus. One was reinoculated by the same route 6 months later with homologous virus, same dosage. Another was twice challenged within the ensuing 7 months by feeding homologous virus. Neither animal became reinfected from the homologous challenge. One of them did become a virus carrier again on oral challenge with a heterologous strain (NC).

Howe, Bodian and Morgan (75) vaccinated 15 chimpanzees intramuscularly with Lansing virus. Ten received live virus throughout, 7 doses per animal of 1 ml each of 10% suspension followed by a series of 4 doses, 2 ml each, of 20%. The entire vaccination period covered 3.5 months. The remaining 5 animals each received initially 5 doses, 1 ml each, of formalin treated 20% suspension at weekly intervals. This was followed by 7 injections, 1 ml each, of 10% suspension of live virus, the entire vaccination period covering 5 months. All the animals were bled at intervals and serum antibody titrated by the method referred to above (p. 354). All the vaccinated animals developed titers of $10^{-2.0}$ to $10^{-3.3}$. Whether any became virus carriers as a result of the parenteral inoculation was not determined. No paralytic infections resulted from the vaccination.

One of the vaccinated chimpanzees was lost from dysentery. The remaining 14 were challenged by feeding with heterotypic (Brunhilde) virus. Three became paralyzed from this challenge. Six others became alimentary virus carriers al-

though they remained symptomless. Five showed no signs of infection, and virus was not found in their stools.

2. *Use of adjuvants to enhance antibody response. Agar.* Aycock and Kagan (60) gave to 8 monkeys repeated subcutaneous injections of live poliomyelitis virus in agar. The number of injections varied from 3 to 17 per animal, the total dosage (concentration not stated) 20 to 96 ml. Two developed paralytic infection from the vaccine. Four were challenged intracerebrally, dosage and titer of virus not given. Two of the 4 were paralyzed. Neutralization tests on these animals are not reported.

Vaccinia virus. Thompson and McKinley (76) reported that intracutaneous vaccination of monkeys with a mixture of poliomyelitis (Aycock strain) and vaccinia viruses would protect against subsequent intracerebral challenge with the homologous poliomyelitis virus. Vaccination with the poliomyelitis virus alone, in the same dosage, was found ineffective. No protocols are given, and it is not possible to gather from the published report the factual basis for the conclusion drawn.

Mucin, hyaluronidase. Pinto (77), in studies referred to below (p. 366), added mucin to the live virus used to vaccinate certain groups of cotton rats intraperitoneally. Hyaluronidase was added to the virus used for other groups intramuscularly. In neither case was any evidence found of an enhancement of antibody response over that obtained with equal doses of virus alone. The groups vaccinated with virus plus mucin developed antibody in lower titer than did those vaccinated with virus only.

Paraffin oils. Morgan (78) used with live poliomyelitis virus an adjuvant mixture previously shown by Freund and McDermott (79) to enhance the antibody response of guinea pigs to horse serum. The ingredients of the vaccine were mixed in the following proportions; 2 mg of heat killed *Mycobacterium tuberculosis*, 0.5 ml of paraffin oil, 0.25 ml of falba and 0.25 ml of 20% suspension of poliomyelitis infected CNS tissue. Monkeys received repeated subcutaneous injections of this mixture, 1.0 ml at each dose, 2 or 3 injections. In a group of 9 monkeys so vaccinated, 8 developed allergic encephalomyelitis. The one survivor developed serum antibody, the titer ranging from 1:20 to 1:200 over a period of 7 months (71). Other animals, vaccinated intramuscularly with live virus without adjuvant, showed a better antibody response.

A group of 5 monkeys was vaccinated subcutaneously with the virus-adjuvant mixture minus tubercle bacilli. Each received 1 ml at each injection. None developed allergic encephalomyelitis, but 3 died of other causes during the course of immunization. The survivors received 4 doses over 3½ months. Neither developed serum antibody as tested at 1 and 3 months. Both became paralyzed on intracerebral challenge.

It may be noted that Morgan had not used adjuvants for intramuscular vaccination. Ward, Rader, Lipton and Freund (80) were the first to report the efficacy of paraffin-oil adjuvant for intramuscular vaccination against poliomyelitis. They vaccinated 3 groups of 6 monkeys each. Each animal, in all 3 groups, received 3 intramuscular doses, each dose 10 ml of 10% suspension of

Lansing infected monkey cord. The injections were given at 0, 5 and 11 weeks. The only difference of treatment among the 3 groups was in regard to the suspension medium used for the first dose of vaccine. The amount of virus given at each dose was uniform, 1 g. For Group I, it was suspended in 9.0 ml of saline; for Groups II and III, in an emulsion containing 4.0 ml of saline, 4.5 ml of paraffin oil ("bayol F"), and 0.5 ml of "arlacel A", an emulsifying agent. A small amount of killed acid-fast bacteria (*Mycobacterium butyricum*) was added to the inoculum for Group II but not for III.

The animals were bled for serum neutralization tests one month after the first injection, one week after the second, and one week after the third. Neutralization tests were run on mice, using serial dilutions of serum mixed with equal volumes of a constant dilution of virus. One virus pool was used throughout. It was titrated for infectivity when each set of neutralization tests was run, and the PD_{50} end point varied from $10^{-3.1}$ to $10^{-4.0}$. The number of PD_{50} doses used in the tests varied from about 10 to 100. Eight or 9 mice were used for each dilution.

Of the 6 monkeys in Group II, 4 developed allergic encephalomyelitis. Two died before the first bleeding, and 2 more between the second and third.

Neutralizing titers rose more rapidly and reached higher levels in the second and third groups (adjuvants used) than in Group I (vaccinated without adjuvant). For the first and third tests, results were:

GROUP	FIRST BLEEDING—1 MONTH Range of Titers	Mean	THIRD BLEEDING—12 WEEKS Range of Titers	Mean
I	$10^{-1.2}$–$10^{-2.5}$	$10^{-1.9}$	$10^{-2.9}$–$10^{-3.7}$	$10^{-3.4}$
II	$10^{-2.5}$–$10^{-3.8}$	$10^{-3.3}$	$10^{-4.2}$–$10^{-4.6}$	$10^{-4.3}$
III	$10^{-2.8}$–$10^{-3.3}$	$10^{-3.1}$	$10^{-3.5}$–$10^{-3.9}$	$10^{-3.7}$

It thus appeared that paraffin-oil adjuvant did enhance somewhat the antibody response to intramuscular vaccination. While some further enhancement was obtained from the addition of killed acid-fast bacilli, allergic encephalomyelitis has so frequently followed use of that ingredient that the paraffin-oil adjuvants have generally been used without bacilli in subsequent work. The suspension medium has contained bayol F and arlacel A in the same proportions as used by Ward *et al.* A modified adjuvant combination has been used for human vaccination (p. 400, below).

Salk, Lewis, Youngner and Bennett (81) compared the antigenic effects of live virus (Lansing) vaccines prepared with and without adjuvants. Serial 10-fold dilutions of virus (monkey cord) were prepared. Each dilution was emulsified with adjuvant in the proportions indicated above. Four groups of 6 monkeys each were inoculated intramuscularly with the respective preparations, a single dose of 4 ml divided between the two calf muscles. A fifth group was inoculated in the same manner with a 10^{-1} dilution of the virus in saline.

Neutralization tests were made on pooled sera of the respective groups, using serial dilutions of serum against 100 LD_{50} of virus in mice. At the eighth week following vaccination antibody titers were:

VACCINE	DILUTION OF VIRUS IN VACCINE	50% NEUTRALIZING END POINT
Virus-adjuvant	10^{-1}	1:3,200
	10^{-2}	1:120
	10^{-3}	1:5
	10^{-4}	0
Virus and saline	10^{-1}	1:16

The intracerebral titer in monkeys, of the virus pool used for these vaccines, was $10^{-4.5}$.

The same group has made extensive use of the mineral oil adjuvant for obtaining antisera to be used in virus typing (55). Various other laboratories have employed the method, but the only other published data found that offer a comparison between vaccines prepared with and without adjuvant are those of Rhodes, Clark and Shimada (82). In one experiment a large group of monkeys was subjected to intramuscular vaccination, four or more doses to each animal at 2 week intervals. Each dose was 2 ml of a 12.5% suspension of Lansing infected cord. The animals were distributed in 3 subgroups. One received only virus in water suspension. The second received a first dose of virus in adjuvant, vaccination being continued with virus in water. The third group received virus in adjuvant throughout. Serums were obtained at 2 week intervals and titered in mice against 17 or 21 LD_{50} of Lansing virus.

Of 28 animals initially included in the 3 subgroups, only 11 survived to the end of the vaccination period. Antibody titers on these were as follows:

VACCINE	NUMBER OF MONKEYS	ANTIBODY TITER AT WEEK INDICATED 2	4	6	8	11	13
Virus in water	4	$10^{-1.2}$	$10^{-2.13}$	$10^{-3.05}$	$10^{-3.17}$	$10^{-4.04}$	$10^{-3.7}$
Adjuvant first dose only	3	$10^{-2.15}$	$10^{-2.63}$	$10^{-3.55}$	$10^{-3.48}$	$10^{-4.25}$	$10^{-3.9}$
Adjuvant all doses	4	$10^{-1.7}$	$10^{-3.02}$	$10^{-3.77}$	$10^{-3.65}$	$10^{-4.5}$	$10^{-4.95}$

Serum titers of the monkeys vaccinated with adjuvant were thus somewhat higher on the average than titers in monkeys vaccinated without adjuvant. Other data in the same report show a similar difference.

"Pendil". This agent, a mixture containing a cholesterol derivative, peanut oil and beeswax, has been used as an adjuvant by Pollard (see p. 370).

3. *Primates: intranasal and other miscellaneous routes of vaccination. Intracerebral.* Reference was made above (pp. 343–344) to immunity following a first paralytic attack in laboratory animals. The first attack in such instances has usually been induced by intracerebral inoculation. The question would naturally arise whether immunity developed wholly as a consequence of the paralytic infection or partly from the simple antigenic action of the original inoculum. In laboratory work the question has had some practical importance. Monkeys inoculated with virus alone occasionally show no signs of infection, and a greater

number remain well after inoculation with serum-virus mixtures. Uncertainty regarding their immune status following such an experience has made it common practice to discard them although it would save animals and expense if they could still be considered normal, nonimmune monkeys and used again as such.

Zappert, von Wiesner and Leiner (58) refer to "about 150" monkeys inoculated intracerebrally with virus known to be active. Of these, 17 had remained symptomless. On reinoculation 15 of the 17 developed paralysis. While the percentages of "takes" at first and second inoculation were approximately the same, no information is given regarding uniformity of dosage.

Jungeblut (30) reinoculated 34 monkeys that had previously suffered paralytic attacks and 20 that had once been inoculated intracerebrally but failed to develop symptoms. All received the same challenge dose, 1.0 ml of 10% virus suspension. The 34 convalescents all resisted. The 20 that had not shown previous infection all developed paralysis. The virus strain or strains used are not identified, so it is not clear that the successive inoculations were made with homotypic viruses. The same applies to the data of Zappert *et al.* (58).

Kessel and Stimpert (83) carried out neutralization tests on monkeys that had once been inoculated intracerebrally with serum-virus mixtures and had remained symptomless. Ten had been inoculated with the McK strain of virus, 11 with MV. Neutralization tests against both strains were made on each serum. Subsequently, these two strains have been typed and classified, respectively, as Type 1 and Type 2 (35).

In the following tabulation, the denominator of each fraction represents the number of animals tested; the numerator, the number showing neutralization:

TEST	PREVIOUSLY INOCULATED WITH MV	McK
Against MV virus	4/11 (36%)	2/10 (20%)
Against McK virus	2/10 (20%)	4/10 (40%)

Two groups of monkeys with histories corresponding to those above were challenged intracerebrally with McK or MV virus, and the outcome compared to that of normal controls similarly challenged. Paralytic rates were:

CHALLENGE VIRUS	PREVIOUSLY INOCULATED WITH MV	McK	Controls
MV	12/20 (60%)	8/9 (89%)	21/24 (87.5%)
McK	5/9 (55.5%)	8/16 (50%)	48/52 (92%)

The challenge dosage is not stated.

The authors conclude that monkeys remaining symptomless after an intracerebral inoculation may sometimes develop immunity, manifest both by serum antibody and by resistance to challenge. That conclusion was not borne out by a subsequent study from the same laboratory (19). The animals used had all resisted one or more inoculations (intracerebral) with doses of virus that pro-

duced paralysis in other normal animals. Those resisting, called "no-takes", were subsequently reinoculated with the homologous virus, and the outcome compared with that of normal monkeys being inoculated for the first time.

Three strains of virus were used, each in two or more dilutions, 1.0 ml inoculum. In the following table, each of the "no-takes" had resisted a previous inoculation with the strain indicated:

STRAIN	DILUTION	HISTORY	NUMBER INOCULATED	PARALYTIC RATE	PER CENT
MV	1:10	Normal	9	9/9	100
		No-take	7	7/7	100
	1:1,000	Normal	7	4/7	57
		No-take	8	5/8	63
McK	1:10	Normal	30	30/30	100
		No-take	12	11/12	92
	1:100	Normal	10	6/10	60
		No-take	9	6/9	67
	1:1,000	Normal	6	4/6	67
		No-take	5	2/5	40
Ba	1:10	Normal	13	10/13	77
		No-take	13	13/13	100
	1:100	Normal	10	6/10	60
		No-take	7	6/7	86

Data on the incubation period and on severity of the disease failed to show any difference between the no-takes and the previously uninoculated monkeys. The figures above also fail to show any significant difference in paralytic rate. Serum neutralization tests for this series are not reported.

Morgan (44) used 5 monkeys that had once been inoculated intracerebrally with doses of Lansing virus sufficient to produce paralytic poliomyelitis in the majority of a group of monkeys similarly inoculated at the same time. These 5 had resisted. They were sacrificed at 21 days to 5 months after the original inoculation. Quantitative tests for neutralizing antibody were made on blood, CSF and various tissues of these animals. Two of these monkeys, sacrificed at 21 days, showed low-titer neutralization by tissue from the thalamus (site of original inoculation). All other fluids and tissues failed to neutralize (p. 347).

To summarize, there is no clear evidence that unmodified live virus, injected into the brain in a dosage that fails to infect, leaves any measurable immunity. The quantities of virus that can be so introduced without infecting, however, are generally so small that little if any antigenic effect could be expected if they were introduced by any other route. It should be noted further that intracerebral inoculation of monkeys occasionally results in infection not manifest by clinical symptoms and revealed only by histologic examination of the CNS (84, 85). Presumably such inapparent infections with definite involvement of the CNS would confer resistance to intracerebral challenge, though direct evidence of this appears to be lacking.

Intranasal. Jungeblut and Hazen (86) treated 4 monkeys by spraying of the nose and throat with 10% suspension of virus, strain unspecified. About 1.0 ml of the suspension was sprayed on the surfaces at each treatment. This was repeated twice weekly for a total of 20 treatments per animal. One was lost by intercurrent infection. The 3 survivors were bled for neutralization tests and challenged intracerebrally with 1.0 ml of 10% virus. The 3 treated monkeys and one control were all paralyzed. Each serum was mixed with an equal volume of 1% virus suspension and test monkeys inoculated with 1 ml of the mixture. All failed to neutralize.

Flexner (87) made observations on several groups of monkeys subjected to repeated nasal instillations of poliomyelitis virus (various strains). One ml of a virus suspension of unspecified concentration or titer was dropped into each nostril. This was repeated at 1 to 3 day intervals. The number of such doses varied from 2 to 10 per animal, and some of the monkeys were subjected to repeated courses of inoculation. Flexner notes that the CSF cell count generally rose following a course of intranasal inoculations, whether any symptoms of illness appeared or not. Tests of immunity were made on 3 monkeys, 2 cynomolgus and 1 rhesus, that had survived repeated intranasal inoculation with MV virus. One cynomolgus had shown transient muscle weakness after the first but recovered completely and resisted 4 later courses of intranasal instillations. This animal resisted intracerebral challenge, and the blood serum neutralized MV virus. The 2 other monkeys had remained symptomless throughout 4 or 5 courses of intranasal inoculation. They both succumbed to intracerebral challenge, and their sera failed to neutralize. The challenge dose for all 3 monkeys intracerebrally was 0.1 ml of filtrate from a suspension of MV virus of unspecified concentration. Two control monkeys were paralyzed by this dosage. Neutralization tests were made with 0.1 ml of the virus filtrate against 0.9 ml of undiluted serum. Immunity in the one resistant monkey seems most likely to have been a consequence of the previous mild infection. Flexner found it difficult to understand why the others failed to develop immunity, in view of "the many instances of general immunity arising spontaneously in human populations from the chance entry of virus into the nasal passages".

Kramer, Grossman and Parker (88, 89) used "adrephine" (a mixture containing 2% ephedrine and 1/10,000 adrenalin) and pituitrin S for pretreatment of the nasal mucosa to protect against subsequent instillation of poliomyelitis virus. Treatment consisted of instillation into each nostril of 0.5 ml of pituitrin S, followed 5 minutes later by 1.0 ml of adrephine. This was repeated once or twice daily and was followed by instillation of 1 ml of a 5% suspension of poliomyelitis virus (Armstrong strain) into each nostril. Fifteen series of experiments are reported with varying time schedules of drug and virus administration that will not be recounted here. Of 69 monkeys in the experimental groups, 35 survived the treatment and resisted one or more intranasal challenges in which the Armstrong virus (5% suspension) was instilled into both nostrils on 3 successive days. Neutralization tests were made on these survivors. One ml of a suspension of a heterologous virus strain (VM 11), representing two "infective doses", was mixed with 1 ml of undiluted serum and 1.2 ml of the mixture used for inoculation of

test monkeys. Of the 35 serums tested, 25 neutralized. Thirty-four of the survivors were challenged intracerebrally with one "infective dose" of VM 11 virus in 1 ml of inoculum. Twenty-five of the 34 resisted. The protocols list 107 controls, of which 106 succumbed to challenge. It is not clear how many of these were used in intranasal and how many for the intracerebral challenge. The same papers give data on 9 animals that had survived repeated intranasal inoculations following the application of alum sulfate-picric acid, or zinc sulfate, to the olfactory mucosa. Only two of these developed neutralizing antibody and 8/8 succumbed to intracerebral challenge.

No further attempts at active immunization by the intranasal route have been found on record. While a considerable degree of immunity evidently was produced in Kramer's animals, the percentage paralyzed during the immunizing procedure is higher than that reported for intramuscular or intracutaneous vaccination.

Intraperitoneal. Pettit (90) immunized adult *cynocephalus hamadryas* by intraperitoneal injection of infected monkey cord, 2, 3 or 4 doses at weekly intervals. Serum of animals so treated was tested for neutralization of the homologous virus strain, using 0.5 or 1.0 ml of serum against 1.0 ml of a virus suspension. The mixtures were inoculated intracerebrally into test monkeys which were protected, while controls inoculated with the same dose of virus alone were paralyzed. The concentration and titer of virus, used for immunizing and for challenge, are not stated. The numbers of animals immunized, and of test monkeys and controls in neutralization tests, are also unstated.

Jungeblut and Hazen (63) gave to each of two rhesus monkeys a series of 4 injections of a 20% virus suspension, 2 ml per dose, intraperitoneally. Both became paralyzed on subsequent challenge with 1.0 ml of 5% virus intracerebrally. Four other monkeys each had a collodion sac, containing 3.0 ml of 20% virus, placed in the abdominal cavity. They were similarly challenged, and 3 of the 4 became paralyzed with all of 3 controls.

Intraperitoneal vaccination has been used more extensively with rodents (see pp. 364–366) than with primates.

Other routes. Jungeblut and Hazen (63) tried intrapleural injections of live virus. Three monkeys each received a series of 3 injections of 20% virus, totalling 1.5 to 3.0 ml. Two of these resisted intracerebral challenge. Dosage and controls are mentioned above.

Römer and Joseph (57) tried vaccination of one cercopithecus monkey by inoculation of 0.2 ml of 5% virus suspension into the sciatic nerve. The animal remained well but suffered transient paralysis on intracerebral challenge 33 days later.

Jungeblut and Thompson (91) inoculated 8 rhesus monkeys by injection of virus into the testicle. The testicles were removed at varying intervals thereafter, 24 hours to 15 days, and tested for the presence of virus by subinoculation into other animals. The authors state that some of the inoculated testicles were left *in situ* but in how many animals is not clear. The monkeys did not develop poliomyelitis from the testicular inoculation, nor did they acquire active immunity,

"as evidenced by their full susceptibility to subsequent intracerebral reinfection with potent virus" (no further details).

A report published in abstract by Brebner (92) states that "many infective doses" of poliomyelitis virus may be injected into the spleen of normal monkeys with little risk of developing the disease, and that later neutralization tests on such animals showed them to have acquired immunity. No protocols are given.

4. *Vaccination of rodents.* Transmission of the Lansing strain of poliomyelitis virus to cotton rats and mice, reported in 1939, make it possible, for the first time, to carry out immunity studies on nonprimates.

Two brief papers by Levaditi (93, 94) report intraperitoneal vaccination of mice against Lansing virus. The vaccine was an emulsion of live virus of unspecified concentration, titer of the virus in mice $10^{-1.0}$. Each animal received 6 to 8 injections, each of 0.5 ml, spaced 3 or 4 days apart. Four groups, totalling 46 mice, were so vaccinated. All resisted intracerebral challenge given 6 or 7 days after vaccination was complete. In two groups of controls the paralytic rates were respectively 4/5 and 8/10. A third group of controls, number not stated, all became paralyzed. One group of 8 vaccinated mice, having resisted the first intracerebral challenge, was again challenged 78 days later. Five resisted, three succumbed, although in two of the latter death was delayed (at 30 and 63 days, respectively) and the CNS did not show characteristic lesions.

Kramer and Geer (95) ran parallel experiments on vaccination of mice with live and formalin treated Lansing virus (see p. 394). The vaccine was supernate from a centrifuged 10% suspension of infected mouse and cotton rat CNS tissue. Charts are shown of the results in 9 groups of mice, 6 to 44 per group, vaccinated with live virus. One group of 21 was vaccinated subcutaneously, 8 doses of 0.1 ml each, biweekly, per animal. They were challenged, with 18 controls, 10 days after vaccination was completed. The intracerebral challenge dose was 0.03 ml of 5% virus. Paralytic rates were 9/21 in the vaccinated and 18/18 in the control group. Eight other groups of mice were vaccinated intraperitoneally, 3 to 10 doses of 0.15 to 3 ml each per animal. The time of challenge was varied from 2 to 19 days after vaccination was completed. As a rule the challenge dosage was 0.03 ml of 0.5% virus, but 5.0 and 0.05% suspensions were used in preliminary experiments. In 3 of the vaccinated groups protection was complete (0/11, 0/44, and 0/40). In the other 5 it was incomplete, but the highest paralytic rate in any of the vaccinated groups was 14/33 (42.5%). In the control groups paralysis developed in 80 to 100%.

Weaver (96) inoculated cotton rats with Lansing virus by various routes. All animals were 62 days old at first exposure. Those failing to show symptoms of infection were challenged 25 days later by 3 way inoculation of a 10% suspension (0.1 ml intracerebral, 0.06 ml intranasal and 0.5 ml subcutaneous). Those resisting were again challenged in the same way 25 days later and observed for a final period of 25 days. Animals symptomless at the end of that time were listed as immune. Half of the animals were on deficient diets and half on an adequate diet, but no differences either of susceptibility at first exposure or of resulting immunity were noted in relation to diet.

One large group of 70 cotton rats was simply challenged by the method out-

lined above. All became paralyzed, 66 at first and 4 at second challenge. These may be considered as controls for the groups listed below, all of which were exposed to virus, by the various routes indicated, prior to challenge. With the one exception indicated, each group originally numbered 24 rats. The virus used for initial exposure or inoculation was a 10% suspension except where otherwise specified. Results of first and second challenge were here combined.

IMMUNIZATION, ROUTE AND DOSAGE	PARALYTIC RATE, FROM Immunizing Exposure	Challenge
Feeding virus (one cord and brain stem of infected cotton rat)	0/24	22/24
Submucosal injection, tonsillar area, 0.5 ml	2/24	0/22
Intragastric, 1.0 ml, one instillation	0/24	18/24
Same, 3 successive days	0/24	19/24
Intracolonic, 1.0 ml, 3 successive days	0/24	19/24
Intraperitoneal, 0.5 ml, once	0/24	5/24
Intraperitoneal, 1.0 ml, 3 successive days	1/24	1/23
Intranasal, 0.05 ml in each nostril, twice daily, 3 days	1/24	19/23
Intrapulmonary, 0.08 ml, concentrated virus. Dosage equivalent to 1 ml of 10%	0/24	11/24
Subcutaneous, 1.0 ml, once	0/24	3/24
Subcutaneous, 1.0 ml, 3 successive days	0/24	2/24
Intracardiac, 1.0 ml, once	1/10	0/9
Intracardiac, 0.5 ml, once	1/24	0/23
Intracardiac, 0.08 ml, concentrated	0/24	10/24
Contact with infected cotton rats	0/24	20/24

These observations were in part repeated in subsequent studies by the same author (97, 98) with similar findings. While cotton rats inoculated by various parenteral routes developed substantial immunity, it may be noted that little or none was acquired by contact of the virus with intact mucosa of the gastrointestinal tract.

Sá-Fleitas (99) inoculated cotton rats by various peripheral routes with the Y-SK strain of virus. The infectivity titer of the virus, tested intracerebrally in cotton rats, is given as about 10^{-4}. Vaccinated rats that showed no symptoms of infection were subsequently challenged intracerebrally with 20 LD_{50} units of virus. Each animal received a single immunizing inoculation. Routes and dosage of primary inoculation, and outcome of challenge, are summarized below:

NUMBER OF ANIMALS	IMMUNIZING INOCULATION Route	Dosage, 10% Suspension	Paralytic Rate	CHALLENGE, PARALYTIC RATE
		ml.		
6	Intragastric	1.0	1/6	5/5
5	Intranasal	0.06	0/5	5/5
5	Intra- and subcutaneous	1.0	0/5	0/5
4	Intraperitoneal	1.0	1/4	0/3
5	Intracerebral	0.03	5/5	—

Pinto (77) inoculated groups of mice, 6 to 7 weeks old, with Lansing virus. The pool of virus used titered $10^{-4.6}$ by intracerebral inoculation in mice. Each

immunized animal received a single dose, 0.5 or 1.0 ml intraperitoneally or 0.5 ml subcutaneously, of a 10% suspension. Of 39 mice inoculated intraperitoneally and 46 subcutaneously, none developed paralytic infection. Other groups of younger mice (aged 1 to 14 days) were similarly inoculated with no infections resulting. Subgroups of the immunized mice were challenged intracerebrally with serial dilutions of Lansing virus, together with controls in similar numbers. For the controls, the LD_{50} end point for the challenge was $10^{-3.6}$. For the several immunized groups it varied from $10^{-2.0}$ to $10^{-3.1}$. Some resistance to challenge was thus evident in the immunized mice. Those immunized peritoneally were more resistant than those immunized subcutaneously. Pools of serum from each group were tested for neutralization against 32 LD_{50} of Lansing virus. Serum from only one group neutralized. This serum was from mice that had received one ml dosage of virus intraperitoneally.

Pinto also carried out intraperitoneal, subcutaneous and intramuscular vaccination of adult cotton rats. These animals also received each a single dose intraperitoneally, a single dose intramuscularly, or two doses 12 hours apart subcutaneously. Total amount given to each animal was in most instances 1 ml of 10% suspension (130,000 LD_{50} intracerebral mouse doses) although larger amounts were given to some groups intraperitoneally (up to 3 ml of 20% suspension). Two of 60 cotton rats inoculated intraperitoneally developed paralysis. Of 36 inoculated by other peripheral routes, all remained well. (Concurrent tests on infant cotton rats showed them to be more susceptible to peripheral inoculation.) The surviving adult animals that had been immunized were challenged after 23 days intracerebrally, with 0.06 ml of $10^{-1.5}$ or $10^{-2.5}$ suspensions of virus. The $10^{-1.5}$ inoculum contained 3,200 LD_{50} as measured by normal cotton rat controls. All the mice immunized intramuscularly resisted this dosage, and those immunized by the other two routes showed a considerable degree of protection. Pooled sera from the various groups were tested for neutralization, serial dilutions of serum against 32 LD_{50} of Lansing virus in mice. All the sera neutralized. Titers ranged from $10^{-3.8}$, for the group immunized intramuscularly, to $10^{-1.6}$, for those immunized intraperitoneally. Neutralizing antibody first appeared at 2 to 4 days following vaccination, and maximum titer was reached in about 21 days.

Schwerdt and associates (100) immunized cotton rats with three intramuscular injections, 1 ml of suspension of Lansing virus at each dose, injections spaced 1 week apart. Serial dilutions of the vaccine were tested in different groups of animals. All were challenged intracerebrally, one week after the final dose of vaccine, with 100 LD_{50} of virus. The object was to obtain an antigenicity titer of the vaccine expressed as the maximum dilution that would protect half the vaccinated animals against the standard intracerebral challenge. Two different pools of virus, thus titered, gave end point dilutions of $10^{-3.8}$ and $10^{-4.2}$.

In summary, cotton rats can be effectively immunized with live poliomyelitis virus by various parenteral routes. The risk of paralytic infection from peripheral inoculation is relatively small. Simple exposure of the gastrointestinal mucosa to live virus rarely infects in this species and has little or no immunizing effect.

5. *Primates: immunization by way of the alimentary tract. Rhesus monkeys.* Landsteiner, Levaditi and Pastia (101) instilled into the rectum of one rhesus monkey, daily for 27 days, 5 ml of a mixture of two parts milk and one part virus suspension (concentration unspecified). The animal remained well. The day following the last treatment it was challenged (0.5 ml intracerebrally, 5 ml intraperitoneally) and developed paralysis. The same authors report daily administration of 20 ml of the milk-virus mixture by stomach tube to one *Macacus sinicus*. Two days after the last treatment it was challenged and succumbed.

Schultz (102) used 2 monkeys, species unstated but presumably rhesus. Each was fed 21 g of virus infected monkey cord in 450 ml of milk, divided into 3 feedings over 2 days. Both animals remained well. They were challenged intracerebrally 71 days later with 0.75 ml of 5% suspension of homologous virus (Aycock strain). Both succumbed.

Thompson (103) subjected one monkey to fasting for 18 hours, then administered 15 ml of bile by stomach tube followed 24 hours later by 15 ml of 20% suspension of virus (Aycock strain). The procedure was repeated 51 days later. Finally after 23 days more the animal was challenged intracerebrally. It developed mild poliomyelitis; a control was severely paralyzed. The same procedure was repeated with two additional monkeys, except that volumes of bile and virus suspension were reduced to 10 ml. The bile-virus administration was repeated 4 times in 18 days on each animal. Both resisted intracerebral challenge with 1 ml of 20% virus, while 3 controls were paralyzed. The challenge was repeated 26 days later. One of the treated monkeys was paralyzed; the other developed a mild illness.

Jungeblut and Hazen (63) gave to each of two rhesus monkeys a series of 4 instillations, 4 ml each time, of 20% virus suspension *per rectum*. Both succumbed to subsequent challenge with 1 ml of 5% homologous virus suspension intracerebrally.

Kolmer and Rule (68) gave to each of two monkeys 2 ml per kg of 2% suspension of MV virus daily, by stomach tube, for 10 days. They remained well but succumbed to intracerebral challenge (0.2 ml of 5% suspension). Two controls were similarly paralyzed.

No later attempts to immunize rhesus monkeys by way of the gastrointestinal tract have been found on record. This species also is generally refractory to infection from the alimentary tract although occasional exceptions have been reported. Recent studies on immunization by this route have employed species naturally susceptible to oral infection (chimpanzee, cynomolgus, man). Reinoculation, to test immunity, has generally been by the same route rather than intracerebral.

Chimpanzees. Horstmann and Melnick (38) report findings on 18 chimpanzees exposed to different strains of poliomyelitis virus by feeding or in 5 instances by combined intra- and subcutaneous inoculation. Total exposures of the group to live virus numbered 50, including successive inoculations with the same strain. The dosage, by mouth, was varied from 6.5 ml of 10% suspension to 30 ml of 20%. The dosage by injection was 2.0 ml of 10%. Excepting the first two animals studied, neutralizing antibodies against Type 2 virus (Lansing or Y-SK) were

titered on serum specimens drawn before first exposure and at intervals thereafter.

Sixteen of the 18 chimpanzees became intestinal carriers of virus after the first exposure, 13 by feeding and 3 by cutaneous inoculation. The two that failed to become infected had both been previously exposed to Theiler's FA virus, one by feeding and one by cutaneous inoculation. Of 12 chimpanzees that had once been intestinal carriers and were reinoculated one or more times with the homologous virus, only one became a carrier a second time. The intervals between successive inoculations with the same strain varied from 2 to 28 months.

Development of the carrier state following a first exposure to Type 2 virus (Y-SK) was associated with appearance of neutralizing antibody against that type. Serum samples obtained from two of the chimpanzees 7 days after an initial feeding of Y-SK virus were negative, but serum obtained at 16 days from the same animals both neutralized. Two other chimpanzees developed neutralizing antibody against Type 2 virus before any known exposure to it. These were considered instances of infection by accidental contagion.

Serum samples from most of the animals were titered against Type 2 virus before and after each oral inoculation. Where infection with Type 2 virus had once occurred, subsequent reinoculation with homologous or heterologous virus had no detectable effect on the serum titer against Type 2. Neutralization tests against heterotypic viruses were not made. Of 10 chimpanzees that had once become carriers, 7 became carriers a second time when reinoculated with a heterotypic virus.

None of the chimpanzees inoculated orally by Horstmann and Melnick developed paralytic infection. Howe, Bodian and Morgan (75), however, report 3 paralyzed chimpanzees (19%) of 16 first exposed to poliomyelitis virus by feeding Type 1 strains (Brunhilde or Kotter). All the rest showed evidence of infection, either by virus carriage in the stool (14/16, 88%), appearance of serum antibody (serologic tests were not made on all), or both. Eleven chimpanzees first exposed by feeding Type 2 virus (Lansing) all remained well. All developed serum antibody although difficulty was encountered in demonstrating intestinal carriage of Lansing virus.

Nine of the chimpanzees thus immunized orally against Type 1 and 9 immunized against Type 2 were challenged orally 2 to 3.5 months later. To these 18 were added 14 chimpanzees previously immunized against Type 1 (Lansing) by intramuscular vaccination (p. 356). Challenge, in each instance, consisted of a single oral inoculation with 5 or 10 ml of 20% suspension of a Type 1 virus (Brunhilde, Kotter or Frederick). Several of the animals were repeatedly challenged with these in varying sequence. Chimpanzees, once infected with Type 1 virus and fed the same strain a second time, did not develop paralysis. Only one in 9 (11%) became a carrier. In 22 instances animals once infected with Brunhilde virus were fed Kotter or Frederick (homotypic) strains. No paralytic infections resulted. Four animals became carriers of the homotypic strain. In the instances where reinfection with the same or a homotypic strain did occur, the period of virus carriage was relatively brief.

Of the 23 chimpanzees first immunized against Lansing virus and subsequently fed Brunhilde, 5 became paralyzed (22%) and 17 in all (74%) became virus carriers. These percentages were of the same order as were found in normal, nonimmunized animals and indicate little or no cross-immunity. The 5 chimpanzees paralyzed on challenge with Brunhilde included two that had been immunized orally, and 3 by intramuscular vaccination, against Lansing.

Sera from all the animals immunized against Lansing virus were titered. Those that became paralyzed following Brunhilde challenge had antiLansing titers as high as the average for the group.

Cynomolgus monkeys. The early appearance of neutralizing antibody, in cynomolgus monkeys infected by mouth, was noted by von Magnus and Melnick (39). Melnick and Ledinko (40) inoculated a large group of cynomolgus monkeys by mouth. Observations were made on (a) the paralytic and nonparalytic infection rates, (b) development of serum antibody, and (c) in the nonparalyzed animals, the outcome of intranasal or intracerebral challenge in relation to antibody level. Sixty-seven monkeys each received 4 to 20 daily feedings of Y-SK virus, total dosage per animal 5 to 60 ml of 10% suspension. Sera of the inoculated animals were titered against Lansing (homotypic) virus, serial 5-fold dilutions of serum being mixed with equal volumes of 5% suspension of a virus pool titering $10^{-3.5}$ in mice.

Of the 67 monkeys inoculated by mouth, 21 (31%) developed paralytic infection. Twelve of the paralyzed animals all showed neutralizing antibody on the first day of paralysis. Tests on the remaining paralyzed monkeys are not reported. All the 46 nonparalyzed monkeys were bled about one month after the last virus feeding, and 29 (63%) were found to have neutralizing antibody. Twenty-two of those with positive serum and 11 that failed to develop antibody were challenged. The virus used for challenge was Y-SK infected monkey cord, titering $10^{-4.5}$. The dosage was 0.8 ml of 1.0% suspension intracerebrally, or intranasal instillation of 0.25 ml of 20% suspension, into each nostril daily for 4 days. The paralytic rate in normal controls thus challenged was 30/32. In the orally inoculated monkeys that showed no antibody, it was 8/11. In the orally inoculated monkeys that had escaped paralysis but developed antibody, it was 4/22. In the latter group there were 15 with serum antibody titers of 1:100 or higher. Only one of these was paralyzed. Titers of the 3 other paralyzed animals were 1:5, 1:5 and 1:50.

These observations indicate that oral immunization with live virus can induce active immunity of a degree sufficient to protect a fairly high percentage of animals against intracerebral or intranasal challenge. There is as yet no evidence, however, that virus so introduced can confer active immunity except as a consequence of actual infection. Melnick (104) fed to cynomolgus monkeys Lansing and Y-SK virus inactivated by heat (p. 374). No antibody development resulted from this exposure. It was not demonstrated, however, that the heat-inactivated virus retained its original antigenic property. Heat-inactivated vaccines administered by other routes have not shown any definite antigenic action (pp. 371–374).

B. *Immunization with Inactivated or Modified Virus*

Under this heading will be included studies on the antigenic action of viruses treated in various ways to reduce the hazard of paralytic infection. Treatment with physical or chemical agents has generally been aimed at inactivation, that is, at rendering the virus incapable of multiplication in any host. Treatment with one chemical agent (ricinoleate) admittedly did not inactivate the virus in that sense but was believed to render it nonpathogenic to man and reduce pathogenicity toward the monkey. A limited amount of work has been done with viruses adapted to nonprimate hosts. This treatment was believed to have reduced pathogenicity toward monkeys and, by inference, toward man.

1. *Vaccines treated by physical agents. Drying.* Levaditi and Landsteiner (105, 22) [see also Levaditi (106)] used infected monkey cord dried over caustic potash, making up emulsions freshly as used (concentration not stated). They give protocols of two monkeys, each vaccinated by 8 daily subcutaneous injections of 2 ml. Applying a procedure used with rabies vaccine, the first two injections were of cord material dried for 9 days, the subsequent doses dried for 6, 6, 5, 5, 4 and 3 days, respectively, in the order given. Both monkeys were challenged intracerebrally (dose not stated), one 10 days and one 19 days after vaccination was finished. Both resisted. Two controls became paralyzed. Two other monkeys, vaccinated with cord material dried for a longer period of time (21 to 25 days), both developed paralysis during the vaccination procedure. Virus dried for 9 days was found to be infectious by intracerebral inoculation.

Aycock and Kagan (60) also used infected monkey cord dried over caustic potash for periods varying from 1 to 26 days. Seven monkeys were vaccinated, starting with material dried for 26 days and working down to one day. The volume given, the concentration, and route of injection are not stated although the last was apparently subcutaneous. Two monkeys developed paralysis during vaccination. One died accidentally. The 4 remaining were challenged intracerebrally (dosage not stated). Two resisted; two became paralyzed. No controls are mentioned for this experiment specifically, but the same report gives protocols on two normal monkeys that became paralyzed on intracerebral challenge (no details as to dosage).

While these observations indicate that dried vaccines are capable of immunizing, the methods used obviously did not inactivate the virus. Pollard (107) tested the infectivity of poliomyelitis virus before and after drying from the frozen state. Freeze-drying reduced the titer by 2 to 3 logs but did not inactivate completely and consistently. Pollard vaccinated monkeys with the following preparations:

(a) Live virus (MEF_1) in oil adjuvant.

(b) Live virus in saline.

(c) Frozen-dried virus, rehydrated, in oil adjuvant. The adjuvant used was "pendil" (see reference for source). Two animals were vaccinated intramuscularly with each vaccine, two doses two weeks apart, each dose equivalent to 1,000 PD_{50} of the original untreated material. All were challenged intracerebrally (with 100 PD_{50} of homologous virus) 4 weeks after the second dose of vaccine.

The monkeys that received vaccine (a) both resisted. Of those that received (b) and (c), one of each pair became paralyzed. The second in each case showed mild symptoms, but histologic examination showed poliomyelitic lesions in the CNS. The dried material thus appeared to be inferior in antigenic potency to untreated virus when both were given with adjuvant.

Faber, Dong and Silverberg (108) dried suspensions of poliomyelitis virus from the frozen state and stored them *in vacuo* for 4½ to 7½ months. Two of 6 samples of this material, tested by intracerebral inoculation of monkeys, produced paralytic infection. The same authors report that poliomyelitis virus in human stool or in monkey cord suspensions is inactivated within a few hours by drying at room temperature. No studies on the antigenicity of such material have been reported.

Heat. Levaditi and Landsteiner (105) mention negative experiments with heat-treated vaccines, without giving any data. In the same year (1910) Flexner and Lewis (109), in a brief note, refer to an experiment with heated virus in the following words:

> "A considerable quantity of an emulsion of virus-containing spinal cord which had been warmed to 55 or 57°C for one hour, or to 60°C for half an hour, was injected beneath the skin at the same time that a usual intracerebral injection of virus was given. The two monkeys employed in the experiment developed paralysis in the usual manner."

Römer (110) used a virus emulsion, concentration not stated. In the first experiment, the emulsion was heated to 45 C for 30 minutes. One monkey received two subcutaneous injections, each of 2 ml, two days apart. One was inoculated intracerebrally with the heated virus and suffered a paralysis which he survived. Thirty-four days after the vaccination of the first monkey was completed, both were challenged intracerebrally with 0.6 ml of unheated virus suspension. Both remained well. One control monkey became paralyzed. In a second experiment the virus was heated to 50 C for 30 minutes. One monkey received a single subcutaneous injection, amount not stated. One was inoculated with the heated virus intracerebrally and remained well. Both were challenged as in the first experiment. Both resisted. One control became paralyzed. Finally, one monkey received two doses, 5.0 ml each, subcutaneously, 4 days apart, of virus heated to 55 C. He succumbed, with one control, to intracerebral challenge 35 days later. From these observations Römer inferred that heated virus was effective as a vaccine provided the temperature of treatment was kept below 55 C.

Levaditi and Danulesco (111) used two lots of virus, one heated two hours at 60 C, the other for the same time at 50 C. One monkey received 3 doses subcutaneously, 4 ml each, of the first preparation, and another 3 doses of 5 ml each of the second. The same time schedule was followed with both, 8 days between the first and second injections, one day between the second and third. Both were challenged intranasally, with one control, 11 days later. All three became paralyzed.

Landsteiner, Levaditi and Pastia (101) followed Römer's procedure of heating virus to 50 C for 30 minutes. One monkey received 0.5 ml subcutaneously and

was challenged intracerebrally, with one control, 15 days later. Both became paralyzed.

The same authors tried virus heated to 50 C for one hour. One monkey inoculated intracerebrally with this material became paralyzed. The heated virus was then used to vaccinate two monkeys, by daily subcutaneous injections of 2 ml, from February 15 to March 6. One of them received a second course of injections, March 8 to 16. The first was challenged intracerebrally March 12, the second March 25, each with one control. Both the vaccinated monkeys resisted. Both controls developed paralysis.

Abramson and Gerber (112) carried out two series of vaccination experiments with heated virus. In the first, 11 monkeys received daily injections of a 10% suspension of a potent strain received from the Rockefeller Institute (not otherwise identified). The daily dose was 5 ml. The first two doses had been heated to 55 C, the third to 45 C, the fourth to 37 C, all for 30 minutes. The fifth and final dose was of unheated virus. No paralytic infections resulted from the immunizing procedure. Two monkeys died of other causes before they were challenged. The infective titer of the virus is not stated. The following table shows the time interval from completion of vaccination to challenge; challenge dose, intracerebral, of 5% virus suspension; and the ratio of paralyzed to challenged animals.

NUMBER OF MONKEYS	TIME INTERVAL	CHALLENGE DOSE	PARALYZED TOTAL
	days	*ml*	
1	9	0.3	0/1
4	21	0.3	2/4
3	21	0.15	1/3
1	24	0.6	1/1
2	Controls	0.15	2/2
2	Controls	0.3	2/2
Total vaccinated			4/9
Total controls			4/4

Serum was obtained from 7 of the vaccinated monkeys just before challenge. Each serum was mixed with an equal volume of 5% virus, incubated two hours at 37 C and stored overnight on ice. One test monkey was inoculated with 0.6 ml of each mixture. All the 7 sera neutralized. In the second series of experiments, 5 monkeys were used. These also received each 10 daily injections of 5 ml of 10% virus suspension. With 3 monkeys, virus heated to 55 C for 30 minutes was used throughout. These were challenged 3 weeks after vaccination was complete. The challenge dose was 0.15 ml of 5% suspension for two monkeys, 0.3 ml for the third. All developed paralysis, as did two controls. In tests on sera of the 3 vaccinated monkeys, obtained just before challenge, one neutralized, two did not. Two monkeys received each 7 doses of the vaccine heated to 50 C, followed by 3 doses heated to 45 C. Both of these developed paralytic poliomyelitis from the vaccination procedure.

Levaditi (106) in a monograph published in 1922 reviews earlier work on

heat-treated vaccines and adds protocols on two monkeys. They received subcutaneous inoculations of virus heated to 55 C for 30 minutes (concentration not stated). Injections of 3 ml were given on two successive days, followed by two more of 5 ml each at 3 day intervals. Nine days later they were challenged intracerebrally (dosage not stated) with one control. All became paralyzed.

Shaughnessy, Harmon and Gordon (113), using the Aycock strain of poliomyelitis virus, carried out the most extensive study reported to that time on the inactivation of the virus by heat. Monkeys were inoculated with the heated virus, each animal receiving intracerebrally an amount corresponding to 4 paralytic doses, and intraperitoneally 10 paralytic doses. After heating to 50 C for 30 minutes the virus was still infective by this test but not after heating to 52.5 C for the same time. In an accompanying paper (114) the same authors report studies on immunization with heated virus.

The first experiment included 5 monkeys that had remained well after intracerebral inoculation with 2.0 ml of virus suspension heated at 55 C, for 5, 10, 20, 30 and 60 minutes, respectively. These were reinoculated 31 days later, by the same route, with 2.0 ml of 5% emulsion of unheated virus. Four of the five became paralyzed, the fifth resisted two further challenges. One control was paralyzed by 0.2 ml of the same emulsion.

Nineteen other monkeys had remained well after intracerebral inoculation with 2.0 ml of virus emulsion subjected to varying heat treatment: 55 C, from 5 to 30 minutes (5 monkeys); 52.5 C, 30 minutes (2); 50 C, 20 minutes to 1 hour (6); 47.5 C, 30 minutes (2); 45 C, 30 minutes (2); and 42.5 C, 30 minutes (2). These were all challenged 40 days later, intracerebrally, with 0.5 to 0.75 ml of unheated virus. Thirteen became paralyzed. Of the remaining 6, 5 became paralyzed at a second challenge; one resisted 3 challenges. Of 5 controls, only one developed definite paralysis following the first challenge, and one a questionable nonparalytic illness. One control became paralyzed at the second challenge, and two each resisted 3 challenges.

In a second series of experiments, 10 monkeys each received 14 inoculations of 2 ml each at 5 day intervals of Berkefeld V filtrates of virus. The first 5 doses had been treated to 50 C, and there followed 3 doses at 47.5 C, 3 at 45 C and 3 at 42.5 C. Six of the animals were vaccinated subcutaneously, two intradermally and two intracerebrally. The last two both developed paralytic poliomyelitis from the heated vaccine. Of the 8 vaccinated subcutaneously or intradermally, 7 became paralyzed at the first intracerebral challenge and the remaining one on second challenge. Of 3 controls, only one became paralyzed at the first, two at second challenge. The authors conclude that there was no evidence of immunity from the vaccinations.

Kolmer and Rule (68) used a 2% virus (MV) suspension, heated to 55 C for 30 minutes. Tricresol was added to 0.5% concentration. Monkeys were vaccinated daily for 10 days, 0.1 ml per kg each dose. One received the vaccine subcutaneously, two intracutaneously. All were finally challenged intracerebrally with 0.2 ml of 5% virus suspension, with two controls. All became paralyzed.

Levaditi (94) vaccinated 19 mice with an emulsion of Lansing virus 3 times heated to 50 C for one hour. Each mouse received 8 intraperitoneal injections

of 0.5 ml each. Another group of 19 was vaccinated in a similar manner with unheated virus. Six days after vaccination was completed, they were all challenged intracerebrally with 0.05 ml of the unheated emulsion. Of the mice vaccinated with unheated virus, all resisted challenge. Of those vaccinated with heated virus, 5/19 developed paralysis and died. Of 10 controls, 8 were paralyzed.

Melnick (104) inactivated Lansing and Y-SK virus by heating to 55 C for 30 minutes. Six cynomolgus monkeys were fed the treated virus in daily dosage of 3 ml of 10% suspension (infected mouse CNS). Each animal received a course of 22 doses of heated Lansing virus. A similar group of 6 cynomolgus received live untreated virus in the same dosage. No animal of either group developed symptoms of infection, and none developed neutralizing antibody. The experiment was repeated 3 months later with the same animals and the same result. Finally it was again repeated, substituting Y-SK for Lansing virus. No paralytic infections resulted, but 5 of the 6 monkeys fed live virus developed neutralizing antibody. Those fed the heat-inactivated virus remained seronegative.

No further work on vaccination with heat-treated virus has been reported. From the data cited it would appear that inactivation by heat destroys most if not all of the antigenic property of the virus.

Ultrasonic treatment. The only report found on vaccine prepared by this treatment is a brief one of Kasahara (115). Three grams of infected monkey cord were ground in 30 ml of saline, filtered through gauze and then through a Berkefeld V filter. The filtrate was treated with ultrasound, details of the technique being outlined. Three to 5 doses, 0.5 ml each at 2 day intervals, were injected intraspinally into cynomolgus monkeys. At 17 to 47 days after the last injection, the animals were challenged with 0.5 ml of live virus intraspinally. Protocols are given of 9 monkeys. Five resisted the challenge, 4 did not. No controls are mentioned. None of the monkeys was paralyzed by the vaccine.

Ultraviolet irradiation. Milzer, Oppenheimer and Levinson (116) describe a procedure for inactivation of Lansing virus by ultraviolet irradiation and for testing the product (in mice) for residual infectivity. A suspension of the irradiated virus (0.4% mouse CNS in water) was used as a vaccine. Seven groups of mice were vaccinated intraperitoneally with 1, 2 or 3 injections of 0.5 ml. They were challenged intracerebrally with 0.03 ml of Lansing virus, varying dilutions, one week after the last dose of vaccine. One group of mice received a single dose of vaccine. No protection was observed in these. In the other six groups, given each 2 or 3 doses of vaccine, the paralytic rate varied 2/12 (17%) to 4/8 (50%). In the controls, with the same virus dosage, the paralytic rate was 100%. The PD_{50} of the virus used was not calculated precisely, but from the data was evidently $>10^{-3.3}$.

Pooled serum from mice of each vaccinated group was tested for neutralization of Lansing virus (equal parts of undiluted serum and 10-fold serial dilutions of virus suspension, 0.03 ml of each mixture inoculated intracerebrally into 8 mice). Antibody of low titer was found in serum from the group that had received a single dose of vaccine. Serum of the other groups gave complete protection against 10% virus suspension. In further tests, serial dilutions of serum were used against a constant 10% virus suspension. PD_{50} end points were 1/5

serum dilution for mice given two doses of vaccine, 1/20 and 1/15 for two groups that had received 3 doses.

Morgan, Howe and Bodian (71) vaccinated 15 monkeys with ultraviolet irradiated virus (Riley strain). A 5% suspension of infected monkey cord was irradiated by the method of Milzer *et al.* (116). Three preparations were used, irradiated for 1, 3 and 5 seconds, respectively. These were separately tried as vaccines, 5 monkeys for each preparation. Each monkey received 2 ml subcutaneously, repeated at weekly intervals, 7 doses in all. Two of the 15 died of causes other than poliomyelitis. Five months after the beginning of vaccination, the 4 survivors of the first group (vaccine irradiated, 1 second) were bled and neutralization tests run. Equal volumes of undiluted serum and 20% Riley virus were mixed, and 0.8 ml of each mixture inoculated intracerebrally into two monkeys. The inoculum contained somewhat more than 1,000 PD_{50} of virus. Serum of one monkey neutralized, 3 did not. The 13 survivors vaccinated with ultraviolet irradiated vaccine were all finally challenged intranasally with homologous virus, 0.1 ml of 10% suspension into each nostril. All became paralyzed except the one monkey on which the positive neutralization test had been obtained.

While only one monkey of this series gave any evidence of immunity, parallel experiments with live, untreated virus, reported in the same paper, show that the subcutaneous route is relatively ineffective for vaccination. The small effect achieved with the irradiated vaccine cannot therefore be attributed entirely to low antigenic potency.

Dick *et al.* (117) used vaccines prepared by irradiation of Lansing virus (10% suspension). Three pools of virus were used, titered for infectivity by intracerebral inoculation (0.05 ml) into cotton rats. Titers of the 3 pools were $10^{-4.2}$, $10^{-3.7}$ and $10^{-4.2}$. Preliminary tests were made by inoculation of cotton rats and monkeys to determine the minimum irradiation required to render the vaccine noninfective. Vaccination consisted of 3 intramuscular injections, 1.0 ml each at weekly intervals, in cotton rats. Serial dilutions of the vaccine were used with different groups of animals. All were challenged intracerebrally, one week after the final vaccinating dose, with 100 PD_{50} of Lansing virus. The antigenicity of the vaccine was titered as the PD_{50} end point dilution protecting against the standard challenge. The antigenicity titer of the irradiated vaccine, on this basis, averaged $10^{-1.7}$. In an accompanying paper (100) the antigenicity titer of live virus, used as a vaccine, was $10^{-3.8}$ and $10^{-4.2}$ for two separate pools.

It therefore appears that the irradiated vaccine retains some antigenicity although it is a poor antigen in comparison with live virus. It was noted that antigenicity is destroyed by over-irradiation and that the margin of safety is quite limited.

Some observations on ultraviolet irradiated vaccines are reported by Casals *et al.* (see p. 396).

Electron bombardment (capacitron). Dick *et al.*, in the paper cited above, tried vaccines of Lansing virus treated by high speed electron bombardment. The methods of testing for infectivity, the vaccination procedure, and measurements of antigenicity were all the same as those employed with ultraviolet

irradiated vaccines. With electron bombardment the margin of treatment between inactivation and complete loss of antigenic potency was found to be somewhat wider than with ultraviolet irradiation. The antigenicity titer, however, was somewhat lower with the first method, averaging $10^{-1.35}$. Both methods were found to be more cumbersome and to have narrower margins of safety than chemical inactivation by means of formalin.

In summary, of the 5 physical methods of inactivation tried, only two have given definite evidence that they are capable of yielding noninfective vaccines with any antigenic potency. These have not been found to have any practical advantage over chemical inactivation.

2. *Vaccines treated by chemical agents. Phenol.* Levaditi (106) gives protocols on two monkeys treated with a phenolized vaccine. This experiment is briefly referred to in an earlier paper by Landsteiner and Levaditi (22). Phenol was added (0.5%) to a virus suspension of unstated concentration. The mixture was held 3 days on ice. Two monkeys each received a single subcutaneous injection of 6 ml. They were challenged respectively 11 and 12 days later, challenge dose not stated. Both became paralyzed, one of them two days after challenge.

Kraus (118) gave each of two monkeys a single subcutaneous injection of 6 ml of virus suspension (monkey cord, concentration not stated), treated with 0.5% phenol. One of these had received 5 ml of untreated suspension 15 days earlier. Both were challenged intracerebrally (dosage not given) 10 days after receiving the phenolized vaccine. Both resisted. One control became paralyzed.

In a second paper (119) Kraus reports inoculation of monkeys with unfiltered virus, concentration not given. The inocula had been treated with 0.5% phenol for 5 days, or with 1% for 1, 3, 4, and 6 days. He concludes that 0.5% phenol, or 1.0% with treatment up to 3 days, did not abolish infectivity. No infections occurred with preparations treated with 1.0% phenol for 4 days or longer. Only 3 monkeys, however, were inoculated with the 1%, 4 day preparation; two of these subcutaneously, one intracerebrally, dosage unstated. Suspensions of infected brain and cord, combined, were treated with phenol as indicated below and used as vaccines, a single dose being given subcutaneously to each animal:

MONKEY	PHENOL, CONC. %	TIME OF TREATMENT	DOSAGE OF VACCINE	INTRACEREBRAL CHALLENGE First	Second	Third
		days	*ml*			
1	0.5	5	5.0	Resisted	—	—
2	0.5	5	8.0	Paralyzed	—	—
3	0.5	3	5.0	Resisted	Paralyzed	—
4	0.5	3	6.0	Resisted	Resisted	Resisted
5	0.5	5	5.0	Resisted	Paralyzed	—
6	0.5	5	10.0	Resisted	—	—
7	1.0	3	5.0	Resisted	Resisted	
8	1.0	3	5.0	Paralyzed	—	—
9	1.0	6	10.0	Resisted	Resisted	—
10	1.0	6	10.0	Resisted	Resisted	—
11	1.0	6	10.0	Resisted	Resisted	—

Five controls all became paralyzed on challenge.

The first 8 monkeys in the table above received a suspension of vaccine of unstated concentration. The last 3 received vaccines prepared from the supernatant fluid of a 5% suspension. Challenge dosages are not given.

A third report by Kraus (120) deals with a vaccine prepared by grinding two infected monkey brains in a suspension medium composed of 200 ml saline, 50 ml glycerol and 2.5 ml of phenol. Protocols are given of 10 monkeys inoculated subcutaneously (8) or intraperitoneally (2) with single doses of 3 to 5 ml. Seven were challenged by combined intracerebral and intraperitoneal inoculation of untreated virus (dosage not stated) 16 to 66 days after vaccination. Four remained well, 3 became paralyzed. The absence of unvaccinated controls leaves it uncertain whether the vaccine had any protective effect. Two monkeys became paralyzed from the vaccination procedure. One of them had received vaccine stored in the cold for a month; hence the phenol treatment evidently did not inactivate the virus under the conditions described.

Flexner and Amoss (121) used 0.5% phenol (and glycerol) in attempts to render bacteriologically sterile a human brain from a fatal case of poliomyelitis. Sterility was not achieved, but by inoculation of the brain material into monkeys a strain of virus ("Buffalo") was isolated. Protocols are given of 9 monkeys inoculated with second or third passage virus of this strain. All developed characteristic symptoms of poliomyelitis, but in relatively mild form, and all recovered more or less completely. Four of them were subsequently challenged intracerebrally with MA virus and all resisted, while two controls became paralyzed. The authors speculate that treatment of the source material with phenol might have attenuated the Buffalo strain.

Aycock and Kagan (60) added to suspensions of virus (concentration not stated) phenol in concentrations of 1.0, 0.75, 0.50 and 0.25%. The 4 preparations were stored in a refrigerator for 7 days. They were then used to vaccinate 4 monkeys, subcutaneously. Each monkey received a first injection of 8 or 10 ml of the vaccine treated with 1.0% phenol, followed by similar injections of the other three, in order of descending phenol concentration. The injections were spaced at 2 day intervals. Two of the four monkeys became paralyzed from the vaccine itself. The two remaining were challenged intracerebrally; one resisted, one became paralyzed.

Cowie (122) prepared a vaccine consisting of 5% virus suspension in 0.7% phenol treated "according to the Pasteur vaccine technique". Monkeys were vaccinated with daily subcutaneous injections, starting at 0.1 to 0.3 ml; gradually increased to 3.5 or 4.0 ml. This was continued for 28 or 29 days. Three monkeys were thus vaccinated. They were challenged intracerebrally at different times up to 30 days from the completion of vaccination. A "proved paralyzing dose" (not otherwise defined) of homologous virus was used for challenge. Two monkeys resisted the first challenge, one became paralyzed. Both the resistant monkeys became paralyzed at a second challenge, one at one month, one about 4 months later.

Brodie (17) made preliminary tests on the infectivity of virus preparations treated with phenol in varying concentrations and for varying periods of time.

The original virus suspension was assayed for infectivity, in terms of "minimal completely paralyzing doses" (MCP). The various phenol treated samples were each tested by intracerebral inoculation of one monkey with inocula equivalent to 40, 80, 320, 400, or 800 MCP of the original suspension. Concentrations of 0.5 and 1.0% phenol did not inactivate the virus completely. The virus in a 10% tissue suspension was inactivated by treatment with 2% phenol, in the cold, for 1.5 days. (Test animals resisted inoculation with amounts equivalent to 80 and 320 MCP of the untreated suspension.)

Two monkeys were vaccinated with a 10% virus suspension treated with 2.0% phenol under refrigeration for 10 days. Each monkey received a single dose of 10 ml intracutaneously. Subsequently they were challenged intracerebrally and bled at the same time for neutralization tests. One monkey resisted successive challenges with 1 MCP and 2 MCP of virus but became paralyzed at a third challenge with 3 MCP. The second monkey became paralyzed at the first challenge with 1 MCP. Four controls, inoculated with 1 MCP, all became paralyzed.

Neutralization tests were made by adding to a small volume of virus suspension (0.05 ml or less) enough undiluted serum to make the total volume 1.0 ml. After this incubation was inoculated into one test monkey. The serum of one vaccinated monkey (the one that resisted first challenge) failed to neutralize 1 MCP of virus. The serum of the other vaccinated monkey neutralized 1 MCP but failed to neutralize 2 MCP. Brodie concludes that phenolized vaccine is relatively weak as an antigen. He adds that the concentration of phenol required to inactivate the virus is highly irritating to tissues at the site of injection. No report of any later work with phenol treated vaccines has been found.

Aluminum hydroxide. Rhoads (123, 124) treated 5% suspensions of infected monkey cord, or filtrates from such suspensions, with alumina gel. A solution of the latter, 22.5 g per liter, was added in varying proportions to the virus, and the mixtures allowed to stand for 30 minutes at room temperature. Tests for infectivity of the mixtures were made by intracerebral inoculation of monkeys as follows:

MONKEY	$Al(OH)_3$	INOCULUM, ML VIRUS FILTRATE	RESULT
1	0.05	0.05	Resisted
2	—	0.05	Paralyzed
3	0.1	0.2	Resisted
4	0.02	0.2	Paralyzed
5	—	0.2	Paralyzed

It was concluded that the gel inactivates the virus when equal volumes are mixed but not in a volume ratio of 1:10. The effect was found dependent on pH. Mixtures of 0.2 ml each of $Al(OH)_3$ and virus filtrate were prepared, one at pH 8.8, the other at 5.5. Both were centrifuged and the supernates each inoculated into one monkey. The supernate at pH 8.8 produced paralysis; the other did not.

In further experiments, alumina gel solution was mixed with 10 parts of a 5% glycerolated virus suspension. The latter was said to be of lower infectivity before admixture than fresh virus and to have become noninfective after treatment. Tests establishing these points are not described. Four rhesus monkeys were vaccinated subcutaneously with the treated virus. Each received 5 doses of 15 ml each, at 3 day intervals, totalling 75 ml per animal. One month after vaccination was complete, all were bled. Neutralization tests were made, 0.99 ml of undiluted serum against 0.01 ml of virus filtrate. All the sera neutralized. One monkey was lost by intercurrent infection. The 3 survivors were challenged intranasally. All resisted; one control became paralyzed. One month later the vaccinated monkeys were again challenged, intracerebrally. One became paralyzed, also one control; two resisted.

Sabin (125) showed that virus treated with alumina gel at low pH became adsorbed but could be eluted in alkaline solution, and that infectivity was retained. He mixed equal volumes (5 ml) of alumina gel solution and filtrate from a 5% virus suspension, adding 1 ml of M/15 KH_2PO_4. This mixture was shaken, stored 3 to 4 hours, shaken again and finally centrifuged. The supernate was tested as described below. The sediment was washed with water. It was then resuspended in 5 ml of M/15—Na_2HPO_4, shaken, stored, shaken again and centrifuged. A sample of the original virus filtrate was then diluted 1/20. The first supernate referred to above and the eluate were diluted to correspond, and each was tested by intracerebral inoculation into two monkeys, 1 ml inoculum. The first supernate produced no evidence of infection, all monkeys receiving the other inocula developed paralysis.

Schaeffer and Brebner (126) made similar observations. Their brief account omits experimental data. Quoting,

> "at pH 7.0, adsorption is incomplete. The optimum range is apparently pH 5.4 to 6.4, for within it the supernatant liquid is nearly always free from virus. As the pH approaches 7.0 or goes toward 5.0, the supernatant becomes infectious. Contrary to the observations of Rhoads, we have found the gel infectious when virus was adsorbed on it, both at pH 5.4 and at 6.2, so that adsorption in these instances did not seem to inactivate the virus . . . . Elution takes place above pH 7.0."

Kramer, Grossman and Hoskwith (127) vaccinated monkeys with virus adsorbed on alumina gel. The supernate from a centrifuged 10% suspension of virus (monkey cord) was mixed with an equal volume of alumina gel solution, and the pH adjusted to about 6.0. Details of further treatment are here omitted. The sediment at the final centrifuging, gel with virus adsorbed, was diluted so that 1 ml contained the equivalent of 0.5 g of the original infected cord. This concentration was used as a vaccine. A sample further diluted was used, in a dosage equivalent to 0.02 ml of the original 10% virus suspension, for intracerebral inoculation of two monkeys to test its infectivity. Both animals became paralyzed.

In the vaccination experiments, neutralization tests were made using 0.5 ml of undiluted serum, mixed with an equal volume of virus suspension con-

taining 1 MID. Intracerebral challenge of the vaccinated animals was with varying dosages expressed in MID units. Vaccination was subcutaneous.

I. a. Six monkeys each received a single dose equivalent to 0.5 g of infected cord. They were bled 3 to 4 weeks later. Serums of 2/6 protected. Three were lost by intercurrent infection, 3 succumbed to challenge with 2 MID or less.

b. Six monkeys, same procedure except that two doses of vaccine were given one week apart. Serums of 3/6 neutralized. 5/6 succumbed to challenge with 2 MID or less.

c. Six monkeys, 3 doses of vaccine totalling 2.0 g, procedure otherwise the same. Three monkeys were lost by intercurrent infection. Serums of 4/5 neutralized. Of 3 remaining to be challenged, 2 succumbed to 2 MID, the survivor resisted re-challenge with 8 MID.

II. Ten monkeys, given 1.5 g each, divided into 3 weekly doses. Serums of 7/10 neutralized. The same number survived repeated challenges up to 64 MID.

III. Ten monkeys, given 2.0 g each, divided into 4 weekly doses. Serums of 8/10 neutralized. Two were lost by intercurrent infection. Three survived repeated challenge, up to 64 MID, 5 succumbed with varying dosages.

Gordon, Hudson and Harrison (69) used the supernate from a lightly centrifuged emulsion (6%) of MV virus, treated with an equal volume of alumina gel solution. Both virus-gel complexes and eluates were prepared by procedures essentially similar to those followed by Sabin (125). Both were used as vaccines, and untreated live virus used in equivalent dosage for comparison. Each dose was of 10 ml, equivalent to 0.3 g of infected cord, given subcutaneously. Controls were vaccinated with normal monkey cord treated with alumina gel. The vaccinated monkeys were bled one month after the last dose of vaccine. Neutralization tests were made using 1.5 ml of undiluted serum mixed with 0.5 ml of 1.0% virus suspension. The vaccinated monkeys also were challenged by intranasal inoculation (one exposure).

I. Six monkeys were vaccinated with virus-gel, 1 to 5 doses per animal. One developed paralysis from the vaccine. Of the survivors, all developed neutralizing antibody, but only 1 of 4 resisted challenge. Six monkeys were similarly vaccinated with eluate, 1 to 5 doses, all developed neutralizing antibody. Only one of 6 resisted challenge.

II. Six monkeys each received a single dose of vaccine. Only one developed neutralizing antibody, all became paralyzed on challenge. Two controls each received 5 doses of normal CNS tissue-gel. Their serums failed to neutralize and both succumbed to challenge.

III. Nine monkeys were vaccinated with a 6% untreated virus suspension in 5 ml doses, given once (4 monkeys), 3 times (3 monkeys) or 5 times (2 monkeys). Two became paralyzed from the vaccine. Serums of 6 were tested and only two neutralized. Of 7 challenged, 4 became paralyzed and 3 showed indications of possible mild infection.

IV. Serums from I and II above were respectively pooled. Each pool was

tested for neutralization, undiluted and in dilutions of 1:5 and 1:25. Neutralization occurred only with undiluted serum of I.

The authors found no evidence that virus-gel vaccines had any advantage over eluates or untreated virus.

Levaditi (93, 94) used a vaccine prepared by adding to an emulsion of Lansing virus alumina gel (16%) and treatment with 0.5% formalin. The precipitate was washed and resuspended in 5 volumes of saline. The product was used to vaccinate 10 mice, 0.5 ml intraperitoneally at each dose, 8 doses spaced 2 to 4 days apart. Another group of mice was simultaneously vaccinated with a vaccine similarly prepared except that formalin treatment was omitted. Both groups, with 10 unvaccinated controls, were challenged intracerebrally one week after the final dose of vaccine. Deaths in the two vaccinated groups were, respectively, 7/10 and 7/9; in the controls, 7/10.

Miscellaneous chemical agents. a. Glycerol. Aycock and Kagan (60) prepared a series of suspensions of infected monkey cord in varying concentrations of glycerol, increasing from 5 to 50% in steps of 5%. Eight monkeys were vaccinated with daily injections, 11 in all, starting at 5% and progressing to 50% glycerol. Because of the known stabilizing effect of glycerol on the virus in storage, this procedure was designed to begin with an attenuated dose and progress to more potent doses of virus. Three monkeys developed paralytic infections from the vaccine; one died of irrelevant cause. The 4 survivors were challenged. Three became paralyzed, one resisted. No details are given on virus concentration in the suspensions used, on the dosage of vaccine, or on the method of challenge. The vaccine was given subcutaneously.

b. Chloroform. Kolmer and Rule (68) treated a 2% suspension of MV virus with chloroform (1%). The mixture was stored cold for two weeks, shaken 3 times daily. Two monkeys were vaccinated with this preparation, one subcutaneously, one intracutaneously. Ten daily doses of 0.1 ml each were given to each animal. Both succumbed on intracerebral challenge with 0.2 ml of 5% suspension of untreated virus.

c. Tannic acid. Olitsky and Cox (128) treated 5% virus suspensions with tannic acid to precipitate the virus. Protocols are given of 8 monkeys that received subcutaneous injections of 2 ml of virus so treated. Two developed paralysis from the first immunizing injection. Two died from intercurrent infections. Four completed the series of 3 injections, given at intervals of 7 to 14 days. All were challenged with homologous virus (MV or Philadelphia strain), 3 intranasally, 1 intracerebrally. Three of the 4 became paralyzed. Four additional monkeys were vaccinated with a more dilute suspension of virus (0.1%) treated with tannic acid. Three doses, 1, 1 and 2 ml, were given at 9 or 10 day intervals. Two were challenged intracerebrally and both succumbed. Two resisted a first intranasal challenge but succumbed to a second 260 days after the first. Of the series treated with 5% virus antigen, pooled serum of two monkeys, and individual sera of the other two, all neutralized. Of the series treated with 0.1% virus, pooled serum of two neutralized, individual sera of the other two

were both negative. Titration data on the viruses used are not given. Intracerebral challenge of the immunized monkeys was with 0.2 ml of filtrate from a 5% suspension, and in neutralization tests the same virus dosage was mixed with 0.8 ml of undiluted serum. It is stated that "only exceptionally" did normal monkeys resist intracerebral inoculation with the 0.2 ml dose of virus filtrate.

d. Potassium chlorate. Contat and Spycher (129) used a virus strain obtained from the Pasteur Institute at Paris. It is stated that 0.2 ml of 1% suspension was sufficient to paralyze rhesus monkeys, but actual protocols of the tests are not given. In a first series of experiments, 40% virus suspension was mixed with an equal volume of 5% $KClO_3$ solution. After two hours of contact, 0.4 ml of the mixture was inoculated intracerebrally into each of two rhesus monkeys. They remained well. Fifteen days later both were reinoculated with 0.3 ml of a 20% suspension of untreated virus. Both resisted; two controls became paralyzed in 10 days. In a second series of experiments, virus was treated with $KClO_3$ in the same concentrations as above. Monkeys were vaccinated intraperitoneally in groups of 3 with virus treated with $KClO_3$ for (a) 30 minutes, (b) 1 hour, (c) 2 hours, or (d) 4 hours. Each animal received 3 doses, of 2.5 to 3.5 ml each, at 10 day intervals. From group (a), 2/3 became paralyzed from the vaccination procedure, from group (b) 1/3, none from (c) or (d). The 9 survivors were challenged intracerebrally with 0.2 ml of 20% untreated virus 10 days after the final dose of vaccine. One became paralyzed, 8 resisted; two controls were both paralyzed.

The authors state that monkeys inoculated intracerebrally with untreated virus, and given $KClO_3$ medication daily, not only remained well but resisted later challenge. It is not clear from the text how many such instances of immunity actually were demonstrated. Later reports from other sources (130, 131) failed to confirm the protective action of $KClO_3$ *in vivo*. No other reports on the use of $KClO_3$ for preparation of vaccines have been found.

e. Nitrogen and sulfur mustards. Schwerdt and associates (100) treated 10% suspensions of Lansing virus with varying concentrations of a nitrogen mustard, tris(B-chloroethyl)amine, or of a sulfur mustard, bis(B-chloroethyl)sulfide. One preparation, treated with nitrogen mustard, showed no residual infectivity by tests on cotton rats, and as a vaccine titered $10^{-1.4}$. The method of titration is referred to above (p. 366). This promising result could not be repeated using the same or other concentrations of reagent. All other preparations tried were found either to have retained infectivity or to have lost their antigenic property.

Ricinoleate. The first reported experiments with ricinoleated virus were those of McKinley and Larson in 1926 (132). Four monkeys in all were inoculated with a 5% suspension of virus (MA strain) in 1% sodium ricinoleate. The first received 1.5 ml intracerebrally. An immediate hemiplegia developed from the direct action of the soap solution on brain tissue, but no indication of infection. A control inoculated by the same route, with an equal dose of virus in saline, likewise resisted. Five months later the hemiplegic monkey was again inoculated with virus in ricinoleate, 0.7 ml, and again resisted. A control was paralyzed in 5 days. A second monkey, inoculated intracerebrally with soap-virus mixture,

also developed immediate hemiplegia but resisted infection. A control was paralyzed. Two additional monkeys received, respectively, 5 and 4 ml of soap-virus mixture intraperitoneally with no paralysis developing. Finally all 4 of the monkeys treated with ricinoleated virus were challenged intracerebrally with 1 ml of virus in saline. One became paralyzed, 3 resisted. Three controls were paralyzed.

Kolmer and associates carried out extensive experiments with ricinoleated vaccine on monkeys and on human subjects. The MV strain of virus was used throughout. The virus used in their first reported experiments (68) was "of such virulence that 0.2 ml of a 5% emulsion regularly produced paralysis in rhesus monkeys in 7 to 11 days". To a 2.0% suspension of this virus, sodium ricinoleate was added to 10% concentration. The mixture was stored cold for 2 weeks, shaken 3 times daily. Five monkeys were vaccinated with this preparation, 2 subcutaneously and 3 intracutaneously, 10 daily doses of 0.1 ml per kg each. All were challenged intracerebrally with 0.2 ml of 5% untreated virus suspension. Two developed typical paralytic poliomyelitis, two mild symptoms only, one remained well. Two controls were paralyzed.

In all subsequent studies, Kolmer used a vaccine of 4.0% virus suspension containing 1.0% sodium ricinoleate. Other details of preparation were modified from time to time. The final technique described (133) included preliminary storage of infected cord material in 50% glycerol for one week, "as this appears to reduce the virulence of the virus" (grounds for this belief are not stated); then addition of 1.0% sodium ricinoleate and phenyl mercuric nitrate to a concentration of 1/80,000, the latter as a precaution against bacterial contamination. Finally the material was stored for 8 or 10 days, at 4 to 8 C, before use.

Although the two monkeys inoculated intracerebrally by McKinley and Larson (132) with ricinoleated virus had not shown signs of infection, Kolmer's vaccine did induce paralytic disease by this route. He considered completely inactivated virus to be ineffective for immunizing, citing in evidence observations of Levaditi, Kling and Haber (*vide infra*) and of his own (134). He argued, however, that ricinoleate treatment did reduce the pathogenicity of the virus for monkeys by intracerebral inoculation. Evidence cited to support this hypothesis is as follows (135):

> "Intracerebral inoculation of monkeys with 0.3 ml of a vaccine 5 months after its preparation produced mild paralysis about 12 days later and showing thereby the survival of devitalized virus in the vaccine."

The number of animals used in this test is not stated, nor is any reference made to controls. Another paper (136) reports a relatively long incubation period after intracerebral inoculation with ricinoleated virus. Three monkeys each inoculated with 0.2 ml became paralyzed after 11, 9 and 12 days, respectively. One control, inoculated with 0.1 ml of 5% suspension of fresh untreated virus, was paralyzed in 5 days.

Kolmer further suggested that the MV virus used in the vaccine had through repeated passage in monkeys lost, in part or entirely, its original pathogenicity

for man (134). Actually no evidence for this appears to have existed prior to use of the vaccine on man. The outcome of the human trial did indicate a low infectivity of ricinoleated MV virus for man by the route of inoculation employed. It would be difficult to say, however, whether this was due to characteristics of the human host species, of the original strain of virus, or whether it resulted from modification of the virus by monkey passage or by the process of preparing the vaccine.

Finally, Kolmer held that ricinoleate treatment reduced infectivity of the virus by peripheral routes of inoculation, even for monkeys. At one time he had vaccinated 42 monkeys by subcutaneous injection of the vaccine, 0.5 ml per kg, repeated for a total of 5 doses at 5 day intervals. None had become paralyzed. In a control group of 20 monkeys, similarly inoculated with untreated virus, one paralytic infection occurred (135). Subsequently, however, some monkeys were infected by the vaccine given subcutaneously. A final review (137) lists 183 monkeys vaccinated with ricinoleated vaccine in his laboratory, 124 subcutaneously, 59 intracutaneously. Three became paralyzed by the vaccine. All of these received it subcutaneously, there being no infections among the 59 vaccinated intracutaneously.

Kolmer and Rule (135) vaccinated two groups of monkeys. The first group each received 10 injections at 5 day intervals. Six were vaccinated subcutaneously, 0.05 to 1.0 ml per kg at each dose. Two received 0.1 ml per kg intracutaneously at each dose. All were challenged intracerebrally one month after vaccination was complete. The challenge dose, 0.2 ml of 5% suspension, was said to represent about 18 MID of virus. All resisted. An unstated number of controls all became paralyzed. The second group was similarly vaccinated, except that only 5 doses were given and the minimal dose was 0.1 ml per kg. Seven received the vaccine subcutaneously, 3 intracutaneously. These likewise all resisted intracerebral challenge while an unstated number of controls all became paralyzed. Both groups of vaccinated monkeys were re-challenged 7 months later and all resisted. At a third challenge, after 3 months more, one became paralyzed.

The same authors (138) studied in monkeys the effectiveness of ricinoleated vaccine given during the incubation period. Monkeys were inoculated intracerebrally with 0.5 ml of 5% virus, stated to represent a dosage of 5 to 10 MID. The vaccine was given subcutaneously, 3 daily doses started at varying time intervals after inoculation, with results shown in the following table:

NUMBER OF DAYS AFTER INOCULATION BEFORE VACCINE WAS GIVEN	NUMBER OF MONKEYS TESTED	NUMBER OF MONKEYS PARALYZED
1	4	2
3	3	1
5	3	2
8	1	1
9	1	1
10	1	1
12	1	1
Controls	4	4

These data suggest that the vaccine may have had some protective action when given early in the incubation period following intracerebral inoculation. They are somewhat scanty, however, to warrant any definite conclusion, and no confirmatory evidence has been found.

Schultz and Gebhardt (139) reported immunization of a series of monkeys (number not stated) with the Kolmer vaccine. The serums of these animals 'neutralized 30 MID doses of virus per ml", but on intranasal challenge all developed typical poliomyelitis.

Olitsky and Cox (128) vaccinated 6 monkeys with a 4% suspension of virus, treated with 1% sodium ricinoleate and stored for one month. Five doses at 5 day intervals were given to each animal, 0.27 to 0.35 ml subcutaneously at each dose. Pooled serum from two of the vaccinated monkeys and individual sera from the 4 others, all neutralized. Two were challenged intracerebrally, and both became paralyzed. Four were challenged intranasally, two becoming paralyzed. Of the two resisting, one became paralyzed on re-challenge (see reference, p. 381, for details on neutralization test and challenge procedures).

Kramer and Grossman (140) used 3 lots of ricinoleated vaccine, one supplied by Kolmer, the others prepared by the authors following Kolmer's directions. Infectivity of all 3 was tested by intracerebral inoculation. The first lot, after storage for 4 months, produced paralysis in one monkey in 0.2 ml dosage and in 3 others with doses of 0.6 ml. The authors' own preparations paralyzed 5 monkeys, in dosages varying from 0.13 to 0.6 ml. They failed to find any evidence of "attenuation".

Three groups of monkeys were vaccinated. All were bled 4 to 6 weeks after vaccination was complete, and neutralization tests carried out as described in a previous reference (127). All were challenged intracerebrally, minimal dosages being employed. The first group of 6 monkeys (one of these was lost from pneumonia) each received 3 subcutaneous injections of 0.1 ml per kg at weekly intervals. Only 1 serum of 5 neutralized. The immunized animals were challenged with 1/5 MID of virus, 1 of 5 becoming paralyzed. Only 1 of the 4 resisting survived a second challenge with 1 MID, and the 1 survivor became paralyzed at a third challenge with 2 MID. The second group of 12 monkeys each received 5 injections, same dosage and time schedule. None of them developed neutralizing antibody. All were challenged with 1 MID of virus, only 1 resisting. The survivor became paralyzed on re-challenge with 2 MID. The third group of 18 'was vaccinated in the same manner as the second. Sera of 7 neutralized, 11 did not. All succumbed in a series of challenges with 1/5 MID, (8/18); 1/2 MID, (5/10); 1 MID, (4/5); and 2 MID (1/1).

Levaditi, Kling and Haber (141) used 4 different vaccines, all 4% virus suspension in 1% sodium ricinoleate and prepared by Kolmer's procedure. These were used to vaccinate different groups of monkeys subcutaneously as follows:

1. A Pasteur Institute strain of virus, with ricinoleate obtained from a French source. Six monkeys were vaccinated. Five developed paralytic poliomyelitis after 2 to 3 0.1 ml doses of the vaccine. One survived a course of 10 doses and resisted intracerebral challenge.

2. A supply of Kolmer's vaccine obtained from him. The vaccine proved noninfective by intracerebral test on monkeys. Four were vaccinated with it, 5 doses of 0.5 ml each. All 4 succumbed to intracerebral challenge. The vaccine was considered as having probably deteriorated in transit.

3. The Pasteur Institute virus, ricinoleate obtained from Kolmer's source of supply in the United States. Five monkeys were vaccinated of which 3 developed paralysis from the vaccine. Two survived 5 doses of 0.5 ml each, and both resisted intracerebral challenge.

4. Virus (MV) and ricinoleate obtained unmixed from Kolmer. Six monkeys were vaccinated. Two developed paralysis from the vaccine. Four survived 5 doses of 0.5 ml each and resisted intracerebral challenge.

Controls were included with each challenged group of monkeys, and all controls succumbed. The diagnosis of poliomyelitis in the paralyzed animals was verified by histologic examination. The authors found the vaccine capable of immunizing but found no evidence that it differed in pathogenicity from untreated virus.

The final report on animal vaccination by Kolmer (133) reviews the work on ricinoleated vaccines in his own laboratory and elsewhere. New data are reported to show that finely divided virus suspension is more effective as a vaccine than either a filtrate or the supernate from centrifuged suspensions. All three materials were treated with ricinoleate and used to vaccinate different groups of monkeys. Five doses were given to each animal at 5 day intervals, each dose 0.05 to 0.25 ml per kg subcutaneously or intracutaneously. All the monkeys were challenged intracerebrally, 0.5 ml of 5% virus. Results of challenge were:

VACCINE	RATIO OF PARALYZED ANIMALS AT CHALLENGE
Filtrate	10/10
Centrifugate	3/8
Suspension	2/8
None, controls	3/3

Data also are shown on a group of 18 monkeys immunized with the regular vaccine, employing varied doses and routes. Each monkey received 5 doses at 5 day intervals. All were challenged intracerebrally, first with 0.2 ml and survivors 18 days later with 0.5 ml of 5% virus suspension.

NUMBER OF MONKEYS	ROUTE OF INOCULATION	DOSE	OUTCOME OF CHALLENGE First	Second
		ml/kg		
4	Subcutaneous	0.1	0/4	0/4
4	Subcutaneous	0.25	0/4	1/4
2	Subcutaneous	0.5	0/2	0/2
4	Intracutaneous	0.1	0/4	1/4
4	Intracutaneous	0.25	0/4	1/4
Controls 2			2/2	
Controls 2				2/2

A tabulation is given of 58 monkeys vaccinated subcutaneously at 5 day intervals, 5 or 10 injections, dosage varied. All were challenged intracerebrally with 0.5 ml of 5% virus suspension.

NUMBER OF MONKEYS	DOSAGE	NUMBER OF DOSES	PARALYZED DURING VACCINATION	CHALLENGE RATIO PARALYZED
	ml/kg			
11	0.05	5	0	4/11
1	0.05	10	0	0/1
8	0.1	5	0	3/8
2	0.1	10	0	0/2
18	0.25	5	2	2/16
3	0.4	5	0	1/3
10	0.5	5	0	0/10
1	0.5	10	0	0/1
3	1.0	5	1	0/2
1	1.0	10	0	0/1
Controls 6				6/6

Including data reported in earlier papers, the results of subcutaneous and intracutaneous vaccination of monkeys with Kolmer's vaccine in his laboratory are summarized as follows:

NUMBER OF MONKEYS	DOSAGE	NUMBER OF DOSES	PARALYZED DURING VACCINATION	CHALLENGE RATIO PARALYZED	IMMUNE
	ml/kg				*per cent*
I. Subcutaneous					
14	0.05	5–10	0	5/14	64
16	0.1	5–10	0	4/16	75
26	0.25	5	2	5/24	73
3	0.4	5	0	1/3	67
15	0.5	5–10	0	1/15	93
6	1.0	5–10	1	0/5	100
II. Intracutaneous					
10	0.05	5	0	6/10	40
15	0.10	5–10	0	3/15	80
16	0.25	5	0	1/16	94
2	0.5	5	0	0/2	100

Kolmer concludes that for a given dosage of vaccine intracutaneous inoculation is more effective than subcutaneous. This is in accord with results obtained in immunizing with untreated virus by the same routes.

Kolmer and one of his associates (136) subjected themselves to vaccination. Three doses of 0.5, 1.5 and 2 ml, respectively, were given at intervals of 5 days. The vaccine was then given to a group of 25 children. Finally, it was prepared in quantity and given to a large number of other human subjects during 1935. The recommended dosage for adults consisted of three subcutaneous injections, 0.5, 1.0 and 2.0 ml at weekly intervals, with smaller doses on the same time

schedule for children. Numbers and age distribution of the vaccinated persons were given as follows (134):

Under 4 years	3,313
4 to 15 years	6,444
Over 16 years	995
Total	10,752

Nine cases of paralytic poliomyelitis developed in subjects who had received the ricinoleated vaccine (134). During the same year another poliomyelitis vaccine (Brodie's, formalin-treated) was being tried on human subjects. Leake (142) lists 9 paralytic cases that developed following administration of "vaccine A", and 3 after "vaccine B". The interval between the first dose of vaccine and onset of illness varied from 8 to 31 days. In 7 of the 9 cases associated with "vaccine A" the site of primary paralysis was correlated segmentally with the site of vaccination.

Because of the hazard thus revealed, use of both vaccines on human subjects was stopped. It was naturally suspected that the source of these paralytic infections was live virus present in the vaccines. The ricinoleated vaccine admittedly contained viable virus, and there appears to be no real evidence that this virus had been rendered any less pathogenic for man than it had been when originally isolated. On the other hand, paralytic poliomyelitis, correlated in a similar way with the time and site of injection, has occasionally been observed following administration of other immunizing agents which presumably did not contain poliomyelitis virus in either active or inactive form (reviewed in references 143 and 144). We do not know whether injection of a completely inactivated poliomyelitis vaccine is or is not capable of such a nonspecific effect.

Among the 10,752 persons said to have received the ricinoleated vaccine, one case of poliomyelitis two years later was reported (145), in addition to the 9 earlier cases mentioned above. The vaccinated subjects were distributed over 36 states (133). No controlled study was attempted, and direct proof that the vaccine had any protective effect in man is lacking.

Some data were obtained on the serologic response of human subjects to the ricinoleated vaccine. Kolmer and Rule (136) made neutralization tests on their own serums before and two weeks after a course of 3 injections. The first test on each was negative; the second, positive. Data are given on 25 children, bled before and one week after completion of a series of 3 weekly injections. Ages of the children ranged from 8 months to 15 years. In the tests, 0.5 ml of undiluted serum was mixed with 0.5 ml of 10% virus suspension. Positive tests were repeated with 50% virus suspension, and the results indicated as + or ++. Control monkeys received 10% virus mixed with saline.

Ten of the prevaccination sera are listed as +, 15 as 0. Eleven post-vaccination sera are listed as ++, 10 as +, and 4 as 0. None of the sera initially positive reverted to negative.

Kolmer's own data thus indicate a fairly consistent antibody response to his

vaccine in monkeys and in man. Other investigators reported an antibody response to the vaccine in monkeys, consistently (128, 139) or irregularly (140). The only neutralization tests reported by others on human subjects receiving this vaccine are inconclusive (146).

At the time when the Kolmer and Brodie vaccines were tried, the existence of distinct immunologic types of poliomyelitis virus had not been definitely established. It is now known that immunity conferred by vaccination is type-specific (74). Since both Kolmer and Brodie used the MV ("mixed virus") strain, the history of that strain is pertinent. Quoting Flexner (147),

> "The specimen now called mixed virus was first successfully passaged in monkeys in 1909, and as its activity fluctuated, strengthened by additions of active specimens as they were secured up to 1920 or thereabouts. Mixed virus is therefore a polyvalent specimen."

Brodie (148) briefly refers to cross-immunity tests of the MV virus against two strains more recently isolated. "Sera of 2 animals actively immunized and 1 convalescent" (MV antisera) "were tested against 1 strain. . . . The serum neutralized only about 1/10 as many infective doses of the recent passage as of the fixed virus". Three MV-immunized monkeys and 3 MV-convalescents were challenged intracerebrally with the second heterologous strain. The paralytic rates were respectively 2/3 and 0/3. The tests are not described further.

Bodian (149) has inoculated monkeys with a mixture of Brunhilde (Type 1) and Lansing (Type 2) viruses and investigated the survival of the two types during serial passage. Ten monkeys were inoculated intracerebrally with the mixture. From the spinal cords of two of these animals, Brunhilde virus alone was isolated; from two others Lansing alone. Cord tissue of the remaining 6 yielded both types. From the 6 cords in which the dual infection was demonstrated, passage was made to another series of 12 monkeys. Cord tissue from each of the 12 was inoculated into cotton rats as a test for the presence of Lansing virus. Seven of the 12 samples failed to infect cotton rats, while 5 did. Reciprocal neutralization tests on the 12 samples have not been completed, but 3 were shown to contain Lansing alone and 1 Brunhilde alone. From these observations it seems unlikely that a mixture of two or more virus types would continue to represent all its original constituents after a series of passages.

The constituent strains added to MV from time to time may have included representatives of the 3 immunologic types now known, but this is conjectural. When the MV strain was finally typed in 1949 (16, 35), its behavior was that of an unmixed Type 2 virus. Available evidence suggests that this type contributes a relatively small percentage to the total number of cases reported as poliomyelitis. Therefore it would probably be difficult to evaluate any unmixed Type 2 vaccine from the morbidity statistics in a human experiment, even with controls that were lacking in 1935.

Formalin. Römer (110) mentions one monkey inoculated intracerebrally with formalin treated virus (no details). The animal remained well. Twenty-four days later it was challenged with a virus suspension of unstated concen-

tration, 0.6 ml intracerebrally and 3.0 ml intraperitoneally, one control being similarly inoculated. Both became paralyzed.

Abramson and Gerber (112) treated a 10% suspension of infected monkey cord with 0.5% formalin for 4 hours at icebox temperature. The formalin was then removed by dialysis. Two monkeys each received 4 subcutaneous injections of 10, 15, 15 and 15 ml, respectively, spaced over 8 days. One monkey died 3 days and one 21 days after the vaccination was completed. No paralysis was noted, but histologic examination of the cord postmortem showed lesions considered typical of poliomyelitis. Formalin treatment was not further investigated.

Brodie (17), attempting to find the minimal treatment with formalin required to inactivate poliomyelitis virus (MV), treated 10% suspensions with concentrations of formalin varying from 0.2 to 1.0%, for periods varying from 1.5 to 10 days (icebox temperature). Test monkeys were inoculated intracerebrally with dosages of the product equivalent in amount to 80 to 1,600 "minimal completely paralyzing doses" (MCP) of the original untreated virus. The MCP of this pool is given as 0.00125 ml of a 5% suspension. Treatment for 1.5 days with formalin in concentrations up to 1.0% did not inactivate completely. Treatment with 0.3% formalin for 10 days rendered the virus noninfective in dosages of 1,600 MCP. Four monkeys are listed as resistant to this dosage, two of them to repeated inoculation. Vaccines were then prepared by treating 10% virus suspension with 0.3% formalin for 10 days. Seven monkeys received each a single dose given intracutaneously in multiple piqûres. Amounts given were 2.5 ml (1 monkey), 5.0 ml (3 monkeys) or 10 ml (3 monkeys). All of these resisted a first intracerebral challenge with 1 or 2 MCP doses of virus. Six succumbed on re-challenge with 2 to 4 MCP. The seventh was lost by intercurrent infection. An attempt was made to measure quantitatively the level of neutralizing antibody in sera obtained from the vaccinated monkeys at the time of challenge. The undiluted serum was tested against increasing doses of virus beginning with 1 or 2 MCP (p. 378). Six of the sera neutralized, one did not. End points for the positive sera were 1 to 15 MCP doses.

Twenty monkeys each received two intracutaneous doses of the formolized vaccine, spaced 10, 14 or 20 days apart. The amount given at each dose varied from 2.5 to 10 ml. For comparison, 7 monkeys were similarly vaccinated with untreated virus. Of the latter, one became paralyzed from the vaccination procedure. No paralysis resulted from giving the formol treated vaccine. One of the group receiving it died of intercurrent infection. All survivors were challenged intracerebrally with 1 to 3 MCP doses of virus. Of 19 that had received formalin treated vaccine, 14 succumbed at first challenge. Of the 5 that resisted, 3 became paralyzed on re-challenge with 3 or 4 MCP, 2 were not re-challenged. Of the 6 surviving monkeys vaccinated with live virus, two became paralyzed at first challenge and the remaining 4 on re-challenge. None resisted a dosage higher than 3 MCP. Sera from 16 monkeys given formalin treated vaccine neutralized, sera of 3 did not. Titers of the positive sera ranged from 1 to 30 MCP. The 6 monkeys vaccinated with live virus all showed positive neutralization, serum titers ranging from 20 to 90 MCP.

Brodie (148) later tried vaccines prepared from 10% virus suspension, again treated with 0.3% formalin at icebox temperature, but for 3 days instead of 10. Protocols are given of 4 monkeys vaccinated with single doses of 5 ml, and 4 given repeated doses of 5 ml each at intervals of 11 or 14 days. These all resisted challenge with 1 or 2 MCP doses of virus, except for one monkey which showed symptoms of a mild infection. Neutralization tests on all serums were positive, titers ranging from 3 to 20 MCP.

Because of local irritation produced by 0.3% formalin, Brodie (148) and Brodie and Park (33) adopted the procedure of treating virus with formalin at 37 or 37.5 C rather than under refrigeration. It was reported that at the higher temperature inactivation of the virus could be accomplished with 0.1% or 0.2% formalin, acting for a shorter period of time. The first paper cited mentions four groups of monkeys vaccinated with 10% virus suspension treated with formalin at 37.5 C. Concentration of formalin, time of treatment, and dosage were as follows:

GROUP	MONKEYS	DOSAGE	FORMALIN	TREATMENT
			%	hr
1	6	1 or 2, 5 ml	0.2	6
2	6	1 or 2, 5 ml	0.2	24
3	3	2, 5 ml each	0.1	24
4	3	2, 5 ml each	0.1	72

Detailed protocols are not given. It is stated that the monkeys of group 1 "developed an immunity quite comparable with that developed by those receiving virus suspension inactivated at icebox temperature". The second group "developed less immunity". In groups 3 and 4 the sera of all neutralized, and 5/6 resisted intracerebral challenge.

The second paper (33) gives protocols of 7 monkeys, used for testing infectivity of a 10% cord suspension treeted with 0.1% formalin for 6, 12 or 24 hours. The monkeys were inoculated intracerebrally with the product in amounts equivalent to 3,200 to 6,400 MCP of the untreated virus, larger amounts being given intraperitoneally at the same time. One monkey became paralyzed from inoculation with virus treated with formalin for 6 hours. The other 6 received material treated for 12 hours or longer, 5 of them being inoculated 2 or 3 times. None showed any evidence of infection.

The same two papers report the first trial of formalized vaccine on human subjects. Preparation is described as treatment of 10% virus suspension with 0.1% formalin, at 37 C, for 8 to 12 hours. The authors cite earlier work (67) indicating that the maximum antibody response is reached in about 20 days after a single injection of antigen. The vaccine was given to children in one or two doses of 5 ml each, of which 1 to 2 ml was injected intracutaneously and the rest subcutaneously. Where two doses were given, the second was 11 to 13 days after the first.

Brodie and Park (150) estimated that during 1935, 9,000 persons, about 7,000 of them living in "epidemic areas", received the Brodie vaccine. Unfortunately observations on control groups were quite limited. Gilliam and Onstott (151)

report an attempt at a controlled study, set up in 3 communities of North Carolina (Greensboro, Washington, Wilmington) and 1 in Virginia (Petersburg). It was estimated that there were in these 4 communities 20,145 children below the age of 11. From this age group of 1,452 candidates for vaccination, 766 were selected to receive it and 686 to serve as unvaccinated controls. Actually only 458 were vaccinated. No cases of poliomyelitis developed in either the vaccinated or the control group. From the total number at risk in the age group concerned, 15 cases were reported. The inconclusive results in Greensboro were noted also by Reynolds and Knox (152). The situation was complicated by the simultaneous trial of another vaccine (Kolmer's, ricinoleated) on a somewhat larger scale. Neither vaccine was used for human prophylaxis in subsequent years since some cases of paralytic poliomyelitis developed among the subjects vaccinated in 1935 (p. 388). Any protective action of the Brodie vaccine, as of Kolmer's, must therefore be inferred from the available data on its antigenic potency; from what is known of the virus types represented in it; and from information acquired in later years regarding the antibody levels necessary to protect against paralytic poliomyelitis in man.

Neutralization tests, before and after administration of the Brodie vaccine, were made on a number of human subjects. Brodie (148) and Brodie and Park (33) refer to tests on "about 75". Data are given on one group of 12, with neutralization tests before vaccination and at 1, 5 and 8 months after. Another tabulation lists pre- and post-vaccination tests on 17 subjects. Undiluted serum was used against increasing dosages of virus, and the titer expressed as the highest number of MCP doses neutralized. Of the 17 subjects 4 had initially negative reactions, and one of these did not change. Serums of the remaining 16 either changed from negative to positive or increased in titer.

Aycock and Hudson (146) reported neutralization tests on blood samples from 95 children obtained in Greensboro, North Carolina, in 1935. Of these, 41 had been vaccinated, 26 with the Brodie and 15 with Kolmer vaccine. In the tabulation of data these are not treated separately, but as one vaccinated group. There are 54 unvaccinated controls, said to be of comparable age, but the actual age range is unstated. Each subject was bled prior to vaccination and again 31 to 77 days after (average interval 61 days). Samples from controls were taken at the same time. Virus used in the tests was a 5% suspension of infected monkey cord, titer unstated. It was mixed with an equal volume of undiluted serum. Controls were run with (a) normal monkey serum and (b) convalescent serum (not further described). Inoculation of test monkeys was presumably intracerebral, but the volume of inoculum and the number of monkeys used in each test are not stated. Pre- and post-vaccination sera were both tested on 28 vaccinated and 35 control subjects. Only one sample was tested from each of 13 vaccinated and 19 controls. In the latter instances, where the pre-vaccination sample was first tested and found positive, it was assumed that the post-vaccination sample would likewise have been found positive. Similarly, where the post-vaccination sample was tested first and proved negative, a negative reaction of the prevaccination sample was assumed without actual test.

On this basis of actual and assumed reactions, 20 (48.8%) of the 41 vaccinated subjects had neutralizing antibody before vaccination, and 28 (68.3%) afterward. During the corresponding period positive reactions among the 54 controls increased from 22 (40.7%) to 30 (55.6%). The authors calculate that the percentage of acquired positive reactions was 19.5 for the vaccinated and 14.9 for the control group. It is inferred that a number of the controls acquired immunity by natural exposure during the period of observation, and the difference between the groups was not considered to be clearly significant.

Kramer (153) made neutralization tests on serums from 14 vaccinated children and 17 controls. The vaccinated group, all aged 1 to 8, included 9 children at an institution in New York City and 5 in North Carolina. Each had received a single dose of 5 ml of Brodie's vaccine. The controls, aged 3 to 12, included 7 children in another institution of New York City and 10 in North Carolina. Initial serum samples were obtained prior to vaccination and at about the same time from the controls. Second samples were obtained 1 to 2 months after vaccination or, from the controls, 1 to 3 months after the first sample.

The virus used for neutralization tests was titered, 3 monkeys being inoculated with each of a series of dilutions. With 0.1 and with 0.05 ml of a 5% suspension, the paralytic rate in test monkeys was 3/3, with 0.01 ml 2/3. In the neutralization tests, undiluted serum was mixed with an equal volume of 5% virus suspension and 1.2 ml of the mixture given intracerebrally. Sera of all the children, in both vaccinated and control groups, were initially negative. Following vaccination 7 of the 14 children developed neutralizing antibody, 7 did not. Of the 17 controls, 7 developed neutralizing antibody during the period of observation. Changes from negative to positive were thus 50% in the vaccinated and 41.1% in the control group, a difference not considered significant.

In the same paper, Kramer reports vaccination experiments with monkeys. Vaccine was prepared by treating a 10% virus suspension with 0.1% formalin at 37 C for 10 to 12 hours. Tests were made for residual infectivity by combined intracerebral and intraperitoneal inoculation of normal monkeys on 3 successive days. No evidence of infection was found (number of test monkeys not stated). Two groups of 18 monkeys each were vaccinated. The dosage was 5 ml, part given intracutaneously and the rest subcutaneously. One group received each a single dose, the other two doses 10 days apart. Serums were tested as described above before vaccination, and all found negative. Tests were repeated 26 or 28 days after vaccination was completed. At 7 weeks challenge inoculation (0.1 ml of the titered virus referred to above, in 0.5 ml of saline) was given; repeated one month later on those resisting the first. Fifteen monkeys died from intercurrent infections at various stages of the experiment. Of the monkeys vaccinated with a single dose, 13 remained seronegative, 2 changed to positive. Ten survived until challenged. All became paralyzed. Of 4 controls, 2 became paralyzed, 2 resisted. Of the monkeys vaccinated with two doses, 13 remained seronegative, 4 became positive. Twelve were challenged, paralytic rate 9/12. Two of the 3 that resisted were rechallenged and both became paralyzed.

Kramer's neutralization tests, also those of Aycock and Hudson (146), were

made against unidentified strains of virus, which may not have been related to that composing the vaccine. Brodie's tests apparently were made against the homologous strain.

Schultz and Gebhardt (139) report observations as follows:

> "We recently undertook to immunize 15 monkeys with a 10% virus suspension inactivated with 0.1% formaldehyde (Brodie vaccine). Three animals received this vaccine by the subcutaneous route; four by the intramuscular route; and four by the intradermal route, a total of 5 injections of 0.1 ml per kilo being administered to each animal at intervals of one week. Four animals received 1 ml of the vaccine per kilo by the intravenous route for 5 injections. Twenty-four days after the final injection all of the animals were inoculated intracerebrally with about 3 MID of virus. All developed paralysis in about the same length of time and with about as extensive paralysis as the controls, despite the fact that their serums seem to have acquired slight but definite virucidal properties . . . . In still another experiment we repeatedly injected 4 monkeys with 1 ml of formaldehyde inactivated virus by the intracerebral route. Two months after beginning the immunization they were all given an intracerebral injection of 1 ml of a virus suspension treated with 0.005% formaldehyde for 24 hours at 37°C. All developed extensive paralysis in the usual length of time. Finally, we instilled formaldehyde inactivated virus intranasally into 4 monkeys, giving 5 instillations at intervals of a week, each instillation being preceded by an intranasal lavage. All of these animals came down following an intranasal instillation with active virus."

Olitsky and Cox (128) immunized 8 monkeys with a vaccine of 10% virus suspension, treated with 0.1% formalin at 37 C for 16 hours. Each monkey received two doses of 5 ml each, intradermally, 13 days apart. Thirty to 50 days after the second dose they were challenged, 4 intracerebrally (0.2 ml of filtrate from a 5% suspension of untreated virus), 4 intranasally. Seven became paralyzed. One resisted two intracerebral challenges. Neutralization tests were made on the sera of the 8 monkeys, 4 serum pools representing two monkeys each. Undiluted serum, 0.8 ml, was mixed with 0.2 ml of filtrate from a 5% virus suspension. One serum pool was considered clearly positive, one clearly negative, and two doubtful.

Levaditi's observations on vaccine prepared with formalin have been already mentioned (p. 381).

Kramer and Geer (95) centrifuged 20% suspensions of Lansing infected mouse CNS tissue. The supernates were divided into two equal parts. To one was added an equal volume of 0.2% formalin; to the other, an equal volume of saline. Each of these preparations was divided into 3 parts. One was kept in an icebox, one at room temperature (18–25 C) and one incubated at 37.5 C. Infectivity of each lot was tested at intervals by intracerebral inoculation into mice. Inactivation, by mouse tests, took place within 4 hours in the formalin treated virus incubated at 37.5 C. Inactivation was much slower at lower temperatures. Formolized and active preparations were used as vaccines in parallel experiments on mice. Ten paired experiments in all were carried out. Dosage was varied from 0.1 to 0.3 ml, repeated 3 to 8 times. Intervals between doses varied, commonly 3 to 5 days. Vaccination was intraperitoneal in all experiments but one, where it was subcutaneous. The mice were challenged intracerebrally one to two weeks after the

final dose of vaccine. The inoculum was 0.03 ml, the dilution of virus used for challenge was varied from group to group. The titer of the virus is not given, but the mortality in control groups of mice was 85 to 100%. In the groups vaccinated with live virus it varied from 0 to 41%. In those vaccinated with formolized virus the mortality rate varied over an intermediate range.

Loring and associates (154) used a Lansing virus preparation from infected cotton rat CNS tissue, partially purified by two cycles of differential centrifugation. The product was treated with formaldehyde, 0.1 or 0.2% by volume, in phosphate buffer. It was stored in a refrigerator at approximately 0 C and the infectivity tested at intervals. Serial 10-fold dilutions were inoculated intracerebrally into groups of 5 cotton rats per dilution, 0.05 ml dosage. By this method of testing, minimal treatment required for complete inactivation was 12 days of contact with 0.1% formalin. The purified live virus preparation was titered, the ID_{50} end point being expressed in terms of grams of N per ml. The preparation used for challenge of immunized animals titered $10^{-11.7}$ in normal cotton rats, intracerebral dosage 0.05 ml. The first series of 32 vaccinated cotton rats each received two intraperitoneal injections of formalin treated virus. Four days after the last injection all were challenged with 100 ID_{50}. Paralytic rate was 26/32 (82%); controls all paralyzed.

In a second experiment, two groups of 30 cotton rats were used. Both received formalin treated virus, same dosage and schedule, but for one group the vaccine was given in paraffin-oil adjuvant. Each rat received 3 doses, 1 intracutaneous, 1 intramuscular and 1 subcutaneous, spaced one week apart. The ID_{50} titer was determined for normal rats ($10^{-11.7}$). For the group immunized with oil adjuvant, it was $10^{-6.5}$, and for the group immunized without adjuvant, $10^{-6.2}$.

In a third experiment, 24 cotton rats were vaccinated without adjuvant by the same procedure just described. One week later, all were challenged with 1,000 ID_{50} of virus. Only one developed paralysis (recovered); of 24 controls, 20 became paralyzed. The vaccinated rats were re-challenged 3 weeks later, with 10,000 ID_{50}. The paralytic rate was 8/24, as compared to 21/24 in controls.

Morgan, Howe and Bodian (71) vaccinated 5 monkeys with formalin treated virus (Riley strain). A 20% virus suspension was treated with 0.5% formalin at 4 C for 11 days, then diluted to 5% virus. The animals each received 2 ml subcutaneously each week for 7 weeks. Three died of causes other than poliomyelitis before any tests of immunity were made. Five months from the beginning of vaccination, neutralization tests were made on sera of the two survivors. Undiluted serum was mixed with an equal volume of virus suspension, and 0.8 ml of the mixture, containing 1,000 PD_{50} of homologous virus, inoculated intracerebrally into test monkeys. Both sera failed to neutralize. The two monkeys were challenged intranasally, 0.1 ml of 10% Riley virus suspension into each nostril. Both became paralyzed.

Morgan (155), in preliminary tests, studied minimal treatment required for inactivation of virus by formalin. Lansing infected mouse CNS, in 10% suspension, was treated with 0.1, 0.3 or 0.5% formalin at 1 to 4 C for 11 days. Samples were removed at intervals and tested for infectivity by intracerebral inoculation

of 0.03 ml into 6 to 8 mice. With 0.1% formalin, inactivation was not complete in 11 days. Both 0.3 and 0.5% inactivated in 5 days, but not earlier. At 37 C, 0.3% inactivated in 3 hours. A vaccine was prepared by treatment of 20% suspension of Lansing infected monkey cord with 0.3% formalin at 4 C for 13 or 14 days. This proved noninfectious when tested by intracerebral inoculation into 11 rhesus monkeys. The PD_{50} titer of untreated virus from the same pool was established in monkeys as $10^{-3.6}$–$10^{-3.8}$.

Seven monkeys were immunized by intramuscular injection of the formolized vaccine, 1 ml doses, repeated for a total of 9 injections over 11 weeks. Blood samples were drawn before and at intervals up to 19 weeks from the start of immunization. Neutralization tests were made in mice, serial dilutions of serum being mixed with equal volume of 1/160 dilution of Lansing virus. Neutralizing titers in the vaccinated monkeys rose from 0 before vaccination to 2,000 or 3,000 at the 19th week, except for one monkey whose final serum titer was only 300. All were finally challenged intracerebrally with 6,000 PD_{50} of Lansing virus. Five remained well. They were sacrificed 3 weeks after challenge, and no evidence of poliomyelitis was found on histologic examination of the CNS. Two vaccinated monkeys became paralyzed (including the one with lowest antibody titer). Three controls were paralyzed.

Schwerdt and associates (100) used a vaccine prepared by treatment of a 10% suspension of Lansing virus with 0.1% formalin at 25 C. After varying periods of treatment, the formalin was neutralized by addition of diammonium hydrogen phosphate. Tests for residual infectivity were made by intracerebral inoculation (0.05 ml) into groups of 12 cotton rats. Two monkeys per vaccine were similarly inoculated with 1.0 ml doses. It was concluded that 24 hours of treatment under these conditions was sufficient to inactivate. Serial dilutions of the vaccine were given to cotton rats intramuscularly, 3 injections of 1.0 ml each, at weekly intervals. One week after the final immunizing injection they were challenged intracerebrally with 100 PD_{50} of Lansing virus. The antigenicity titer of the vaccine was calculated as the dilution of vaccine protecting half the animals against the standard challenge 100 PD_{50}. The antigenic titer of live virus used as a vaccine was established on this basis as $10^{-3.8}$ and $10^{-4.2}$ for two separate pools of virus. For the formalin inactivated vaccines it ranged from $10^{-0.5}$ to $10^{-2.1}$, averaging $10^{-1.5}$.

Casals, Olitsky and Brown (156) used inactivated vaccines prepared from CNS tissues of mice infected with the MEF_1 strain of poliomyelitis virus (Type 2). Mice were vaccinated intraperitoneally, each receiving 2 or 3 doses at weekly intervals, 0.1 or 0.2 ml per dose. All were challenged 8 to 10 days after the final dose of vaccine. Details of the procedure and outcome in the several experimental groups are here omitted because of the number of variables involved.

In most instances the vaccines were prepared from the MEF_1 variant, adapted in the same laboratory (157) to newborn mice. Three experiments, however, are reported with vaccines of the standard MEF_1 strain. The inactivating agent was usually formalin in 0.2 to 0.4% concentration, but ultraviolet irradiation was

also employed although the inactivation procedure with UVL is not described. Vaccines treated with formalin were held at 4 C and tested for inactivation at weekly intervals by intracerebral inoculation into groups of test mice. Formalin treatment was not interrupted by addition of a neutralizing agent. Test mice were observed for 28 days, and the vaccines were not ordinarily used until the test was complete. Vaccines were thus subjected to treatment at least 28 days beyond the point of inactivation. Treatment with 0.2% formalin for 33 days did not inactivate completely. Inactive vaccines had been treated with 0.3 or 0.4% formalin, for varying periods. The total dosage of vaccine was varied, from 1 to 60 mg of tissue, by using varying dilutions.

Vaccinated mice were challenged either with homotypic virus (standard MEF_1 strain) or with the heterotypic Leon strain (Type 3) adapted to mice by Li and Habel (158). The latter strain was given intraspinally, and its pathogenicity was so low as to preclude challenge with serial dilutions. Challenge with the MEF_1 strain was done with serial dilutions, ordinarily given intracerebrally but in one experiment intraspinally. Significant protection against the homotypic challenge was demonstrated with both formalin treated and irradiated vaccines. The antigenic potency of the MEF_1 variant was found to be much higher than that of the standard MEF_1 strain. Significant cross-immunity against the Leon virus was conferred by the Type 2 vaccines. Of 141 vaccinated mice, challenged with Leon, only two died as compared to 38 of 114 unvaccinated controls.

Howe (159) studied the antibody response of chimpanzees and of human subjects to formalin treated vaccine in which virus strains (Brunhilde, Lansing, Leon) representing the 3 known immunologic types were combined. Each component virus pool before treatment titered $10^{-4.5}$ or higher by intracerebral inoculation in monkeys. Two polyvalent vaccines were prepared. Each was a mixture of equal volumes of the 3 components in 10% suspension, with formalin added to 0.1% concentration. Treatment was at 25 C for 48 hours (vaccine I) or 72 hours (vaccine II), terminated by neutralizing the formalin with diammonium phosphate.

In vaccine I, the three components were mixed before formalin treatment. The treated product was tested for residual infectivity by inoculation of 10 rhesus monkeys, 2 ml of inoculum per animal, injected into the thalamus bilaterally. One of the test monkeys showed histologic evidence of infection. The other 9 did not, and all had remained symptomless for 4 weeks following inoculation. In vaccine II, the 3 components were separately treated with formalin and separately tested for residual infectivity by the procedure described. No symptoms developed and no lesions were found in any of the 30 test animals.

Vaccine I and the separate components of vaccine II were tested for antigenicity in rhesus monkeys. Each was given intramuscularly, combined with mineral-oil adjuvant. The polyvalent vaccine I was given in a total dosage of 10 ml to each monkey, divided into 5 injections at 0, 1, 2, 3 and 4 weeks. The components of vaccine II were tested individually, total dosage of each 3 ml per monkey, 3 injections at 0, 2 and 4 weeks. All the monkeys were bled at 6 weeks.

Serums were titered against 1 to 32 PD_{50} of Lansing virus (in mice) and against 10 to 100 PD_{50} of Brunhilde or Leon (in monkeys). Serum titers were $10^{-2.5}$ against Lansing (both vaccines), $10^{-2.5}$ against Leon (vaccine II, vaccine I not tested against this strain), and $10^{-1.0}$ (I) and $10^{-1.8}$ (II) against Brunhilde.

Vaccine I was administered to 10 young chimpanzees (12 to 21 pounds), divided into two groups of 5 each. Both groups were vaccinated intramuscularly, 1 ml doses at 1, 3 and 6 weeks, 2 ml at 9 weeks, totalling 5 ml per animal in the initial course. This was given to one group (y) with mineral-oil adjuvants, to the other (x) without it. Vaccination of both groups was continued without adjuvant, 5 ml doses at 11, 12, 15 and 16 weeks. Finally, in the attempt to raise the serum titer against Brunhilde virus a supplementary course of Brunhilde vaccine alone was given combined with adjuvant; 5 ml doses at 20 and 22 weeks, 10 ml at 24 weeks.

The animals were bled before vaccination and at 5 stages during the period of observation. Serums were titered against 10 to 30 PD_{50} of Lansing virus (in mice). Pooled serums of the vaccinated groups were tested against 10 to 100 PD_{50} of Brunhilde and Leon (in monkeys). One chimpanzee of group y had serum antibody initially against Lansing virus, the titer rising from $10^{-2.0}$ at 0 weeks to $10^{-3.8}$ at 6 weeks. Excluding this one from the antiLansing data, titers for the two groups at 17 weeks were:

	SERUM TITER AGAINST		
	Brunhilde	Lansing	Leon
Group x	0.7	1.8	3.0
Group y	1.5	2.7	3.0

After the supplementary course of vaccination against Brunhilde virus, titers rose to 2.4 (group x) and 3.0 (group y) at 25 weeks. The low antigenicity of the Brunhilde strain, illustrated in the table above, has been noted by other investigators (53). The marked response to Leon virus may have been due in part to incomplete inactivation of this component in vaccine I.

Vaccine II was given to 6 children, aged 2 to 5 years. Each received 3.5 ml of gamma globulin solution intramuscularly and 3 ml of the vaccine immediately afterward, without adjuvant. No further vaccine was given. None of the subjects developed any fever or other serious reaction.

Five controls, unvaccinated ward mates of the vaccinated children, were bled near the end of the observation period. Their serums were tested for antibody against Lansing and Leon viruses. All were negative against Lansing, one was weakly positive and one showed a borderline reaction against Leon. No tests against Brunhilde were made.

All the vaccinated subjects were bled at 0, 5, 14 and 18 weeks and at 6 months. Serums were tested against all 3 viruses in the same manner as the chimpanzee serums mentioned above. Omitting the data on other bleedings, the serum titers at 0 and at 18 weeks for individual subjects were as follows:

	SUBJECTS					
	C.P.	C.L.	L.S.	G.T.	P.P.	L.L.S.
I. Lansing						
Titer, initial	0	?	0	1.2	0	0
Titer at 18 weeks	0.3	0.6	1.0	0.8	0.4	1.0
II. Brunhilde						
Titer, initial	0	2.5	0	2.5	0	0
Titer at 18 weeks	0	2.5	0	2.8	0	0.3
III. Leon						
Titer, initial	0	0	0	0	0.9	0.6
Titer at 18 weeks	1.3	1.0	1.3	0.4	1.7	1.5

With one exception and one doubtful instance, antibody that appeared following vaccination was still demonstrable at 6 months. It would appear from these data that the Leon virus is the best antigen of the 3, and Brunhilde the poorest. The two subjects who had antiBrunhilde antibody initially, however, showed high titers that were not appreciably raised by vaccination.

In all the work with poliomyelitis vaccines hitherto cited, infected central nervous tissue was the source material. The recent development of tissue-culture techniques has made it possible to propagate the virus in the absence of nerve tissue. Virus is found in high concentration in the nutrient fluid with a minimal admixture of irrelevant antigens. Salk (160) has used such fluids for preparing formalin inactivated vaccines, employing tissue cultures also for assaying infectivity of the original material and for measuring the antibody response. Although his report is a preliminary account of work still in progress, it presents the most extensive set of quantitative data available on the antibody response to poliomyelitis vaccines in man.

The Mahoney, MEF_1 and Saukett strains of virus representing Types 1, 2 and 3, respectively, were propagated in cultures of monkey kidney or testicular tissue in a protein-free, synthetic medium. Fluids from the infected cultures were collected into pools, clarified by centrifuging and assayed for infectivity. Six pools, two for each virus strain, showed titers in tissue cultures ranging from $10^{-2.4}$ to $10^{-5.2}$, the lowest average titer being recorded for Saukett and the highest for MEF_1.

The several pools were each treated with 0.4% formalin, with excess $MgCO_3$ added to neutralize its acidity, at a constant temperature of about 1 C. Inactivation under these conditions required several days. Action of the formalin was stopped at any desired stage by addition of sodium bisulfite. Finally the pH was adjusted to 7.4 with NaOH.

After varying periods of treatment, tests of residual infectivity were made on each pool by intracerebral inoculation of 0.5 ml into each of 6 to 10 cynomolgus monkeys. Those surviving for 30 days without symptoms were sacrificed and the CNS tissues examined histologically. With Saukett virus, no evidence of

paralytic or nonparalytic infection was found after formalin treatment for more than 3 days; with Mahoney, 5 days, with MEF_1, 7 days. Preparations used for the vaccination of human subjects had all been treated at least 48 hours beyond the time at which inactivation appeared to be complete. Further tests indicated: (a) that the virus did not become reactivated with the passage of time after the formalin had been neutralized; (b) that infectivity remained stable if the formalin and bisulfite were added simultaneously.

Vaccines used on the human subjects were inactivated and tested for safety in the manner described. They were given either (a) without further modification, as monovalent, aqueous vaccines, or (b) as trivalent emulsions combining equal volumes of fluids representing the 3 virus types. The emulsifying agent was an adjuvant mixture modified from that already referred to (p. 358). A specially purified preparation of "arlacel A" was dissolved in 3% concentration in mineral-oil ("drakeol #6"). Omitting details of mixing and sterility tests, the final proportions were 3 parts of tissue-culture fluid to 7 parts of oil adjuvant.

The total number of vaccinated subjects was 161. No illness attributable to the vaccines was noted. Subjects were bled before and at intervals after vaccination. Neutralization tests were made on tissue cultures, serial dilutions of serum against 100 tissue culture infecting doses ($TCID_{50}$) of each type of virus. Some of the sera were tested for Type 2 antibody both in tissue culture and in mice.

Tests on the sera were still in progress, and the published data even on the prevaccination samples are incomplete. Distribution of antibody prior to vaccination in relation to age, clinical history and virus type will not be discussed here. Post-vaccination data cover only certain groups and for the most part only a few weeks interval although a few sera collected 4½ months after vaccination are included:

(a) *Vaccination with aqueous, monovalent vaccines.* These were inoculated intradermally in doses of 0.1 ml. Subjects receiving them had all suffered attacks of paralytic poliomyelitis a year or more prior to vaccination. One group of 27 received vaccines of all 3 types simultaneously, inoculated at separate sites. All 3 vaccines had been treated with formalin for 13 days. Vaccination was repeated 6 weeks later in the same way, except that Types 1 and 3 had been treated only 10 days with formalin.

Concerning the aqueous vaccines of Type 1, the author notes only that some antibody response did occur in many subjects but omits specific data. Five subjects vaccinated with Type 3 are listed. Their sera initially were devoid of Type 3 antibody, and samples obtained 4 weeks after vaccination were still negative. More extensive data are given on the response to Type 2. In 25 of the 27 subjects, no Type 2 antibody was demonstrable before vaccination. At 11 weeks after the first dose of vaccine, 5 weeks after the second, all showed Type 2 antibody titers ranging from 1/8 to 1/256. The two who had Type 2 antibody initially each showed a rise of titer 4-fold and 16-fold, respectively. Data on bleedings obtained from 15 of these subjects show that the antibody rise took place mainly or entirely within the 6 weeks following the first dose, and that a rise

was demonstrable within two weeks in 6 of 8 subjects tested at that interval. Sera obtained at 4½ months from 11 subjects showed no fall below the 11 week level.

To determine the effect of longer formalin treatment on the antigenic property of the vaccines, additional groups of subjects were injected with Type 2 vaccines treated for 17 and for 21 days, respectively. Data on 8 subjects from each group show that antibody responses to these vaccines did occur, but only 2 week and a few 4 week sera had been tested. These data are not sufficient to show whether the longer periods of formalin treatment reduced the antigenicity of the vaccines or not. Retention of at least some antigenic potency after 21 days of treatment tends to discount the possibility that antigenic action is dependent on incomplete inactivation.

(b) *Emulsified vaccines, given intramuscularly.* These were inoculated in a uniform dosage of 1 ml of emulsion, containing 0.1 ml of formalin inactivated virus of each type. Subjects treated with this vaccine had no history of previous attacks of poliomyelitis. The first group inoculated, 16 in number, received a vaccine in which the component viruses of Types 1, 2 and 3 had been treated with formalin for 7, 10 and 7 days, respectively.

Post-vaccination data for this group are limited to 2 or at most 4 weeks, too short a period to show the full antigenic effect of the vaccines. Serologic tests on a few of the subjects, with respect to Type 1 and Type 2 antibody had not been completed. Increase of Type 1 antibody titer was noted in 13 of 15 subjects tested; of Type 2 titer in 10 of 12 subjects; and of Type 3 titer in 13 of 16 subjects. All of this group of subjects initially had Type 1 antibody; 10 had Type 2; and 10 had Type 3.

The author presents data on comparative antibody levels against each of the three virus types in (a) a group of recently paralyzed patients; (b) a group of persons with no clinical history of poliomyelitis; and (c) the vaccinated subjects for whom data are available. Following vaccination with emulsified vaccines the antibody levels were of the same order as those found in the recently paralyzed patients. The aqueous vaccine evoked a Type 2 antibody response nearly as good but failed as noted above to induce any Type 3 response. The author suggests that the Type 3 vaccine may have contained antigen in an amount too small to be effective without the enhancing action of the adjuvant.

Further work is required to supply direct evidence (a) that the formalin treated vaccines are actually effective for human protection against paralytic poliomyelitis, (b) on the duration of the antibody response. Influenza vaccines, prepared and administered in a similar manner, have been shown to produce high antibody levels persisting for as long as two years (161).

3. *Immunization with live, rodent-adapted viruses.* Blanc and Martin (162, 163) report that rabbits are susceptible by various routes of inoculation to poliomyelitis virus. Observations on a series of monkeys (*Macaca sylvanus*) are presented as evidence that rabbit-passage virus produces a relatively mild, immunizing infection in the monkey.

The criterion of infection in rabbits was fever, appearing after an incubation

period of 15 to 17 days on first passage, shortened to 4–6 days with repeated passages. The infection was often fatal although enlargement of the spleen was the only constant finding post mortem. Virus was found in various tissues including blood, spleen and CNS, but neither paralysis nor lesions of the CNS were ever observed.

Poliomyelitis viruses propagated serially in rabbits included 6 monkey-passage strains, one mouse-passage, and strains isolated by direct inoculation of rabbits with stool, blood or CNS tissue from 6 human cases. A tabulation is given of cross-immunity tests on rabbits with various combinations of these strains. The authors also describe a neutralization test using rabbits and give protocols indicating neutralization of rabbit-passage virus by convalescent sera of rabbits, of monkeys and of human cases.

Of 10 monkeys inoculated with rabbit-passage virus, only one developed paralytic poliomyelitis. Five responded with fever only, 4 showed no reaction whatever. The 9 nonparalyzed monkeys were subsequently challenged with monkey-passage virus intracerebrally. Paralytic rates (not counting transient paralysis) were 1/5 in the group that had reacted with fever, 3/4 in those that had not. One normal control became paralyzed. From this outcome the authors infer that a febrile reaction to rabbit virus connotes development of immunity in the monkey.

Aside from the small numbers involved, comparison of the two groups is complicated by several variables. The 10 monkeys that received rabbit-passage virus were inoculated with undefined doses, by various routes, with preparations varying as to strain and number of rabbit passages. The challenge dose of monkey-passage virus is undefined as to volume, dilution, titer, number of infective units or uniformity. No published report has been found of attempts to confirm in other laboratories the findings of Blanc and Martin.

Koprowski and associates (164) inoculated rhesus monkeys intracerebrally with a strain of virus adapted first to mice and subsequently subjected to repeated passage in cotton rats. They state that "an attack rate with mild paralysis was observed in 7 to 10 per cent" of the monkeys inoculated. The entire series of inoculated animals was divided into 3 groups, and each group was challenged with a simian strain of poliomyelitis virus representing one of the respective immunologic types. Results of the challenge inoculation were said to indicate marked resistance of the monkeys to Type 2 virus and to some strains representing Type 1. Resistance to Type 3 was also noticeable though less marked. The publication referred to is an abstract, in which numbers of animals used and other experimental details are omitted.

The strain of virus referred to above, adapted to cotton rats and known as the TN strain, was fed by Koprowski, Jervis and Norton (41) to 20 human subjects of unstated age and sex. The virus was fed in the form of 20% suspension of infected cotton rat CNS. Five subjects each received 10 ml at a single feeding. One received 4 doses of 10 ml each within 6 days. The other 14 subjects each received virus on 2 to 4 occasions, 34 days to 10 months intervening between doses. Initial doses were 10 ml except in two instances where they were 1.0 and

5 ml, respectively. Repeat dosages were 5 or 10 ml. None of the 20 subjects developed any symptoms of illness from the virus feeding.

Three to 6 stool samples were collected from each subject, usually over a 3 week period beginning about 4 days after each feeding of virus. (From one subject only 3 specimens in all were collected after two feedings.) The stool samples were separately processed and tested for virus by intracerebral inoculation into mice. The subjects were all bled before the first virus feeding and at intervals of approximately a month following each dose. Sera were tested in 3-fold dilutions, using 8 mice per dilution. Complete titrations were made against two different dilutions of virus, calculated to provide respectively 15 and 45 LD_{50} or MEF_1 virus (Type 2) in the test inoculum. By actual titer of the virus, run on each occasion, the lower virus dosages ranged from 6 to 27, and the higher 18 to 81 LD_{50}.

Tests on one subject were inadequate to establish his initial serologic status although subsequent tests on his serum showed a high neutralizing titer. Sera of 16 subjects initially failed to neutralize, or neutralized only in dilutions considered too low to be significant. Of these, 15 developed antibody of high titer after the first virus feeding. Three subjects had initially high antibody titers which did not change appreciably after virus feeding.

Virus was recovered from the stool of 14 subjects, on one or more occasions, after the first virus feeding. This number included 12 who were initially seronegative and 2 initially positive. Ten subjects from whom virus was recovered after the first feeding received virus again. Eight failed to become carriers a second time. One became a carrier twice, and one three times, after successive feedings, in the presence of high antibody titer evoked by the first feeding. In these instances virus did not appear in the stool after a final feeding (the third and fourth for the respective subjects). The 6 subjects who never developed the carrier state included 4 who were initially seronegative. Of these, 3 changed to positive. One was the subject whose initial serologic status was doubtful, and the sixth was initially positive. The last subject received four feedings in succession with no demonstrable appearance of virus in the stool and no change of antibody titer.

Sera of 8 subjects were tested for neutralization against Brunhilde and Brockman (Type 1 strains) in monkeys. Sera of 6 were initially negative and remained so after feeding TN virus. Sera of 2 initially neutralized Brunhilde virus. One of these showed a 4-fold rise of titer after feeding TN virus, the other a decrease of the same order.

Subsequently the same group (165) reported feeding the TN virus to 61 additional subjects, all children. Procedures were essentially the same as in the earlier study. Prior to virus feeding, sera of 15 of the subjects were found to neutralize MEF_1 virus in low dilution (titers ranging from 1:2 to 1:27), sera of 46 failed to neutralize at all. In tests on sera collected 30 days after virus feeding, 6 failed to neutralize, 55 neutralized at titers ranging from 1:12 to 1:1,030. In 52 a significant rise of antibody titer was found, in 9, not. Virus was recovered from the stools of 29 subjects, of whom only one failed to show a significant rise of antibody titer.

IV. PASSIVE IMMUNIZATION

Experiments on serum prophylaxis were long handicapped by the same technical difficulties that impeded progress with active immunization, notably a lack of quantitative methods for assay of antiserum or of virus. Even after these were developed, the use of inappropriate animal species and routes for challenge inoculation continued for some time to obscure the potential effectiveness of passive serum protection. The extensive trials of serum therapy are outside the scope of this review. Their outcome, however, negative or at best inconclusive, probably tended to discourage activity in the field of prophylaxis.

Nevertheless passive immunization has been attempted with serum of vaccinated monkeys or apes, serum or serum concentrates from vaccinated animals (e.g., horses) of species naturally refractory to poliomyelitis infection, "convalescent" serum of man and monkey, serum or whole blood of normal human adults, and finally with gamma globulin prepared from human placental or adult donors' blood.

A. *Antibody Levels Resulting from Passive Immunization*

Until recently, few attempts were made to measure the initial level or rate of decline of poliomyelitis antibody in the circulation of passively immunized subjects. Quantitative studies have been facilitated by the use of human gamma globulin of high and relatively uniform potency.

Enders (166) refers to tests on gamma globulin (from pooled adult blood) indicating that it was 10 to 16 times more active than the original plasma against Lansing virus. Bodian (167) assayed gamma globulin (Red Cross) prepared from a pool representing 20,000 or more donors. Serial dilutions were tested against a constant dosage (100 PD_{50}) of virus by intracerebral inoculation in monkeys. Separate titrations were made against 3 virus strains (Brunhilde, Lansing, Leon) representing the 3 known types. The PD_{50} end points, against these respective strains, were 10^{-2}, $10^{-2.4}$ and $10^{-2.5}$, indicating a high and nearly equal potency against all three. Titrations of other lots of plasma gamma globulin against Lansing virus in mice have been reported by Bodian (6) and by Rhodes and Clark (168) with end points ranging somewhat higher ($10^{-2.5}$ to $10^{-4.0}$). These titrations were made against smaller amounts of virus (32 or 21 PD_{50}). The latter authors titered two lots of placental gamma globulin against Lansing virus with similar results.

The course of decline of circulating antibody in monkeys, following its administration as human gamma globulin, was also studied by Bodian (6) who found its half-life to be approximately 8 days. Following a single large dose of gamma globulin (10 ml per kg) intramuscularly, antibody was still detectable at 6 weeks, but not at 8 weeks. Kramer (see p. 414) had previously made studies of a similar kind on monkeys given gamma globulin by other routes.

Wood and associates (169) gave smaller doses of human gamma globulin (0.1 to 0.4 ml per pound) to 10 human subjects selected because their sera initially failed to neutralize Lansing virus. The sera were tested at 4 days and thereafter at weekly intervals. The 4 day samples neutralized, but not when diluted. Groups

of test mice were only partially protected by undiluted serum, and no neutralization was demonstrable for more than two weeks. When gamma globulin was administered by mouth to the same subjects, in the same dosages given intramuscularly 14 weeks earlier, no circulating antibody could be detected.

Earlier observations on levels of passive antibody are referred to in a subsequent section (pp. 409, 412).

B. *Passive Protection Experiments on Animals*

1. *Intracerebral challenge.* Following their observations on the neutralization of poliomyelitis virus by the serum of convalescent monkeys *in vitro*, Flexner and Lewis (21) tried the administration of virus and serum separately. They used serum from monkeys that had survived paralytic attacks and subsequently had been vaccinated to reinforce their immunity. The authors report no attempts to titer the serum, hence "whether any reinforcement is actually accomplished is still an open question". Only 3 protocols of passively immunized monkeys are given. One was inoculated intracerebrally with 0.01 ml of virus filtrate and received 10 daily intraspinal injections of 3 ml each of immune monkey serum, beginning one day after virus inoculation. The animal remained well. No control is mentioned, but it is stated that 0.02 to 0.1 ml of the filtrate produced paralysis "almost invariably" in test monkeys. An account is also given of one monkey inoculated intranasally, with immune monkey serum (dosage unstated) given immediately afterward and later at 3 day intervals. This animal likewise remained well, whereas one treated with immune horse serum and one untreated control both developed paralysis.

In these experiments serum was given by injection into the subdural space, this route being chosen from the belief that infection of the meninges preceded viral invasion of CNS tissues. The method used for production of immune horse serum and tests to establish its potency are not described. Normal monkey serum was tried in passive protection tests (no protocols) and found ineffective.

In 1928, Flexner and Stewart published two papers (170, 171) in which they refer to a prophylactic effect of convalescent serum, without presenting actual experimental data. In summary, they state that "if as much as 15 ml of convalescent serum is injected into the blood of monkeys 24 hours before a suitable dose of virus is injected intracerebrally, infection does not take place"; also that intraspinal injection of 2 ml of monkey or human convalescent serum will protect monkeys against intracerebral challenge 4 days, but not 6 days, later.

Howitt, Shaw, Thelander and Limper (172) and Howitt (173) immunized two goats and one sheep with live poliomyelitis virus. These animals each received repeated injections of supernate from a 10% virus suspension, given over periods of 15 to 26 months and in total amounts of 3,910 to 8,675 ml. Sera from these animals were used in passive immunization experiments on monkeys. It is not clear what tests of potency were made on the specific lots of serum employed for this purpose. Tests were made on samples from various bleedings. Serum was tested in 0.1 to 1.0 ml volume against a fixed volume (0.3 ml) of 2.5 or 5.0% suspension of virus. Most of the samples neutralized, but some did not.

No titration of the virus is mentioned, and protocols of the neutralization tests are not given.

Some of the passively immunized monkeys were challenged by intranasal or intravenous inoculation (see pp. 416, 419). Thirty-two were challenged intracerebrally. One received 20 ml of immune sheep serum intravenously and 18 hours later 0.3 ml of virus intracerebrally. Serum treatment was repeated on 4 successive days after virus inoculation. Another monkey was treated similarly except that the immune serum was given in 5 ml doses intrathecally. Both resisted, one control became paralyzed.

Thirty additional monkeys received immune goat or sheep serum, intramuscularly or intravenously, and were subsequently challenged intracerebrally. Dosage and schedule of serum treatment were varied. The total dose was in some instances given at one time, in others divided over 2 or 3 successive days. The minimal amount given to any monkey was 9 ml, the maximum 150. Challenge was always within 24 hours after the final dose of serum. Detailed accounts of the several groups are here omitted. In two experiments involving 14 treated monkeys, one was lost from dysentery; the others, with 9 controls, all became paralyzed. In two other experiments there were 6 resistant among 16 treated monkeys. Treatment of these 16 and paralytic rates in the respective groups were as follows:

TREATMENT, SERUM	PARALYTIC RATE
5 ml daily, 3 days, intramuscular	4/4
20 ml daily, 3 days, intramuscular	3/4
50 ml daily, 3 days, intramuscular	2/4
20 ml, single dose, intravenous	1/4
Controls	3/4

Howitt (174) later reported use of plasma and serum from convalescent monkeys. One pool of plasma and one of serum were used. Each was tested for neutralizing potency, 1 ml (undiluted) against 0.3 ml of 2.5% virus suspension. While both were considered active, protocols of the tests are not given and the virus was apparently untitered. Six monkeys each received 50 ml of convalescent plasma intramuscularly. All were challenged intracerebrally, dosage unstated. Two were challenged at the time the plasma was given, two one day earlier, and two one day later. One monkey from the second and one from the third pair remained well. All the others, and 4/4 controls, became paralyzed. Six other monkeys each received convalescent serum, 3 to 5 ml intraspinally and 20 ml intravenously. These were similarly challenged in pairs on the same time schedule. All of these, with 5 controls, became paralyzed.

Howitt (175) also tried for prophylaxis a concentrate of serum from immunized horses. Pooled serum from 3 horses, obtained after 2½ months of immunizing, was processed and concentrated in a commercial laboratory. The product is said to have neutralized 16 to 32 MID of virus in a dilution of 1/1000 or slightly

higher. Tests were made with virus and diluted serum mixed in equal volume, but protocols of the titration, of virus, and of serum, are not given. Six monkeys received the concentrate intramuscularly. They were divided into pairs, one of each pair receiving 10 and the other 20 ml. One pair was challenged 24 hours, one 48 hours and one 9 days later. The outcome of challenge is indicated only as "survived" or "died". Four of the treated monkeys and one control died. Two treated monkeys survived, but it is not clear whether they remained symptomless or not. One had received 10 ml of serum concentrate and was challenged at 24 hours; the other 20 ml, challenged at 48 hours.

Meyer and Le Guyon (176) used 4 cynomolgus monkeys, of which 3 received prophylactic treatment as follows:

1. 20 ml human convalescent serum, subcutaneously.
2. 10 ml same serum, subcutaneously and 1 ml intracerebrally.
3. 10 ml spinal fluid from a human convalescent subcutaneously, 1 ml intracerebrally.

The 3 treated monkeys and 1 control were each challenged intracerebrally 3 days later, with 0.2 ml of virus suspension, titer and concentration unstated. All became paralyzed and died. Tests to establish the potency of the serum and CSF are not described.

It is not clear that the sera used, in any of the studies thus far cited, were potent enough to confer any measurable antiviral activity on the blood of the recipient animals. Challenge doses of virus are so ill defined and the numbers of animals studied under any given set of conditions are so small that any conclusion drawn from such data would be hazardous.

Schultz and Gebhardt (177) attempted a quantitative control of virus dosage and of the potency of immune horse serum used for prophylaxis. The stock virus (Aycock strain) was titered from time to time by a procedure not fully described. From illustrative data in a preceding paper the authors apparently used serial dilutions in multiples of 2 or 5, inoculating one monkey per dilution. Various bleedings from the immunized horse (178) neutralized equal volumes of 5% virus suspension at serum dilution titers ranging from 1/100 to 1/240. The MID of the stock virus is given as 1/5,000, hence it is calculated that 1 ml of 5% suspension contained 250 MID and that 1 ml of undiluted serum titering 1/120 contained antibody sufficient to neutralize 30,000 MID. Sera from convalescent monkeys and pooled serum from normal human adults were also used but were untitered so far as the report shows.

Experiments were carried out as follows:

1. Two monkeys each received 1.75 ml of convalescent monkey serum intracranially. Four hours later, they were challenged intracerebrally with 1.5 ml of 5% virus suspension. Both treated monkeys and two controls became paralyzed.

2. Two monkeys each received 20 ml of immune horse serum subcutaneously. They were challenged intracerebrally, with 1 ml of 1/1,000 virus (5 MID) 24 hours later. One control similarly inoculated developed paralysis. Both treated

monkeys remained well. Forty days later they were re-challenged by the same dosage and route. Both succumbed.

3. In the third experiment 18 monkeys each received immune horse serum and were challenged 24 hours later with 1 ml of 1% suspension (50 MID) intracerebrally. The route and dosage of serum given, and the outcome of challenge, were as follows:

MONKEYS	IMMUNE SERUM Dose	Route	CHALLENGE, PARALYTIC RATE
	ml		
2	1	Subcutaneously	1/2
2	5	Subcutaneously	2/2
2	10	Subcutaneously	2/2
2	15	Subcutaneously	2/2
1	20	Subcutaneously	1/1
2	1	Intramuscularly	1/2
2	5	Intramuscularly	1/2
2	10	Intramuscularly	1/2
1	15	Intramuscularly	1/1
2	20	Intramuscularly	2/2
2 Controls	20	Normal horse serum intramuscularly	2/2

One of the 4 surviving treated animals later died from intercurrent infection. The 3 remaining were again challenged, by the same dosage and route, 58 days later. All succumbed.

4. Nine monkeys each received 10 ml of immune horse serum by intracardiac injection. One day later, they and one control were challenged intracerebrally with 1 ml of a Berkefeld V filtrate from a 5% suspension of virus. The control succumbed, as did 7 treated monkeys (3 after 27 days of incubation). Two treated monkeys remained well.

5. Seven monkeys each received 5 ml of immune horse serum, 3 by subcutaneous route and 4 intramuscularly. One day later they were challenged intracerebrally with 1 ml of a Berkefeld V filtrate of 5% virus suspension. The outcome was:

Subcutaneously treated—1/3 paralyzed
Intramuscularly treated—3/4 paralyzed
Control—1/1 paralyzed

6. Eighteen monkeys each received 5 ml per kg of serum intramuscularly. Twelve of them received immune horse serum, 3 convalescent monkey serum and 3 pooled adult human serum ("normal"). One day later, they were challenged intracerebrally with dosages of virus varying from 6.6 to 200 MID. Three controls were challenged with 6.6 MID; all succumbed. Three treated with horse serum were challenged with 6.6 MID, and of these two resisted challenge. The other 9 treated with horse serum were all challenged with larger doses of virus, and all became paralyzed.

The 3 monkeys treated with convalescent monkey serum were challenged

with minimal doses of virus (6.6 MID). Two succumbed; one resisted. The 3 treated with human serum likewise were challenged with only 6.6 MID of virus. All became paralyzed.

7. Nine monkeys each received 1 ml per kg of serum intramuscularly, repeated on 5 successive days. They were divided into 3 groups as follows:

a. 3 monkeys received immune horse serum
b. 3 monkeys received convalescent monkey serum
c. 3 monkeys received pooled adult human serum

All were challenged intracerebrally 24 hours after the final serum injection. Two monkeys of each group of 3 received only 4 MID of virus in the challenge; one received 200 MID. Two controls were each challenged with 4 MID.

The 3 monkeys treated with human serum and the two controls succumbed, as did one treated with monkey serum and two treated with horse serum. One monkey treated with horse serum and two treated with monkey serum resisted challenge with 4 MID of virus.

In all, Schultz and Gebhardt report 65 monkeys that received immune serum (from varying sources and in varying dosage) and subsequently were challenged by intracerebral inoculation. The paralytic rate in the treated group was 48/65, as compared with 10/10 for controls. The immunized group includes 7 monkeys treated with immune horse serum and challenged with less than 10 MID of virus. The paralytic rate among these was 2/7, as compared with 5/5 for controls similarly challenged.

Finally, the authors attempted to find how long transfused antibodies in horse serum persist in passively immunized monkeys. Three monkeys each received 5 ml of immune horse serum, one by intracardiac injection, one subcutaneously and one intramuscularly. The monkeys were all bled 2, 10 and 30 days later. The sera were all tested simultaneously against a Berkefeld V filtrate from a 5% virus suspension. One ml of undiluted serum was mixed with an equal volume of the virus preparation, and 1.5 ml of each mixture inoculated intracerebrally into one monkey. The 2 and 10 day samples all protected, but none of the 30 day samples did.

Kolmer (179) administered immune serum to monkeys during the incubation period, following intracerebral inoculation with 0.5 ml of a 5% suspension of MV virus. This inoculum is said to have been "equivalent to about 10 MID", but titration of the virus is not described.

Five pools of serum were used; convalescent monkey, convalescent human, normal human, vaccinated monkey, and serum from human subjects vaccinated with ricinoleated virus. Each pool was tested, undiluted, against equal volumes of 5% virus suspension. All the sera were considered to be active, but protocols of the tests are not given. All treated monkeys received serum intramuscularly. Seven to 11 monkeys were treated with each pool of serum, and these groups were further subdivided to represent different dosages of serum (0.5 or 5.0 ml per kg), administered at different times (2 or 6 days after virus inocula-

tion). Four controls all became paralyzed on challenge. Nineteen monkeys treated 6 days after virus inoculation, 3 to 7 with each serum pool, all became paralyzed. Among those treated two days after inoculation the paralytic rate was 16/25. The 9 resisting were grouped as follows:

SERUM USED	DOSE	PARALYTIC RATE
	ml/kg	
Convalescent monkey	0.5	1/2
	5.0	0/2
Convalescent human	0.5	2/2
	5.0	1/2
Normal human	0.5	2/2
	5.0	2/3
Vaccinated monkey	0.5	2/2
	5.0	1/3
Vaccinated human	0.5	2/2
	5.0	3/5

These data suggest some protective effect with certain dosages and lots of serum given two days after inoculation, but none after 6 days of incubation.

Gordon, Hudson and Harrison (69) briefly mention intracerebral challenge of two passively immunized monkeys as follows:

> "After taking 70 ml quantities of blood from 2 normal monkeys they were given 90 ml of convalescent monkey serum intravenously and 95 ml intraperitoneally. Both survived subsequent intracerebral inoculations of virus which caused poliomyelitis in a control."

Kramer (48) studied the prophylactic effect of human convalescent serum against Lansing virus infection in mice. Sera of high titer were pooled for use in the experiments, but the titer of the pool is not stated. The serum was given to the test mice intraperitoneally, and controls received an equal volume of normal monkey serum. Treated mice and controls were uniformly challenged by intracerebral inoculation with 0.03 ml of an 0.5% suspension of virus. On repeated titrations, this dosage had been found to produce fatal infection in "half or better than half" of inoculated mice. In the several experiments with serum, the mortality in control groups varied approximately from 60 to 85%. In one series of experiments with different dosages of serum, mice averaging 22 grams in weight were used. In all others the mice were 3 weeks old, averaged 10 grams and received a standard dose of 0.2 ml of serum.

The author presents charts of 28 experiments, each including 24 treated mice and 24 controls. The time of serum treatment was varied from 14 days before to 4 days after inoculation. In one experiment, in which the serum was given 48 hours following inoculation, the mortality in the treated group equalled that of the controls. In all the other experiments it was consistently lower in the treated groups. Protection was maximal when the serum treatment preceded or followed inoculation by an interval not exceeding 8 hours or so. As the interval was increased in either direction the percentage of survivors among treated mice

diminished. At the optimum time of treatment protection was not complete since about 3 of 24 (12.5%) of the treated mice succumbed. When the serum was given 14 days before or 4 days after inoculation, more than half of the treated mice became fatally infected although the mortality was still slightly below that of controls.

Additional experiments showed that: (a) a concentrated globulin fraction of Type II antipneumococcus serum, substituted for the human convalescent serum, did not protect the mice against Lansing virus infection; and (b) the addition of vasoconstricting drugs (adrenalin, ephedrin) to the virus suspension used for inoculating did not prevent the protective action of convalescent serum given intraperitoneally. The latter experiment was carried out on the hypothesis that inoculation intracerebrally might produce local bleeding, and that antibody in the mouse's blood might act locally and directly on the injected virus. It was assumed that local bleeding in the brain could be prevented or minimized by the drugs.

In the experiments with varying doses of convalescent serum, all were given within the optimal period, about 1.5 hours before inoculation. While the largest dose used, 0.5 ml, gave better protection than did 0.2 or 0.1 ml, there were still 4 deaths among the 24 treated mice (16.7%).

Subsequently, in experiments never published, Kramer (49) studied the prophylactic action of human placental gamma globulin (Lederle) against poliomyelitis in mice, cotton rats, and monkeys. In the mouse experiments, groups of animals of the same age were set aside for later inoculation with Lansing virus. In the meantime, each treated mouse received a single intraperitoneal injection of 0.1 ml of the gamma globulin. One group was injected 3 weeks before inoculation with virus; a second group 2 weeks and a third 1 week before. Finally, all the treated mice were inoculated intracerebrally with 0.03 ml of a 0.5% suspension of virus. At the same time, groups of untreated mice were inoculated with 5.0, 0.5 and 0.05% virus, serving both as controls for the treated groups (0.5%) and for titrating the potency of the virus. Results of the first experiments may be tabulated as follows:

TREATMENT	OUTCOME (NUMBER PARALYZED)/(NUMBER INOCULATED)
Treated 3 weeks before inoculation (0.5% virus)	11/15
Treated 2 weeks before inoculation (0.5% virus)	9/16
Treated 1 week before inoculation (0.5% virus)	18/33
Untreated, inoculated with 5.0% virus	5/6
Untreated, inoculated with 0.5% virus, (controls)	26/29
Untreated, inoculated with 0.05% virus	4/5

A second series of experiments was similarly conducted on mice, except that the gamma globulin was given at 96, 72, 48 or 24 hours before inoculation with the virus. The results:

TREATMENT	OUTCOME (NUMBER PARALYZED)/ (NUMBER INOCULATED)
Gamma globulin 96 hours before inoculation (0.5% virus)	19/32
Gamma globulin 72 hours before inoculation (0.5% virus)	21/33
Gamma globulin 48 hours before inoculation (0.5% virus)	14/32
Gamma globulin 24 hours before inoculation (0.5% virus)	8/26
Untreated, inoculated with 5% virus	4/4
Untreated, inoculated with 0.5% virus, (controls)	26/27
Untreated, inoculated with 0.05% virus	3/6

Kramer concludes that the highest degree of protection was obtained when the mice received gamma globulin 24 hours before infection although some protection was demonstrable for at least as long as two weeks.

He attempted to determine how long the antibody persisted in the serum of mice following injections of gamma globulin. Animals that had received the same dosage of gamma globulin used above (0.1 ml) were killed by groups at varying intervals thereafter, and the pooled serum of each group tested in varying dilutions for neutralization of Lansing virus. Serum obtained up to 96 hours after the gamma globulin had been given was active at 1/10 dilution. Details of the technique used are not given, but controls with normal mouse serum showed 9/9 mice paralyzed. With 1/10 dilution of sera from the treated mice the figures were: 24 hour group, 0/6; 48 hour group, 2/5; 72 hour group, 1/6; 96 hour group, 2/6; and 1 week group, 4/5. No antibody could be demonstrated beyond one week at most after the gamma globulin was given.

Kramer's experiments with cotton rats yielded results similar to those obtained with mice. He gave the gamma globulin intraperitoneally, varying the time of administration from 24 to 2 hours before intracerebral inoculation with Lansing virus. He compared also the effects of single doses of gamma globulin with those of repeated doses; of varying the amounts of single doses; and of varying the amount of virus given. Only 3 of the 14 experiments will be outlined here. In these 3, which show the maximum degree of protection obtained with a given dosage, the gamma globulin was given at a single injection 16 hours before the virus.

DOSAGE OF GAMMA GLOBULIN ML/10 GRAMS BODY WEIGHT	VIRUS CONCENTRATION GIVEN I.C.	OUTCOME (NUMBER PARALYZED)/ (NUMBER INOCULATED)
	%	
0.1 ml	0.05	8/19
Untreated (controls)	0.05	18/19
0.1 ml	0.03	7/20
Untreated (controls)	0.03	19/20
0.5 ml	0.03	3/19
Untreated (controls)	0.03	16/18

The percentage of survivals was not increased by giving repeated doses of gamma globulin although the protection afforded by a single dose varied somewhat in relation to the amount given. Kramer comments that in comparison

with unprocessed immune serum, the protective action of gamma globulin did not appear to be in proportion to its presumably higher content of antibody.

Results of Kramer's experiments on monkeys were complicated by unexpectedly low paralytic rates in controls. He suggests that in the first 3 experiments outlined below the virus may have deteriorated in potency during the

Titration of poliomyelitis neutralizing antibody of serums and spinal fluids of monkeys receiving Lederle immune globulin

MONKEY	TIME AFTER GLOBULIN INJECTION	NEUTRALIZATION TEST (0.5% VIRUS SUSPENSION) Outcome: Paralyzed / Number inoculated								
		Serum (dilution)								Spinal Fluid (dilution)
		1:5	1:20	1:40	1:60	1:80	1:160	1:320	1:640	4:5
#482 (iv)	7½ hr.			0/6		0/6	0/5	1/6	3/6	6/8
	24 hr.			0/6		1/6	0/6	3/6	4/6	6/10
	48 hr.			0/6		1/5	0/6	2/6	3/6	6/10
Normal control.....		6/8	6/7		7/8					6/10
#482 (iv)	72 hr.			0/4		1/6	0/6	2/4	3/6	7/9
	96 hr.			0/4		0/5	1/6	3/6	1/6	5/8
	5 days			1/5		1/5	3/4	4/5	3/5	5/10*
	7 days			0/6		0/6	1/5	2/5	1/6	7/9
	11 days			1/5		1/5	2/5	3/6	6/6	4/9
	14 days			1/5		2/4	3/4	2/6	3/6	5/9
Normal control.....		6/7	5/5		8/8					6/9
#482 (iv)	19 days			0/5		1/6	3/6	3/6	3/6	6/9
	26 days			2/5		1/4	2/5	5/6	2/6	7/9
	34 days			2/4		3/3	5/5	3/5	6/6	5/9
Normal control.....		6/8	5/6		5/7					7/9
#483 (ip)	7½ hr.			0/4		1/4	0/6	1/6	0/4	6/8
	24 hr.			1/6		0/6	0/5	1/5	1/6	7/10
	48 hr.			1/4		0/8	0/5	0/5	3/5	7/10
Normal control.....		6/8	5/6		5/7					7/9

* Dilution = 1:2.

Each monkey received 5 injections of 10 ml per kg; ip—intraperitoneal route; iv—intravenous route.

interval between its last titration and its use in these experiments. Challenge was by intracerebral inoculation, volume of inoculum 0.5 ml, in the dilutions indicated. The Aycock strain of virus was used.

Experiment 1. Treated monkeys each received 10 ml of gamma globulin per kg intraperitoneally, 24 hours before challenge. Challenge 5% virus. Outcome: Treated, 5/15 paralyzed. Controls 4/14.

Experiment 2. Treated monkeys each received 5 doses of gamma globulin intraperitoneally, each dose 10 ml per kg. Doses at approximately 24 hour intervals, beginning 24 hours before challenge and ending 72 hours after. Challenge, 5% virus. Outcome: Treated, 0/15 paralyzed. Controls 4/15.

Experiment 3. Treated monkeys each received 3 doses of gamma globulin intraperitoneally, each dose 10 ml per kg. First dose 24 hours before; second, 3–4 hours before; third, 24 hours after challenge. Challenge, 10% virus. Outcome: Treated, 1/15 paralyzed. Controls 3/14.

Experiment 4 was made with a different virus pool. Treated monkeys each received 5 doses of gamma globulin, each dose 10 ml per kg. The first 3 doses were intraperitoneal and given, respectively, 48, 32 and 28 hours before challenge. The last two were intravenous, 1 at 24 hours and 1, 3 to 4 hours before challenge. Challenge, 1.0% virus. Outcome: Treated, 2/15 paralyzed. Controls 12/15.

Experiment 5. In this experiment each monkey received 3 intraperitoneal injections of gamma globulin, same dosage and schedule as in Experiment 4. An intravenous dose of 10 ml/kg was given 22 hours before challenge. Challenge, 2.0% virus. Outcome: Treated, 3/9 paralyzed. Controls 6/9.

While these data indicate that gamma globulin does in some measure protect monkeys against intracerebral challenge with poliomyelitis virus, they do not show whether such protection is effective beyond 24 hours or not.

Finally, Kramer attempted to determine how long the effect of a gamma globulin injection would be manifest from neutralization tests on the serum of the recipient monkeys. Two normal monkeys each received 5 injections each of 10 ml of gamma globulin per kg. It was given intraperitoneally to one, intravenously to the other. Both animals were bled at varying intervals following the final globulin injection, and the serum tested in mice for neutralization of Lansing virus. The results are tabulated on p. 413.

It appears that the serum of the one monkey tested at 26 days was still active in 1/40 dilution at that time and was probably active at 34 days in the same dilution. The very large dosage of gamma globulin should be noted.

Rhodes and Clark (168) used gamma globulin and immune sera from different sources for passive immunization of mice against Lansing virus. Each prophylactic agent was first titered in neutralization tests against 8 to 85 LD_{50} of virus. Procedure and results are outlined in the following table:

INOCULUM ADMINISTERED (0.3 ML PERITONEAL 24 HOURS BEFORE VIRUS)	50% NEUTRALIZING TITER OF MATERIAL	MORTALITY IN MICE CHALLENGED CEREBRALLY WITH FOLLOWING DOSES OF VIRUS	
		25 LD_{50}	5 LD_{50}
Placental gamma globulin	$10^{-3.5}$	8/20 (40%)	4/19 (21.2%)
Immune monkey serum	$10^{-2.5}$	12/20 (60%)	7/20 (35%)
Immune horse serum	$10^{-2.5}$	18/19 (95%)	13/20 (65%)
Normal monkey serum	Nil	19/19 (100%)	15/19 (79%)
Normal horse serum	Nil	19/19 (100%)	19/19 (100%)
Plasma gamma globulin (lot #116)	$10^{-2.63}$	10/18 (55.6%)	5/19 (26.3%)
Placental gamma globulin	$10^{-3.5}$	8/18 (44.5%)	5/20 (25%)
Saline		19/20 (95%)	17/19 (89.5%)

Rhodes and associates (50) carried out passive protection experiments on monkeys, using gamma globulin from human sources and serum from monkeys vaccinated with Lansing virus. One lot of gamma globulin was derived from pooled human plasma, one from placental. Each titered $10^{-3.5}$ against Lansing virus by mouse tests. Two lots of immune monkey serum, similarly tested, titered respectively $10^{-4.5}$ and $10^{-3.86}$. In preliminary experiments, 3 monkeys each received 4 ml/kg of gamma globulin (from plasma) intravenously. Blood samples were drawn at 24, 48 and 72 hours. Serum titers against Lansing virus at 24 hours were $10^{-2.65}$ to $10^{-2.79}$, declining thereafter. Three other monkeys each received 3 ml/kg of gamma globulin intramuscularly and were bled at longer intervals, beginning at 4 days. These showed initial antibody titers of the same order as the first group, with a somewhat slower rate of decline.

Three passive protection experiments were conducted as follows:

Experiment 1 involved 3 groups of 10 monkeys each. Each animal was inoculated intracerebrally with 160 PD_{50} of Lansing virus from a pool titered on monkeys. Two of the groups received placental gamma globulin intravenously 24 hours before the virus inoculation. The dosage of gamma globulin was 0.8 ml/kg for one group and 4 ml/kg for the other. Neither group showed significant protection, paralytic rates being respectively 9/10 and 8/9, as compared to 10/10 for the third group (untreated controls).

In experiment 2, 20 monkeys each received 5 ml/kg of immune monkey serum intramuscularly and were challenged 24 hours later with 50 PD_{50} of virus intracerebrally. The paralytic rate was 4/16, as compared to 18/19 in a group of untreated controls.

In experiment 3, 20 monkeys each received 3 ml/kg of immune serum intramuscularly, and each was challenged 24 hours later with 100 PD_{50} of virus intracerebrally. The paralytic rate in the treated group was 3/18; in a group of untreated controls, 20/20.

(Discrepancies between the numbers of monkeys challenged and those originally assigned to the respective groups are due to losses from irrelevant causes.)

To summarize the results of intracerebral challenge, passive immunization has been proven to confer some protection. It has not been uniformly effective, however, even with the largest doses of immune serum or of gamma globulin employed. In mice only a minimal degree of protection by human serum was demonstrable two weeks after its administration. The duration of the protection in monkeys challenged by this route has not been adequately studied, but presumably would be brief.

2. *Intranasal challenge.* Weyer, Park and Banzhaf (180) conducted experiments on prophylaxis with monkeys, using (a) human convalescent serum and (b) serum from horses immunized with poliomyelitis virus.

(a) Human convalescent serum, 20 ml, was given intravenously to each of two monkeys. Beginning one week later, one of the monkeys was inoculated by intranasal instillation of virus on 3 successive days. The second monkey was similarly inoculated 3 weeks after receiving the serum. Both of these monkeys,

and the one control, died from poliomyelitis. The strain and titer of the virus used are not stated, and *in vitro* tests on the neutralizing potency of the serum are not mentioned.

(b) The tests for potency of the horse serum are described as follows: "Five per cent virus emulsions are prepared by grinding virulent monkey spinal cord with saline. The serums to be tested are added in the desired proportion and after an hour's incubation at room temperature the mixtures are introduced into the cerebral hemispheres of susceptible monkeys. The amount of inoculum is so calculated that it represents at least 2 infective doses. The potency of the virus has apparently no effect upon the amount of a specified serum preparation needed to neutralize it. When using particularly potent strains of virus the inoculum often contains as many as several hundred killing doses of virus." The original sera neutralized the virus in dilutions never exceeding 1:20. The globulin concentrate neutralized in dilutions up to 1:100. No protocols of the tests are given.

Prophylactic experiments with the horse serum concentrate are summarized in the following table. Rhesus monkeys were used. All were treated with a globulin concentrate of immune horse serum given intravenously in the dosage indicated. All were challenged by intranasal instillation of virus on 3 successive days (dose not stated), beginning at the indicated interval after serum.

MONKEY	HORSE SERUM CONCENTRATE	TIME OF CHALLENGE	OUTCOME
	ml		
1	10	1 week later	Survived
1	10	2 weeks later	Survived
1	5	4 weeks later	Survived
1	1	4 weeks later	Succumbed

No controls in the horse serum experiment are mentioned, and in view of the variability of takes in normal monkeys inoculated intranasally the number of animals here used seems too small to be convincing.

Howitt, in the course of studies already cited (172, 175), used intranasal challenge with certain groups of passively immunized monkeys. The first series includes two groups, each of 8 animals, treated with immune goat or sheep serum. All of these resisted challenge, but since only 1 of 9 controls developed paralysis the outcome in the treated groups hardly seemed significant. The second paper deals with the prophylactic effect of a concentrate from immune horse serum.

(1) Six monkeys received the concentrate of immune horse serum intramuscularly; two controls received normal horse serum. The six monkeys were divided into 3 pairs, one of each pair receiving 10 ml and the other 20 ml of horse serum concentrate. All were subsequently challenged by a single intranasal instillation of 10% virus, one pair 1 day, another 2 days, and the third 7 days after receiving the serum concentrate. Two treated animals died from

causes other than poliomyelitis; the others survived. One control died; the other survived.

(2) In another experiment, 3 monkeys each received 20 ml of the horse serum concentrate and 3 others 25 ml of immune sheep serum (for data on this serum see pages 405–406) all intramuscularly. All were challenged intranasally by a single instillation of virus 1, 2 or 7 days later. Three normal monkeys were similarly inoculated as controls. One monkey, treated with immune sheep serum 7 days before challenge, died, as did two controls. The remaining five treated monkeys and one control survived. (Note: The outcome of challenge is indicated only by "survived" or "died". There is nothing to indicate whether any of the surviving monkeys developed poliomyelitis or not.)

Schultz and Gebhardt (177) reported on the results of intranasal challenge of passively immunized monkeys. General details of these experiments have been outlined previously (p. 407).

(1) One monkey received two intracardiac injections, each of 10 ml of immune horse serum, 4 days apart. Starting 24 hours after the second injection, the treated monkey and one control received 3 daily intranasal instillations of virus (MV). The control succumbed. The treated monkey remained well but succumbed to intracerebral challenge with 1 ml of 10% virus two months later.

(2) Two monkeys each received 20 ml of immune horse serum subcutaneously. They and one control were inoculated intranasally 24 hours later. The control became paralyzed. Both treated monkeys remained well. They were again challenged, with another control, by intranasal inoculation 3 weeks later. The control again succumbed; the treated monkeys remained well. Subjected to a third intranasal inoculation, 48 days after the first, both became paralyzed.

(3) Two monkeys each received 10 ml and two others 20 ml of immune horse serum subcutaneously. They were challenged, with one control, by intranasal inoculation 24 hours later. The control and one of each pair of treated animals became paralyzed. One treated monkey of each pair remained well.

(4) Two monkeys each received 20 ml of convalescent monkey serum subcutaneously and were challenged by intranasal inoculation 24 hours later. One treated monkey and two controls became paralyzed. One treated monkey remained well.

(5) Five monkeys each received multiple subcutaneous injections of immune horse serum, totalling 5 ml per pound body weight. The treated monkeys and the 3 controls were challenged intranasally 24 hours later. The 3 controls and 4 treated monkeys became paralyzed. One treated monkey remained well.

(6) Six monkeys each received 15 ml of immune horse or convalescent monkey serum by intracardiac injection, repeated 24 hours later. They were challenged with 3 controls, by intranasal inoculation 24 hours later. The outcome was:

Treated with horse serum—3/3 paralyzed
Treated with monkey serum—1/3 paralyzed
Controls (normal monkey serum)—2/3 paralyzed

The 3 surviving monkeys were again challenged by the intranasal route 22 days later. All succumbed.

(7) Six monkeys each received intramuscular injections of immune horse or convalescent monkey serum on 4 successive days (1, 1, 1 and 2 ml per kg body weight). On the fourth day each was subjected to the first of 3 daily intranasal instillations of virus. Four controls were similarly inoculated. The outcome of challenge was:

Treated with immune horse serum—1/3 paralyzed
Treated with convalescent monkey serum—3/3 paralyzed
Controls—3/4 paralyzed

In summary, of 26 monkeys that received immune serum in varying doses and from different sources, and were challenged by intranasal inoculation, the paralytic rate was 15/26. The paralytic rate for the controls was 14/16.

Gordon, Hudson and Harrison (69) report 2 experiments.

In the first, blood was drawn from 6 convalescent monkeys by cardiac puncture, citrated, and pooled. Normal monkey blood was collected in the same way. Four normal monkeys were bled from the heart, 50 ml from each, and immediately each was given 70 ml of the pooled monkey blood into a leg vein. Two thus received convalescent and two normal blood. During the following two days the 4 treated monkeys and 2 untreated controls each received 3 intranasal inoculations of virus (MV). All developed poliomyelitis.

The pool of convalescent serum used in this experiment was not directly tested for antiviral activity, but a similar pool from the same animals, mixed in the same proportions, had been tested earlier. In the tests, serum (1.5 ml) was mixed with virus in 1% suspension (0.5 ml) and the 2 ml inoculum given to monkeys intracerebrally. The serum pool neutralized when undiluted but failed to do so in dilutions of 1:5, 1:10 and 1:15.

For the second experiment a more potent antiserum was prepared by vaccination of 4 convalescent and 1 previously immunized monkey. A serum pool prepared from these, and tested in the same way as the first pool, neutralized in 1:5 and 1:15 dilutions (higher dilutions were not tried). The second experiment was conducted in the same manner as the first, except that 60 ml quantities of blood were given. The 6 monkeys inoculated (2 untreated controls, 2 treated with immune serum and 2 with normal serum) all developed paralysis.

Bodian (181) challenged treated and control rhesus monkeys with Brunhilde virus. Treated animals uniformly received 10 mg per kg intramuscularly of human gamma globulin. They were divided into 3 groups. One received gamma globulin 8 to 10 days after inoculation at the onset of fever. The other groups received it respectively 1 hour and 24 hours before inoculation. The challenge dose of virus for the third group was 3 intranasal PD_{50}; for the other two groups 10 PD_{50}. The incidence of prostrating paralysis and of fever was noted for each group, also the poliomyelitis rate as determined by presence or absence of lesions in the olfactory bulbs. A part of the data in the author's table 1 is given below:

	POLIOMYELITIS RATE	PROSTRATING PARALYSIS	FEVER
Experiment 1			
Controls	5/6	4/6	5/6
Treated, first day of fever	5/6	5/6	5/6
Experiment 2			
Controls	5/6	5/6	6/6
Treated, 1 hour before inoculation	3/6	0/6	6/6
Experiment 3			
Controls	6/6	3/6	6/6
Treated, 24 hours before inoculation	2/6	1/6	5/6

In these experiments no therapeutic effect of gamma globulin was demonstrated, even when administered at the onset of fever. Some prophylactic effect is evident, but protection was incomplete although the dosage was large and given at presumably the optimum time.

3. *Challenge by other parenteral routes. Intravenous.* Flexner and Amoss (182) used a method of challenge based on their finding that rhesus monkeys, normally resistant to virus inoculated intravenously, became susceptible if a sterile meningitis were first set up by intraspinal injection of foreign serum. Protocols are given of 6 monkeys. Two received respectively 25 and 50 ml of a virus suspension intravenously, without pretreatment. Both resisted. Four received each 3 ml of horse serum intraspinally, followed one day later by 25 ml of virus intravenously. Three of these were otherwise untreated, and all developed paralysis. One received 3 ml of convalescent monkey serum intraspinally immediately after inoculation with the virus, and a similar dose daily thereafter for one week excepting the fourth day. This monkey remained well.

Howitt (172) used a similar procedure. Two monkeys each received normal horse serum "intraspinally" followed 18 hours later by immune goat serum "intrathecally", and virus intravenously immediately thereafter. Dosages of the sera are not given, and the dosage of virus is described only as "large". The two treated monkeys remained well; one control developed paralysis.

Intraperitoneal. Hammon, Cheever and Sather (183) made use of the intraperitoneal susceptibility of infant mice to the MEF_1 strain of virus.

Human gamma globulin (from plasma) was given in 3 dosages, 0.5, 0.1 and 0.02 ml per pound body weight, to different groups of mice. It was administered intramuscularly in a constant volume (0.03 ml) of fluid. Treated mice and controls of the same age were challenged intraperitoneally, 24 hours later, with 0.1 ml of 10% suspension of MEF_1 virus, from pools titering $10^{-3.5}$ to $10^{-4.4}$ intracerebrally in mice. Animals were observed for paralysis or death over a 21 day period after inoculation, but only mortality figures are tabulated.

In the first experiment mice 5 or 6 days old were used. Mortality was 60/103, or 58.3% in the control group; 22/36, or 61.1%, in the group receiving the smallest

dose of gamma globulin; 12/49 or 24.5% in the group treated with 0.1 ml/lb, and 2/36, or 5.6% in the group treated with 0.5 ml/lb.

The second experiment included two control groups, one uninjected and another treated with 0.03 ml of 1.5% gelatin solution intramuscularly. Three groups received gamma globulin, dosages as in the first experiment. Mortality in both control groups was 81.2% (86/106 and 26/32). In the treated groups, in order of increasing dosage of gamma globulin, it was 20/31 (64.5%), 17/64 (26.6%) and 9/35 (25.7%). A parallel series of groups, treated and controls, was not inoculated with virus but simply observed over the 21 day period as a check on the mortality rate in noninfected mice with and without intramuscular injections. The mortality rates varied from 10 to 35.5%, with no evidence that protective inoculations had any influence upon them.

The third experiment included 4 treated groups, receiving respectively 0.1, 0.05, 0.025 and 0.0125 ml of gamma globulin per pound. Two control groups were included, one challenged with the treated groups, the other left uninoculated. Mortalities were 44/47, or 93.7%, for the inoculated and 15/47 (31.9%) for the uninoculated controls. For the treated groups, in order of increasing dosage of gamma globulin, mortality was 53/59 (89.8%), 52/58 (89.7%), 39/55 (70.9%) and 21/54 (38.9%). Thus 0.1 ml of gamma globulin per pound brought the mortality down nearly to the level of uninoculated controls. Some protection was evident with half that dosage but not with lesser dosages.

Survivors of the various groups were challenged intracerebrally one month after the first injection of virus, gamma globulin or gelatin. Mortalities ranged from 90.4% to 96% in those without previous inoculation with virus (untreated controls, mice treated with gelatin and with varying dosages of gamma globulin). Among survivors of various groups once inoculated with virus intraperitoneally, mortalities from intracerebral challenge were:

a. Mice treated with gamma globulin:

0.02 ml/lb	6/10, 60%
0.1 ml/lb	25/47, 53.2%
0.5 ml/lb	21/26, 80.8%

b. Previously inoculated controls.

No intramuscular injection	19/36	47.6%
Gelatin treated	1/6	

It was concluded that gamma globulin in dosage adequate to protect against intraperitoneal challenge did not prevent the development of active immunity in consequence of such challenge. The results suggest that larger doses of gamma globulin may have prevented development of active immunity.

Intramuscular. Bodian (6) tested the protective action of human gamma globulin against poliomyelitis virus inoculated intramuscularly in rhesus monkeys. Four strains of virus were used: Lansing and MV of Type 2, Frederick and Leon of Types 1 and 3, respectively. All the virus pools were titered for infectivity by intracerebral inoculation in monkeys, titers ranging from $10^{-4.5}$ for Lansing to $10^{-6.0}$ for Leon. Rhesus monkeys were inoculated, into the calf muscles of one side, each with 1.0 ml of 10% virus suspension. Gamma globulin

was injected into muscles of the opposite side, usually at the same time as the virus and in a dosage of 2 ml per kg. Controls received the same dosage of virus without gamma globulin.

Of 20 monkeys passively immunized and inoculated with Frederick virus, none became paralyzed. In controls inoculated at the same time the paralytic rate was only 4/20 although it had been 11/13 in a group of untreated monkeys similarly inoculated with the same virus in an earlier experiment. With Lansing virus paralytic rates were 0/5 in the treated and 1/5 in the control group; with MV, 2/12 treated and 13/21 controls. The experiment with MV was repeated, doubling the dosage of gamma globulin (4 ml per kg). Paralytic rates were then 1/12 treated and 7/12 controls. With Leon virus the schedule was modified. Six monkeys received gamma globulin 2 weeks, six 1 week, and ten 3 days before virus inoculation, ten 3 days after and ten at the same time. Paralytic rates for the entire experiment with Leon virus were 0/42 treated and 9/16 controls.

For the entire series the paralytic rates were thus 3/91 treated and 45/87 controls. Protection was complete against all the viruses except MV, known to be highly infective by intramuscular inoculation.

Moyer, Accorti and Cox (184) adapted the MEF_1 strain of virus to infant hamsters and found it to be infective to mice by intramuscular inoculation. Groups of mice (of 12 to 14 g weight) were treated by injection into one flank of 0.25 ml of human gamma globulin (placental). They were challenged by injection into the opposite flank of 0.25 ml of a 10% suspension of virus (hamster CNS tissue). The time of challenge was varied in different groups. One was challenged immediately after administration of gamma globulin, the others at respective intervals of 24, 48, 72 and 96 hours, 7, 14 and 28 days. Mortality in the treated groups (of 9 or 10 mice each) did not exceed 10% up to and including the 14 day group. In control, unprotected groups, mortality varied from 70 to 100%. No protection was demonstrable at 28 days (mortality 4/6 in treated and 5/8 in control mice).

4. *Challenge by way of the alimentary tract. Virus feeding.* Howe and Bodian (4) inoculated 6 chimpanzees, each on 3 successive days, with infected stool suspension (Kotter strain of virus) given by stomach tube and rubbed into the mucosa of the cheeks. Three of the animals, otherwise untreated, served as controls. The other 3 each received 170 ml of hyperimmune monkey serum intraperitoneally. This was divided into 4 doses of 50, 50, 35 and 35 ml, the first given 26 hours before the first inoculation with virus. The remaining doses of serum were given on successive days, one before each virus inoculation. The serum was of known potency, 0.4 ml neutralizing 500 PD_{50} of virus.

All 6 chimpanzees, treated and controls, became intestinal carriers of the virus. This limited experiment therefore indicates that the immune serum did not prevent the occurrence of inapparent infection, and presumably did not interfere with the consequent development of active immunity. But since paralytic disease did not develop in any of the 6 animals, treated or control, there was no indication whether the immune serum conferred any protection against paralysis or not.

Bodian (56) carried out a series of experiments with cynomolgus monkeys. Sixty-eight each received a large dose of gamma globulin (10 ml per kg) intramuscularly, followed by oral inoculation with Per, Y-SK, Leon or Wfd virus. None of the treated monkeys developed paralytic disease, but only 3/62 paralytic infections resulted in untreated controls similarly inoculated with the same viruses. Sera of all the animals initially failed to neutralize Lansing virus. Among those inoculated with viruses of the same type (Wfd, Y-SK) two controls showed rises of antibody titer 5 weeks later, indicative of inapparent infection. AntiLansing titers in all the treated animals and in noninfected controls had fallen at 5 weeks to $10^{-1.0}$ or $10^{-0.5}$, representing residual activity of the gamma globulin antibody. In a similar experiment with another strain of virus (Mahoney) the paralytic rates were 3/9 in controls and 0/10 in treated monkeys. One group of monkeys, that had received gamma globulin and had resisted inoculation with Wfd virus, was inoculated with Mahoney virus one month later without any further administration of gamma globulin during the interval. Paralytic rates were 0/10 in this group, 2/10 in controls. This suggested that residual activity of the gamma globulin, a month after such a large dose, was sufficient to protect against paralytic infection. In another experiment, Mahoney virus was fed following a smaller dose (2 ml/kg) of gamma globulin. Paralytic rates were 0/7 for the treated and 2/6 for the control group.

Still smaller doses of gamma globulin were then tried with the Mahoney virus. At the same time another variable was introduced by using cynomolgus monkeys of Philippine instead of Malayan origin. In these, with a gamma globulin dosage of 1 ml/kg, paralytic rates were 0/12 (treated) and 7/13 (controls). Eleven of the treated and resistant monkeys were again fed Mahoney virus 26 days later. All again resisted. None had received further gamma globulin treatment during the interval. Sera of 3, obtained at the time of the second challenge, and used undiluted, protected 4 or 5 out of groups of 8 mice against 32 PD_{50} of Lansing virus. The level of antibody, though barely detectable, was still apparently sufficient to protect.

Finally, two experiments were made with respective dosages of 0.01 and 0.1 ml/kg of gamma globulin, followed immediately by Mahoney virus given orally. Paralytic rates in treated animals were 3/10 with 0.01 ml and 0/8 with 0.1 ml of gamma globulin. Rates for the corresponding control groups were 5/10 and 4/9.

Tonsillectomy has been shown to increase the susceptibility of cynomolgus monkeys to paralytic infection if poliomyelitis virus is given by mouth during the immediate postoperative period (185). Adams and associates (186, 187) studied the effectiveness of human gamma globulin as a prophylactic under these conditions. Four experiments were carried out, each including one group of cynomolgus monkeys tonsillectomized and treated with gamma globulin intramuscularly; also two groups of untreated controls, one tonsillectomized and one unoperated. Virus (Y-SK) was fed daily to all on successive days, beginning 8 hours after operation. The dosage of virus and the dosage and time

schedule of gamma globulin were varied from one experiment to another as follows:

	EXPERIMENT I	II	III	IV
Virus, daily dose	100 PD_{50}	100 PD_{50}	5 ml, 10%	2 ml, 2%
Number of doses	7	7	5	3
Gamma globulin, ml per pound total	1.0	2.0	4.0	2.0
Single or divided dose	1	2, 3 day interval	3, 2 day interval	1
Time of first dose, in relation to tonsillectomy	same day	same day	same day	1 day before

The various groups were observed over a period of one month. Animals showing symptoms suggestive of poliomyelitis were sacrificed, and attack rates were calculated on the basis of positive histologic evidence of infection in CNS tissues. Attack rates were as follows:

EXPERIMENT	TREATED ANIMALS	OPERATED CONTROLS
I	2/11	3/11
II	2/12	2/12
III	1/7	5/6
IV	0/11	9/11

Ten survivors of the treated group in Experiment IV were rechallenged, 7 weeks after the first challenge, with an attack rate of 1/10. It seems possible that the low rate at the second challenge may have been unrelated to passive or active immunity but rather to healing at the site of operation. In unoperated controls, of the entire series, the attack rate was 5/38.

More recently, Bodian has used oral inoculation in studies on combined active and passive immunization of chimpanzees (p. 430).

5. *Combined active and passive immunization. Mixtures of virus with immune serum.* Landsteiner and Levaditi (22) mixed equal volumes of a virus emulsion (concentration and titer unstated, although the emulsion is described as "virulent") and serum from a sheep which had received "many" subcutaneous injections of virus. Five ml of the mixture were given subcutaneously to one rhesus monkey, and the dose repeated two days later. The animal resisted successive intracerebral challenges at 11 and 23 days. Two controls became paralyzed.

Römer and Joseph (188) inoculated two monkeys intracerebrally with mixtures of virus and immune serum (no details). They remained well and were challenged 36 days later, intracerebrally, 0.5 ml of 5% virus suspension. Both resisted, one control was paralyzed. In his monograph, Römer (110) refers to 5 monkeys in all that had remained well following one intracerebral inoculation with mixtures of virus and immune serum. All were subsequently challenged

intracerebrally with virus alone and all resisted, while 3 controls became paralyzed.

Thompson (103) inoculated one monkey intracerebrally with 1 ml of a mixture of 20% virus suspension (Aycock strain) and convalescent monkey serum. Four subcutaneous injections, 1.0 ml each, of a mixture of serum with 20% virus were given over the ensuing 30 days. Ten weeks later the animal was challenged intracerebrally, 1 ml of 20% virus, and was paralyzed.

Goldbloom, Brodie and Moffatt (189) vaccinated 5 monkeys with mixtures of virus and immune serum (dosage and potency not stated). Three weekly injections were given. Two of the monkeys died of tuberculosis; the remaining 3 succumbed to intracerebral challenge with 0.1 ml of 5% virus suspension. Brodie (190) later reported neutralization tests on the serum of one monkey vaccinated intradermally with a serum-virus mixture (1.5 g of infected cord, 6 ml of serum). The entire dose was given at one time. Serum from this monkey neutralized 2 MCP of virus, failed to neutralize 5 MCP. One control received 2.0 g of normal monkey cord, one received 9.0 ml of immune serum. Sera from these failed to neutralize 1 MCP dose. The interval between treatment and bleeding was 2 to 3 months.

Rhoads (191) gave serum-virus mixtures by different routes to 5 groups of 4 rhesus monkeys each. The mixtures used are stated to have been noninfective by intracerebral inoculation, but the tests of infectivity are not described. The virus used was a 5% suspension of "pooled mixed" (MV?). All were challenged about a month after immunization was complete, and some of them were bled at the same time for neutralization tests. In the tests 0.99 ml of undiluted serum was mixed with 0.01 ml of virus filtrate (from 5% suspension).

Group A each received intradermal injections of a mixture of virus with human convalescent serum; four ml each time, repeated 20 times at 3 day intervals. On challenge with 2 MID of virus intracisternally, 2/4 became paralyzed and sera of 2/4 neutralized. (The positive neutralization tests were obtained on the two resistant monkeys.) One control was paralyzed.

Group B received a similar mixture subcutaneously but in two sets of injections, 30 ml each time, 5 days apart. These were challenged intranasally. Paralytic rate was 2/4 for the immunized group, 2/2 for controls. Serum tests were made only on the two resistant monkeys (both positive) and one control (negative).

Group C was treated precisely as Group B, except that the serum-virus mixture was given intradermally. Paralytic rate on challenge, 2/4 (controls under B). Sera of the two resistant monkeys neutralized; the others were not tested.

Group D received a mixture of immune globulin (horse), 1 part, and virus 10 parts. Each dose was 15 ml intradermally, repeated for a total of 5 doses at 3 day intervals. On intranasal challenge, one immunized monkey was severely paralyzed, one had mild paralysis but recovered, two resisted. The 3 survivors were challenged intracerebrally a month later. All resisted. One control was paralyzed.

Group E received a mixture of virus with human convalescent serum, 1 ml instilled into each nostril daily for two weeks. On intranasal challenge with live virus, 3/4 of the treated monkeys and 4/4 controls were paralyzed. No serum tests were reported in this group.

Kramer, Schaeffer and Park (8, 192) attempted to balance the dosages of serum and virus in mixtures used for immunizing. In preliminary experiments 12 monkeys were inoculated intra- and subcutaneously with mixtures in which the potencies of virus and serum were "only roughly" approximated. The proportions of virus to serum, the number of injections, total dosage and time schedule were varied and will not be recounted. Sera of all the 12 were initially negative. After immunization 4 neutralized, 8 did not.

The virus (strain unstated) and human convalescent serum used were both titrated by intracerebral inoculation of monkeys using a 5% virus suspension. Dosages and results of the infectivity tests were:

DOSE	PARALYTIC RATE
ml	
0.001	2/4
0.005	2/3
0.01	3/3

The MID was considered as 0.01 ml, and varying amounts of serum were tested against 0.1 ml (10 MID). Results were:

VOLUME OF SERUM	MONKEYS PROTECTED
ml	
1/200	2/3
1/160	3/3

On this basis it was calculated that 1 ml of serum should neutralize 16 or 20 ml of 5% virus suspension, and mixtures of these proportions were given in repeated doses to 12 monkeys. Six received a 1:16 mixture, 6 a 1:20. Three of each group received a total dosage each of 30 ml, three 60 to 69 ml. These totals were divided into 4 or 7 injections at weekly intervals. The first injections were intramuscular, but because of local swelling the route was changed to subcutaneous. All the monkeys were bled before and one to two months after the course of injections. Neutralization tests were made using 0.5 ml of serum against 0.1 ml (10 MID) of virus. All the sera were initially negative. None of the 12 became positive after immunization. Challenge inoculations were not reported.

Kramer (193) later carried out additional experiments with balanced serum-virus mixtures, testing not only antibody response but also resistance to intracerebral challenge. Neutralization tests and challenge inoculation were made with virus titered in the manner outlined above. For immunization, two different lots of virus were used, neither of which was separately titered. Each was tested in constant dosage (0.1 ml of 10% suspension) against serial dilutions of serum

(3 monkeys per dilution), and the end point of complete protection thus estimated. The proportion of serum to virus, at this end point, was used in mixtures for immunizing. With the first lot of virus (Experiment 1) the proportion was 1:16; with the second lot (Experiment 2), 1:6.

In Experiment 1, 12 monkeys each received 3 weekly injections (intra- and subcutaneous) of 5 ml of serum-virus mixture (total, 1.5 g of infected tissue). The monkeys were bled before and 3 to 6 weeks after immunization. Neutralization tests were made using 0.5 ml of undiluted serum mixed with 0.5 ml of saline containing 1 MID of virus. All sera were initially negative. After immunization 2/10 neutralized, two monkeys having been lost from tuberculosis. Two more were similarly lost before challenge. On challenge with 1 MID, the paralytic rate was 5/8. The three survivors were successively challenged at 2 to 4 week intervals with progressively increasing doses. One succumbed to the third (4 MID), one to the fifth (16), and one to the sixth challenge (24).

In Experiment 2, 11 monkeys each received 4 weekly doses of 5 ml of serum-virus mixture, totalling 2.0 g of infected tissue. Otherwise the procedure was essentially the same as described above. Sera of 7 became positive after immunization. All were challenged, beginning with 1/5 MID, which brought down 2/11. Nine resisted successive challenges with 1/2 and 1 MID. Three succumbed at 2, 2 at 4, and 1 at 8 MID. Two resisted at 8 but died of intercurrent infection before further challenge. One had been similarly lost earlier.

Bodian (6) immunized monkeys with a mixture containing equal volumes of human gamma globulin solution and 20% suspension of Lansing virus. The virus had an intracerebral titer in monkeys of $10^{-5.0}$. The gamma globulin had a neutralizing titer of $10^{-2.8}$ against 32 PD_{50} of Lansing virus in mice. Hence it was estimated that the mixture should be almost exactly neutralized.

Five monkeys each received four 2 ml doses of the mixture intramuscularly at 2 week intervals. For comparison, 5 other monkeys were vaccinated with the same dosages of live virus in saline, without gamma globulin. The first group was bled on the day after the first immunizing injection, and both groups were bled 10 days after the last injection. Pooled serum from the first group, undiluted, neutralized 32 PD_{50} of Lansing virus at both bleedings. It failed to neutralize in 1:10 dilution. Post-vaccination serum from the controls, immunized with live virus, titered $10^{-2.4}$.

The data available on serum-virus mixtures thus indicate that such mixtures have at best a feeble antigenic effect compared to that of virus alone, or to that of virus and serum given separately.

Virus and immune serum, separately administered. Goldbloom, Brodie and Moffatt (189) tried immunization of monkeys with a 5% suspension of infected monkey brain tissue. The rationale of using brain tissue is not made entirely clear. It was given intracutaneously to 5 monkeys, each with a different dosage or time schedule. Immune serum (tests of potency not described) was given to each animal, sometimes simultaneously with the virus and sometimes two days later. Some of the monkeys received multiple doses of brain tissue, serum or both, 5 to 20 ml of serum per dose. A sixth monkey received 12 ml of serum

only. All were bled 6 weeks after the last injection. Their sera were tested individually, first in 0.8 ml volume, undiluted, against 0.2 ml of the brain tissue used for immunization. Each mixture was inoculated into one monkey intracerebrally. All remained well, but so did the one control inoculated with brain tissue alone.

The neutralization test was then repeated on the same animals, substituting active cord virus for the brain tissue used in the first test. Sera from two of the immunized monkeys protected. All the other 4 test animals and 1 control were paralyzed. The authors conclude that brain tissue, itself noninfective, is capable of immunizing actively in the presence of immune serum if the latter is administered by a different route.

Brodie and Goldbloom (194), in studies on vaccination of monkeys with live virus, attempted to find a dosage of convalescent serum that would protect against infection by the vaccine without interfering with the development of active immunity. The strain used was "Fl. mixed virus", apparently MV. Its potency was such that "0.01 ml of a 5% suspension produced prostration (in test monkeys by intracerebral inoculation) in 10 to 12 days". Pooled human convalescent serum, proven by tests on monkeys to neutralize the virus, was used. Details of assay, on both virus and serum, were omitted. In these experiments, with exceptions noted below, virus was given intradermally and serum subcutaneously, each at a single injection. The virus inoculation varied from 2 to 12 ml, and the authors comment that much of the material must have infiltrated into the subcutaneous tissues.

Experiment 1: Five monkeys received varying combinations of virus and serum as follows:

MONKEY	DAY	TREATMENT	TOTAL VIRUS DOSE
			grams
5-0	0	2 ml 20% virus	
	6	10 ml 10% virus	1.4
	7	10 ml serum	
5-4	0	6 ml 20% virus	1.2
	2	7 ml serum	
5-7	0	4 ml 20% virus	
	0	6 ml serum	
	6	5 ml 10% virus	1.3
	6	3 ml serum	
5-8	0	5 ml 20% virus	1.0
	0	6 ml serum	
1-18	0	12 ml 10% virus	1.2
	4	5 ml serum	
2-1		None, control	

None of the treated monkeys developed paralysis. A month later sera were taken for neutralization tests. Undiluted serum, 0.95 ml, was mixed with 0.05 ml of 5% virus for inoculation into test monkeys. Serum from the first 4 treated monkeys protected. Serum from monkey 1-18 was considered as partially protecting since the test monkey developed mild symptoms. Serum of the control did not protect.

In Experiments 2, 3, 4 and 5 a series of monkeys received each 1 gram of virus intradermally in 8 ml of water. Serum was given in varying dosage and at varying times in relation to the virus inoculation, in attempts to find the minimal dosage required to protect against paralytic infection from the vaccine. The essential data are combined below:

MONKEY	SERUM	TIME, IN RELATION TO VIRUS INFECTION
	ml	
1	None, control	
2	2.5	same time
3	5.0	same time
4	6.0	same time
5	6.0	same time
6	4.0	4 days earlier
7	6.0	3 days earlier
8	6.0	3 days earlier
9	5.0	3 days later
10	6.0	3 days later
11	6.0	3 days later

Monkeys 1, 2, 3 and 6 became paralyzed, as did one additional animal given 1 gram of virus subcutaneously and 6 ml of serum intradermally, reversing the routes of administration used with all the others. The remaining treated monkevs showed no signs of infection.

Finally, 3 more monkeys were treated by the combination of 1 gram of virus intradermally and 6 ml of serum subcutaneously. Serum was given to one of them 3 days before the virus, to one on the same day, and to the third 3 days after. All remained well. Neutralization tests on sera obtained one month after vaccination showed protection by two and "partial protection" bv the third. Three control monkeys used in the tests were paralyzed.

The authors conclude that "for each gram of active cord virus, given intracutaneously as an emulsion, 6.0 ml of human convalescent serum, injected subcutaneously, was required to protect an animal from paralysis", and that some degree of immunity followed its use. Immunity, unpreceded by symptoms of the disease, was obtained when the serum was given at the time of the virus administration, or 3 days prior to it, or three days subsequently.

In a later series of experiments, Brodie (190) found that unprotected monkeys, inoculated intracutaneously with 1.0 g or more of virus, did not develop paralytic infection as regularly as in the series just cited; also that the antibody response, to live virus so administered, was somewhat lower in animals passively protected than in those treated with virus alone.

First, a group of monkeys was immunized intracutaneously with virus of the same strain and potency as that previously used. Human convalescent serum (of a different pool) was given subcutaneously. Serum, drawn 2 to 3 months after vaccination, was tested first against 0.02 ml of 5% virus (2 MCP doses) and then against 0.05 ml. Treatment and results were as follows (the table below omits 3 animals given serum-virus mixtures and one that died of irrelevant cause).

MONKEY	DAY	TREATMENT	NEUTRALIZATION TEST 1 (2 MCP)	2 (5 MCP)
5	0 3	9.0 ml serum 1.5 g virus	−	−
6	0 6	7.5 ml serum 1.2 g virus	+	−
7	0 1	9.0 ml serum 1.5 g virus	+	±
8	0 3	1.5 g virus 9.0 ml serum	+	−
9	0 3	1.3 g virus 7.5 ml serum	+	+
Control	0	2.0 g normal monkey cord	− (1 MCP)	
Control	0	9.0 ml serum	− (1 MCP)	

Since the antibody response was somewhat less than that found in the earlier studies, Brodie suspected that the new lot of serum might be over-neutralizing the virus. The new serum pool and the original were titered. The titration data are here included since they illustrate the fine gradations of potency inferred from tests on single animals.

The virus was first titered, using 2-fold dilutions and 2 monkeys per dilution. The two serum pools were then titered, using diminishing volumes of serum (one monkey for each) against a constant dosage (0.2 ml of 5%) of virus. Results were:

VOLUME OF SERUM	NEUTRALIZATION New Serum Pool	Original
ml		
0.2	+	+
0.1	+	+
0.05	+	+
0.04	+	+
0.033	−	+
0.03		+
0.025		

From this test it appeared that the poor antibody response could hardly be attributed to over-neutralization by the second serum pool in comparison with the first. Tests were accordingly made of the intracutaneous infectivity of several lots of virus of the same strain. Six monkeys each received 1 gram, the dosage previously found to produce paralytic infection regularly. All remained well. One monkey received 1.5 g and remained well; one became paralyzed from a dose of 2 grams. Brodie attributed the weak antibody response, obtained with these lots of virus, to their low infectivity. By intracerebral titer, however, these were found to be as potent as the virus used in the experiments previously reported.

Subsequent work has confirmed Brodie's conclusions regarding the possibility of active immunization in animals passively protected. It now seems obvious, however, that the techniques he used were not adequate for the quantitative measurements he attempted to make.

Bodian (6) studied the effect of gamma globulin on the active antibody response to virus administered at the same time. In preliminary experiments, rhesus monkeys received each a single intramuscular injection of 2 ml of gamma globulin per kg of body weight, with no virus. Serum samples were taken at intervals thereafter and titered in mice against 32 PD_{50} of Lansing virus. One day after injection the titer was 1:25. It declined to a barely detectable level at 3 weeks.

Two groups of 5 monkeys each were then vaccinated with Lansing virus. Each animal received 4 doses intramuscularly, 1 ml of 10% virus suspension plus adjuvants at each dose, distributed over 9 weeks. One group (A) was otherwise untreated. In the other group (B) each dose of virus was accompanied by injection of 2 ml of gamma globulin per kg intramuscularly on the opposite side. In Group A, one monkey developed paralytic infection from the vaccine and another was lost from irrelevant cause. Sera of all the remaining monkeys were tested for neutralization against 32 PD_{50} of Lansing virus in mice. Individual tests on all animals before vaccination were negative. At 5 weeks, pooled sera from Groups A and B showed the same titer ($10^{-3.4}$). At 9 weeks, individual sera from all animals in both groups neutralized in 1:100 dilution (not titered further). At 9 weeks, all were challenged intracerebrally with 1,000 PD_{50} of Lansing virus, and all resisted. At 13 weeks, each monkey of both groups received a single booster injection of virus without gamma globulin. At 15 weeks, serum titers in the two groups were again approximately equal, individual sera ranging from $10^{-3.2}$ to $10^{-4.5}$.

Two later reports by Bodian (3, 195) deal with passive immunization and oral challenge of chimpanzees. Effects on the active antibody response were studied, also the occurrence of paralytic and nonparalytic infections and of viremia. Eighteen animals each received 5 ml of a 20% suspension of Wallingford virus (Type 2) by mouth. Six of them received, just before the virus feeding, an intramuscular injection of 4 ml per kg of hyperimmune monkey serum, titering $10^{-3.8}$ against homotypic virus. Three similarly received a smaller dose of the immune serum, 0.1 ml per kg. Nine controls received virus alone, and one

additional control received 4.0 ml per kg of immune serum without virus. Subsequently it was found that two of the controls had substantial levels of Type 2 antibody before they were fed virus. Excepting these two, all the chimpanzees inoculated with virus developed alimentary-tract infection, as judged by virus recovery from fecal samples. Titrations of virus concentration in the feces failed to show any significant difference between passively immunized and control animals in respect to quantity of virus excreted.

Viremia was demonstrated in 5 of the 7 control animals that showed alimentary-tract infection. Two of these developed paralytic poliomyelitis, 2 and 5 days respectively, after the finding of virus in the blood. Two others were found on histologic examination to have suffered inapparent infection of the CNS. One of the infected controls showed viremia but no evidence of CNS invasion; two showed neither. None of the passively immunized animals developed symptoms of poliomyelitis, and virus was not recovered from the blood of any.

The larger dose of immune serum was calculated to produce a circulating passive antibody titer of about $10^{-2.7}$ on the following day. Titers actually found at this interval in 3 animals were $10^{-2.5}$ to $10^{-2.7}$. In the control given serum alone the antibody level fell thereafter at a rate corresponding to a straight line on a semilogarithmic scale, with an indicated half-life of about 12 days. In the virus-inoculated animals (expecting the two initially immune) a similar decline took place during the first few days. In the untreated controls the antibody level rose rapidly after the viremia stage, reaching levels of about 1/1,000 in 2 to 3 weeks with a slow decline to about 1/300 from the end of the first to the end of the third month. In the treated animals (with two exceptions) a slower rise took place, beginning at about 2 weeks and continuing to a final level about equal at 3 months to that observed in the controls.

Two of the 6 chimpanzees treated with the larger dose of serum showed an uninterrupted decline of antibody level, similar to that noted in the control given serum without virus. Since both of these were intestinal carriers of virus, it appears that alimentary infection may occur without evoking active response in the form of circulating antibody. The observation suggests also that high levels of passive antibody tend to inhibit the active response (*cf* Hammon, Cheever and Sather, p. 420). No such effect was observed in the chimpanzees treated with smaller doses of serum, and in these the passive antibody level was apparently adequate to prevent viremia and CNS involvement.

It seems clear that passive immunization can prevent paralytic poliomyelitis without materially interfering with the acquirement of inapparent, intestinal infection or with the development of active immunity.

C. *Human Trials*

The first trial found on record, of serum prophylaxis against poliomyelitis in humans, was made by Davide (196). The occasion was an outbreak in 1925 in northern Sweden, involving an area of villages and farms. The time course of the outbreak and the time schedule of inoculations are not fully reported.

In the spring there had been an outbreak of "grippe", with catarrhal symptoms, vomiting, leg pains and stiffness of the neck. Later scattered paralytic cases began to appear. These latter had no obvious connection of one to another. But by questioning families regarding recent nonparalytic disease Davide found evidence of radial spread from one or two early foci. He began prophylactic injections "in the fall" in a small area including 3 villages where the disease was just beginning to appear. These were located in the northernmost part of the region involved. The outbreak ended completely "1½ or 2 months" after inoculations were started.

Convalescent serum was used, 5 ml intramuscularly to each subject. The serum was obtained locally and in Stockholm. It came from cases of the same year, bled at least 10 days after the disappearance of fever. Subjects inoculated were all under 25 years of age. Davide wished to divide family groups into treated subjects and controls but was not able in all instances to get the consent of parents to this procedure. He estimates the total population under 25, in the villages concerned, at 157. Of these, 73 were inoculated, 84 not. Subsequently, 1 case developed in the inoculated group, 14 in the group not inoculated. This total includes both paralytic and nonparalytic cases.

Stokes, Wolman, Carpenter and Margolis (197), reporting a prophylactic trial in Philadelphia, give a brief account of two others. Personal communications from Dr. W. H. Park and Dr. R. G. Vogel are cited as the source of data on these earlier experiences. The first refers to an epidemic of 1931 in New York City, during which 200 children, exposed by contact with known cases, each received 25 to 30 ml of convalescent serum or 50 to 60 ml of whole blood from a parent. Two cases of poliomyelitis developed in the group, each with onset 5 days after the injection. No further details are given.

The second refers to large-scale immunizations attempted at Bradford, Pennsylvania, during the epidemic of 1932. All the pertinent information given is as follows:

> ". . . a large free clinic was inaugurated by the Board of Health on September 19, which was continued until October 1. Meanwhile family physicians throughout the town had started to inject parent's blood before the free clinic was opened, and they continued to give injections until October 22. The first case in this epidemic was not reported until August 25, and the last case was reported on October 17. About 1200 children were given injections at the free clinic, and 100 more were given injections by local physicians; the children were between the ages of 6 months and 16 years. Approximately 2500 children in this age group did not receive an injection, thus forming in a certain sense a control group. From 15 to 20 ml of whole blood was injected into each child. Among the 1300 children who received injections the disease developed in only 1 child, without paralysis or weakness, three days after the injection, whereas 32 cases developed among those (2500) in the same age group who did not receive injections. However, many cases had been reported before all the injections were given, and in the latter part of the epidemic the disease was extremely mild in comparison with the severity during the earlier part."

Passive immunization of humans was tried on a fairly large scale during the epidemic of 1932 in Philadelphia. This work was reported briefly by Henry and

Johnson (198) and later in more detail by Stokes and associates (197). The first paper cited states that 2,255 children received some form of prophylactic treatment in 14 hospitals but adds that serum prophylaxis was also, on the advice of the city health officials, being used in private practice. The number of subjects immunized outside the hospitals is unknown. The second paper deals only with subjects immunized at 4 hospitals. The numbers of subjects, age range, and nature of treatment may be summarized as follows:

1. Children's Hospital, 1,341, aged 1 to 10. Each received 60 ml of adult whole blood from an individual donor, ordinarily a parent. The blood was citrated and given intramuscularly.
2. Jewish Hospital, 266, aged 1 to 15. Each received 20 to 40 ml of adult serum.
3. Mount Sinai Hospital, 229, aged 1 to 15. Each received 30 ml of adult blood.
4. St. Luke's Hospital, 343, aged 1 to 15. Each received 20 ml of convalescent serum.

These four groups total 2,179. No further statement is made regarding the sources of the blood or serum used at the last 3 hospitals named, the routes by which they were injected, nor the dates on which the injections were made.

Since the most detailed information is given regarding the subjects treated at the Children's Hospital, this group may first be considered separately. The whole blood used came from individual donors, and its antibody content undoubtedly was variable. But neutralization tests on the donors' blood were made only in instances where the recipient subsequently developed poliomyelitis, and the samples then tested were obtained 8 to 11 months after the original prophylactic injection.

The first injections were given at the Children's Hospital on August 20, and the authors show a tabulation of the numbers of subjects treated daily over a period extending to October 3. Unfortunately the time course of the epidemic is not outlined with the same precision. Henry and Johnson present a chart showing the monthly incidence and state that the epidemic peak was reached during the final week of August. Stokes and his co-authors state that almost one-fourth of all the cases that occurred in the epidemic had been officially reported before August 20 when their first injections were given. Their tabulation shows that half of their 1, 341 subjects had received injections by September 5. It is clear, therefore, that the majority of their subjects received prophylactic treatment during the receding phase of the epidemic.

In the entire city, 521 cases in children below 11 years of age were reported after August 20. The estimated population, in this age group, was 289,455. The attack rate in this age group, for the city at large, was thus about 1 in 555, or 180 per 100,000. Among the 1,341 children given prophylactic treatment at the Children's Hospital, 6 cases subsequently developed. The attack rate in this immunized group was thus about 1 in 224, more than twice the rate for the city at large.

Actually such a comparison is hardly valid. Immunization was purely on a voluntary basis. It seems quite probable that the children brought for prophy-

lactic treatment came largely from neighborhoods where the disease was most prevalent, and that the group immunized was therefore not a representative sample of the city as a whole. Stokes *et al.* note that 620 of the treated subjects came from certain designated census tracts, but the incidence of the disease among untreated subjects in these same areas is not given.

Obviously, these data give no evidence that the risk of incurring clinical poliomyelitis was reduced by the prophylactic treatment used. It must be realized, also, that the attack rate calculated for the 1 to 10 age group at large applies to the entire period after August 20, while the attack rate for the treated group covers, for the average subject, a shorter and later period of time.

In all, 15 cases of poliomyelitis developed in subjects who had received prophylactic treatment at the Children's Hospital or elsewhere. Stokes and his coauthors consider that 4 of these occurred under such circumstances that a protective action could not reasonably have been expected. In one the onset of the disease occurred on the day of treatment, in two others in less than 3 days after treatment. The fourth subject, who had received 10 ml of convalescent serum, developed poliomyelitis 23 days later. The authors consider this dose of serum as probably too small to protect over a period of 23 days. If these 4 are disregarded, 11 cases of poliomyelitis developed following prophylactic treatment. The time intervals between injection of serum and onset of symptoms in these were as follows (in days): 5, 6, 6, 7, 8, 9, 10, 10, 10, 15, and 18. The authors note that of the 11 cases only 4 were paralytic, 2 mild. Nine of the cases recovered completely. One had residual paralysis and one slight residual weakness. The authors suggest that the prophylactic treatment may have mitigated the severity of the disease. They point out that for the entire epidemic the mortality rate was 11.5%, the paralytic rate 67%; a follow-up study showed residual paralysis in 41%.

Epidemic illness diagnosed as poliomyelitis occurred in the Los Angeles area in 1934. Serum was administered prophylactically to employees of the Los Angeles County Hospital who requested it, but no attempt at a controlled experiment was made. Available data on the experience with serum are found in the paper of Kessel, Hoyt and Fisk (199) and in the epidemiologic report of the hospital outbreak by Gilliam (200).

Compared to the general behavior of poliomyelitis outbreaks, this was characterized by (a) relatively high attack rate; (b) low case fatality rate; (c) low paralytic rate; (d) apparent high communicability, and (e) relatively high incidence among adults. Symptoms and spinal fluid findings in a high percentage of cases were atypical. Poliomyelitis virus was recovered from 8 cases in the Los Angeles area during the summer, but not from any among the hospital employees (200). That the hospital outbreak was actually poliomyelitis seems rather doubtful.

The hospital outbreak began early in May and ended in November. The report of Kessel, Hoyt and Fisk covers only the period to September 1. There is no indication how the prophylactic injections were distributed in time. Some subjects received convalescent serum, others pooled normal serum. The majority

received each a single injection of 20 to 40 ml, but some had repeated injections with total dosage varying up to 100 ml. Apparently the route of injection was also varied since a description of the handling of serum refers to some flasks labelled "for intravenous use" and others "for intramuscular use only".

Kessel, Hoyt and Fisk list 115 cases among 3,986 hospital employees (2.9% attack rate). In the communicable disease unit there were 57 cases among 489 employees (11.9%). Employees who received serum numbered 228 in the C.D. unit and 892 in the entire group. The attack rate was considerably higher among those receiving prophylactic serum than among those who did not (14.0 compared to 9.7% in the C.D. unit, 6.8 compared to 1.7% among all employees). The authors suggest that individuals subject to greatest exposure were most likely to apply for prophylactic treatment.

A table on the comparative severity of the disease, in patients who had received serum and those who had not, classifies the cases as showing "no weakness", and "possible paralysis". Only one case of "possible paralysis" is listed among 60 who had received serum, 4 cases among 54 who had not (2% and 8%, respectively). There were no deaths.

Gilliam's report (200) covers the entire period of the outbreak and mentions 198 cases among the hospital employees. Only brief reference is made to the prophylactic use of serum. In two cases the history of prophylactic serum was unknown. Of the remaining 196, 100 had received one or more injections, 96 had received none. Of the 100 who had, 15 received it after the onset of illness and 4 on the day of onset, leaving 81 who had injections one or more days before onset. Total dosage and kind of serum (convalescent or normal) are unspecified. Data are given on the time interval between the last dose of serum and the onset of illness in the 81 cases as follows:

INTERVAL	NUMBER OF PATIENTS
1 to 7 days	20
8 to 14 days	15
15 to 21 days	7
22 days and over	35
Unknown	4
	—
	81

Francis and his associates (201) conducted a small-scale trial of gamma globulin in 1945. During an outbreak of poliomyelitis in Freeport, Illinois, three cases had occurred in a local institution for children. The remaining inmates all received injections on one day, August 26. The total number of subjects was 232, of whom 93 were under six years of age and only 10 over sixteen. Each class and age group was equally divided, half receiving gamma globulin and half saline, intramuscularly. Dosage was 10 ml for each child under six; 20 ml for those over six.

In the entire group, four cases of poliomyelitis occurred after the injections

were given. Two nonparalytic cases developed with onset on the day of injection, another four days later. One paralytic case, with onset three days after injection, and one of the nonparalytic cases occurred in the group that received gamma globulin, two nonparalytic cases among the controls. In addition, six cases of mild illness developed in each group. These were not diagnosed as poliomyelitis, and stool tests failed to show virus. Stool tests from the four cases diagnosed as poliomyelitis were all positive. In addition, virus was recovered from seven of eleven pooled fecal samples, each representing three subjects housed in dormitories where the earlier cases had occurred. Moreover, virus was recovered from the stools of two boys two weeks after they had received 20 ml of gamma globulin.

Bloxsom (202) published a brief report on the prophylactic use of gamma globulin during an epidemic of 1948 in Houston. The data were collected, by questionnaire, from 19 pediatricians.

The preparation chiefly used was obtained from a commercial laboratory. It contained 160 mg of gamma globulin per ml. Its neutralizing potency against poliomyelitis virus had not been determined directly. The usual dose was 2 ml. The age range of persons treated is not stated.

The number of treated subjects and the outcome may be summarized as follows:

Total of Subjects Treated	
Siblings of patients	35
Nonsiblings	806
Subsequent Cases of Poliomyelitis in Treated Persons	
Siblings of patients	3
Nonsiblings	1

Onset, in the 4 cases that occurred following prophylactic treatment, was on the first, second, third and forty-second days, respectively, after the injection. There were reported 8 cases of poliomyelitis in children who were siblings of earlier cases in the same epidemic and who had not received gamma globulin. Comparison between treated and untreated siblings cannot be made on any statistical basis since the attack rate in the untreated group cannot be calculated (total number of untreated siblings unknown). The time when the injections were given, in relation to the time course of the epidemic, is not stated.

Hammon and associates (203, 204, 205, 206) conducted the first large-scale, controlled test of passive immunization against poliomyelitis in man. Trials were carried out successively in 3 communities where outbreaks were in progress. To provide treated and control subjects at equal risk, and to insure objectivity in comparing them, children brought to the clinic for injection were equally divided by a random method of selection into two groups. Half of the subjects received gamma globulin; half received gelatin, but no one knew which agent had been given to any subject until all had been observed for 13 weeks and a 60 day follow-up study had been completed on all cases of paralytic poliomyelitis occurring in the injected subjects. Solutions of gamma globulin and gelatin, indistinguishable in appearance, were packaged in containers of identical type.

These were packed in boxes of 50 units, 25 of each, arranged in random fashion. Each unit was marked with a serial code number identifying its contents, but the key sheets were given to the investigators only after all data on individual cases had been collected.

Injections were given intramuscularly, into the right buttock. Dosages of gamma globulin (or gelatin) were 4, 7, or 11 ml according to body weight. The smallest dose was given to subjects up to 34 lb, 7 ml to those weighing 34 to 61 lb, and 11 ml to those of 62 lb or over. The dosage thus ranged from 0.11 to 0.20 ml per lb, averaging 0.14 ml.

The total number of injected subjects, including controls, was 54,772. Results were presented in terms of (a) incidence of paralytic poliomyelitis by weeks, and (b) comparative severity of the disease at final examination in the two groups. Combining data from the 3 community tests, the incidence of paralytic cases was as follows:

WEEK AFTER INOCULATION	GAMMA GLOBULIN GROUP	GELATIN GROUP	TOTAL
1	12	16	28
2	3	24	27
3	3 } 7	8 } 39	11
4	1	4	5
5	0	3	3
6	3	4	7
7	3	6	9
8	1	3	4
9	4	1	5
10	0	1	1
11	1	0	1
12	0	2	2
13	0	1	1
	31	73	104

These figures appear to show a high though not complete protection by the gamma globulin over a 4 week period extending from the end of the first to the end of the fifth week following its administration. During the first week, also through the sixth to eighth, it is doubtful if the difference between treated and control groups is great enough to be statistically significant, and there is no evidence of protection beyond the eighth week.

Some evidence was obtained, however, that gamma globulin mitigated the severity of the disease in those cases that appeared within the first week after it was given. Of the 12 cases that developed during that period in the gamma globulin group, 6 had recovered completely when examined at 60 days. Of the 16 cases that developed during the same time in the gelatin group only one had recovered completely. The authors present further evidence on the same point by comparing (a) the muscle grade in the most severely affected muscles, and (b) the total amount of muscular involvement in subjects of each group.

The authors consider the possibility that injections of gelatin may have had the effect of localizing or even provoking paralysis, and therefore may have rendered invalid the use of the gelatin-injected group as controls. Data are presented to show that right hip involvement, in paralytic cases among the gelatin group, was no more frequent than in cases that developed among uninoculated subjects during the same periods of time. Other comparisons are made of the uninoculated group with the two groups that respectively received gelatin and gamma globulin. The detailed analysis can hardly be reviewed here.

V. CONCLUSIONS

1. Studies on immunization against poliomyelitis, for more than three decades, were largely vitiated through sources of error that were either unrecognized or could not be excluded by any means available at the time. The most serious of these were:

a. Lack of quantitative methods for assaying virus and antiserum.

b. Ignorance of immunologic diversity among poliomyelitis viruses.

c. Use of inappropriate animal species and routes of inoculation for testing the resistance to infection conferred by immunizing procedures.

2. Recent advances in knowledge and techniques have made it possible to demonstrate in immunized animals, under conditions corresponding closely to those of natural infection in man, a high degree of protection against paralytic poliomyelitis. The protection afforded by passive immunization is brief; with active immunization it lasts for a still undetermined but substantial period of time.

3. Virus, antisera and vaccines can now be assayed, and the dosages given to test animals can thereby be controlled.

4. A critical trial of passive immunization in man, by means of the gamma globulin fraction prepared from immune blood, has demonstrated that it confers significant protection for about five weeks.

5. Active immunization has been shown by direct experiment on man to result in appearance of circulating antibody at levels exceeding the minimum required for protection (as judged by the gamma globulin experiment). Further work is required to establish by direct test in man (a) that active immunization effectively protects against paralytic poliomyelitis; (b) how long the protection and the antibody response persist; and (c) that active immunization can be accomplished without risk of harmful effects.

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