Active Immunization Against Poliomyelitis*

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THE problem that confronts the medical world concerning poliomyelitis is, What can be done for its prevention, or treatment in the acute stage, in the hope of warding off paralysis? In the treatment of the acute stage, we are no further advanced than when Heine described the disease as a clinical entity in 1840. In 1910, when Netter *et al.*[1] first announced that convalescent serum neutralized the virus of poliomyelitis, great hope was held that it had curative powers. Many reports have favored its use. However, as I have pointed out,[2a] several significant controlled studies have failed to uphold its value. Moreover, our own experimental studies on monkeys showed that large amounts of convalescent serum or whole blood had no appreciable effect upon the progress of the disease, even if given during the earliest stages. The reason for this is obvious from studies on the pathogenesis of the disease, which show that it is confined to and transmitted along the nerve tracts of the central nervous system[2b] by certain cells by which the virus is absorbed. This renders it highly improbable that the serum reaches the virus.

To prevent the epidemic spread of the disease by means of isolation is difficult, for it apparently spreads mainly through carriers and abortive cases. As judged by the results of Davide[3] and Brebner,[4] passive immunity obtained by the use of convalescent serum offered some hope, but a recent report from California does not seem to justify this. Moreover, the serum is expensive and the immunity, if present, lasts for only a few weeks. Therefore, in view of the present state of knowledge and the difficulty in controlling the disease by other means, a safe and effective active immunization of the susceptible age group offers the most hopeful means of combating it, especially if immunity can be produced rapidly.

Experience with other virus diseases points to 3 possible methods of immunization. The first of these is by the use of an active virus which, through adaptation to one species, is non-infective for another, yet gives immunity to that species. Perhaps the best example of this is vaccinia virus. The second is by a combination of virus and serum, using an incubated mixture of active virus and antiviral substance, *e.g.*, yellow fever vaccine; or giving virus and serum separately, as in the case of cattle plague. The third method involves the use of germicidally inactivated virus, as in the case of dog distemper, foot and mouth disease, cattle and fowl plague, and rabies. Heat killed virus

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has not proved satisfactory for the prevention of virus diseases.

In our experiments, the various methods of active immunization were investigated. *Macacus rhesus* monkeys were injected intracutaneously, in the abdominal wall, with the antigens. Some of the virus infiltrated into the deeper layer of the skin. Unless otherwise stated, a 10 per cent suspension of infective cord tissue was used prepared as previously described.[6, 8] Similar concentration of virus suspension and methods of inoculation were used in the human cases.

Immunity was tested for some 3 to 6 weeks later by inoculating virus intracerebrally into the treated animals, and by testing for antibody by the neutralization test, which consists of mixing 0.9 c.c. of the serum with varying amounts of virus, incubating for 2 hours at 37° C., and after 8 to 10 hours on ice, injecting intracerebrally into other monkeys. In all instances, the neutralizing power of the serum or the resistance to direct intracerebral injection was estimated in terms of M.C.P. (minimal completely paralyzing) dose, which consists of the smallest amount of virus-containing-tissue causing a complete and rapid paralysis in *Macacus rhesus* monkeys weighing from 2.5 to 4 kilos, within 13 days after intracerebral inoculation of virus.[5]

Since the monkey is the only known experimental animal that is susceptible to the virus of poliomyelitis, one cannot determine whether monkey fixed virus is infectious for man except by direct inoculation, which is attended by risk. In the monkey, active virus produces considerable immunity the degree of which is proportional both to the size [6] and infectivity of the dose.[7] It was found that cord tissue harvested at the height of paralysis gave a better immunity than that obtained later, and a better protection than any other part of the cerebrospinal axis. However, active virus proved to be dangerous, for occasionally animals developed mild non-paralytic and even severe paralytic forms of the disease. In some animals immunity developed in the absence of any tissue reaction as indicated by an absence of symptoms, cerebrospinal fluid, pleocytosis, or temperature changes during the course of immunization.

The specificity of the immunity was demonstrated by the fact that animals which received non-infective cord tissue failed to develop immunity.

The dangers attending the use of active virus in the monkey precluded its use in the human. Work on virus-serum combinations was carried out in conjunction with Dr. Alton Goldbloom, McGill University. The virus-serum combinations were given either as an incubated mixture, or separately, in which case the virus was administered intracutaneously and the serum subcutaneously, either before, with, or after the virus.[8a] Both methods of administration gave immunity, and in the latter it was found that serum preceding virus gave a lower immunity than when administered with or after the virus. Further experimentation [8b] showed that an excess of serum, that is, more than was required to prevent infection, reduced the immunity considerably. Inasmuch as the virus in serial passage changes in potency and since batches of convalescent serum are of different strengths, each must be titrated to arrive at a satisfactory balance. This entails the use of a considerable number of monkeys, rendering this form of immunization impractical.

The results with germicidally treated virus are rather contradictory. Phenolized virus has been reported to be dangerous and unreliable in its efficacy by some,[9a, b, c] whereas Kraus [10] and Erber and Pettit [11] reported favorable results. However, from the data presented, the virus used by the latter did

not appear to have been inactivated. Abramson and Gerber,[12] Römer [13] and Jungeblut and Engle [14] failed to demonstrate immunity with formalized virus.

The results of Abramson and Gerber [12] with heat killed virus and the results of Kraus [10] and Erber and Pettit [11] suggest that the possibilities of vaccination with inactivated virus be reinvestigated. This is further borne out by the results obtained with germicidally treated antigens in other virus diseases.

Throughout this work, the infectivity of the virus was checked by repeated intracerebral and intraperitoneal inoculation of large amounts of the germicidally treated virus. To inactivate virus, various concentrations of the germicide were mixed with the virus and kept either at ice box or incubator temperature. In each instance, double

TABLE I

Concentration of Cord Per cent	M.C.P. Dose Injected Intracerebrally	Temperature Ice Box (Centrigrade)	Days of Contact between Virus and Germicide	Result
10	80	2.5	1.5	Paralysis 19 days
10	3,200 + 16,000 1 perit. retested in 10 days	5–6°	2.0	Slight rise in temperature
10	same	5–6°	3.0	No evidence of poliomyelitis
10	3,200 + 16,000 1 perit. retested for 3 consecutive days	0–4°	3.0	No evidence of poliomyelitis
10	3,200 + 16,000 1 perit. retested in 10 days	5–6°	4.0	No evidence of poliomyelitis
10	same	5–6°	4.0	No evidence of poliomyelitis
10	80	4.5°	3.5	No evidence of poliomyelitis
10	600	1–4°	10.0	No evidence of poliomyelitis
10	1,600	2–4°	11.0	No evidence of poliomyelitis
10	1,600	1–4°	10.0	No evidence of poliomyelitis
10	1,600 + 2,800 1 perit. and in 10 days 1,600 + 6,400 1 perit.	1–4°	10.0	No evidence of poliomyelitis
10	same	1–4°	10.0	No evidence of poliomyelitis
10	1,600 + 9,600 1 perit. retested at 2 day intervals for 6 days	2–4°	10.0	No evidence of poliomyelitis

the required amount of germicide was added to a 20 per cent cord suspension and, during the period of contact, the mixture was agitated frequently.

EFFECTS OF GERMICIDES UPON THE VIRUS OF POLIOMYELITIS

It was planned to treat virus suspension with merthiolate, ether, phenol, and formalin, and keep at ice box temperature. However, both merthiolate and ether were discarded because the virus was quite resistant to their action. Phenol in 2 per cent concentration appeared to inactivate the virus after 10 days.

A mixture of 0.3 per cent formalin and 10 per cent active cord suspension was tested after contact of 1.5 to 11 days at ice box temperature and was found to render the virus non-infective after 3 days. To test for virus in these mixtures, large amounts were repeatedly injected intracerebrally and intraperitoneally into *Macacus rhesus* monkeys. Careful observations of the animals, daily temperature readings, and frequent spinal fluid examinations were carried out, and only when all were negative, was the virus designated non-infective. This is demonstrated in Table I.

ACTIVE IMMUNITY EXPERIMENTS IN MONKEYS

A preliminary experiment showed the formalized virus to be superior to the phenolized, the latter being a cord suspension treated with 2 per cent phenol and kept at ice box temperature for 10 days. The animals received either 1 or 2 doses of the formalized virus, the second being given at 10 to 20 days after the first.

Experiments were then conducted with cord suspension treated with 0.3 per cent formalin for 10 days at ice box temperature, to determine the following points:

1. The best immunizing dose, by injecting animals with 2.5, 5, and 10 c.c. amounts, respectively
2. The comparative value of 1 and 2 doses, using 2.5, 5 and 10 c.c. amounts
3. The relative immunity produced with active and formalized virus

The summary of results is as follows:

1. Of 3 animals that received 2.5 c.c. of formalized virus, only 1 had demonstrable tissue immunity. All had neutralizing substances in their blood sera.
2. Of 12 animals given 5 c.c., 2 failed to show demonstrable tissue immunity, while 2 had a slight degree of immunity. Seven had a tissue immunity equal to or better than that developed by animals receiving a similar amount of active virus. All but 1 of the animals showed neutralizing substances in their sera.
3. Two of 11 animals inoculated with 10 c.c. of formalized virus failed to show a demonstrable tissue immunity. Only two showed resistance to direct intracerebral inoculation of virus comparable with that of the majority of animals which received 5 c.c. of the same material. Humoral immunity was present in the sera of all but 2 animals.

Thus 5 c.c. amounts appeared to give the best results. The use of 2 doses of antigen did not appear to be better than 1.

In the previous experiments 0.3 per cent formalized suspension was used after 10 days' contact with the virus, though it is inactivated after 3 days. In the present experiment, virus that had been in contact with the antiseptic for only 3 days was used. Eight animals were given 5 c.c. of a 10 per cent suspension—4 received a single dose, the others 2 doses 11 days apart.

The results obtained with material formalized for 10 days are given in Table II, and those of virus suspension formalized for 3 days in Table III. There is no obvious difference in the results. Thus, if at ice box temperature formalin is permitted to act for a longer time than necessary to inactivate the virus, it does not reduce its immunizing power.

The degree of tissue and humoral immunity obtained in 20 animals receiving 1 or 2 doses each of 5 c.c. of the

TABLE II

0.3% FORMALIN—10 DAYS—ICE BOX TEMPERATURE

No. Monkey	Course of Immunization	Result of Skin Inoculation	Direct Intracerebral Inoculations				Neutralization Tests			
			Test No. 1		Test No. 2		Test No. 1		Test No. 2	
			M.C.P. Dose	Result	M.C.P. Dose	Result	M.C.P. Dose	Result	M.C.P. Dose	Result
460	5 c.c. of 10% suspension	Erythema	2	No paralysis	4	Paralysis 17 days	6	No paralysis		
405	"	Swelling	2	No paralysis	5	Died 9 days, intercurrent infection	1	No paralysis	2	Paralysis 8 days
589	"	No reaction	2	No paralysis	3	Paralysis 9 days	15	No paralysis	20	Paralysis 9 days
584	5 c.c. of 10% suspension repeated in 10 days	Superficial necrosis	1	Paralysis 11 days			15	No paralysis	25	Paralysis 7 days
590	"	"	1	Mild attack *			15	No paralysis		
588	"	Necrosis	3	No paralysis	4	Paralysis 8 days	20	No paralysis	30	No paralysis
490	"	Induration	3	No paralysis			2	No paralysis	3	Paralysis 27 days
558	5 c.c. of 10% suspension repeated in 14 days	Small necrotic area	1	Paralysis 12 days			5	Mild attack		
580	5 c.c. of 10% and 2.5 c.c. in 14 days	Necrosis	1	Paralysis 16 days			20	No paralysis	30	Mild attack
581	5 c.c. of 10% repeated in 14 days	Slight ulceration	2	No paralysis	3	Paralysis 7 days	10	No paralysis	15	Paralysis 15 days
491	"	Induration	2	Paralysis 8 days			6	No paralysis		
24	"	Induration and slight superficial necrosis	2	No paralysis	3	Mild attack	2	No paralysis	4	Paralysis 6 days

* Rise temperature; cerebrospinal fluid pleocytosis
Average intracerebral resistance 1.7 M.C.P. doses
Average neutralizing power of 0.9 c.c. of serum 13 doses

TABLE III

0.3% FORMALIN AT ICE BOX TEMPERATURE FOR 3 DAYS

No. Monkey	Course of Immunization	Result of Skin Inoculation	Direct Intracerebral Inoculations				Neutralization Tests			
			Test No. 1		Test No. 2		Test No. 1		Test No. 2	
			M.C.P. Dose	Result	M.C.P. Dose	Result	M.C.P. Dose	Result	M.C.P. Dose	Result
657	5 c.c. of 10% formalized suspension, repeated in 11 days	None	1	No paralysis	2	Mild attack	5	No paralysis	10	Paralysis 10 days
678	" "	Superficial necrosis	1	No paralysis	2	Paralysis 11 days	3	No paralysis	5	Paralysis 8 days
668	" "	Skin excoriation	1	Mild attack*			5	No paralysis	10	Paralysis 9 days
677	" repeated in 14 days	Slight necrosis	2	No paralysis	3	No paralysis	20	No paralysis	30	Paralysis 6 days
694	5 c.c. of 10% suspension	None	1	No paralysis	2	Paralysis 9 days	20	No paralysis	30	Paralysis 14 days
676	" "	Superficial necrosis	2	No paralysis	3	No paralysis	3	No paralysis	5	Paralysis 10 days
664	" "	Moderate necrosis	1	No paralysis	2	Paralysis 9 days	15	No paralysis	20	No paralysis
666	" "	Superficial necrosis	2	No paralysis	3	Paralysis 9 days	15	No paralysis	20	Mild attack

* 668 cerebrospinal fluid pleocytosis and rise in temperature
Average intracerebral resistance 1.7 M.C.P. doses
Average neutralizing power of 0.9 c.c. of serum 10 M.C.P. doses

TABLE IV

No. Animals	Previous History	Resistance to Direct Intracerebral Inoculation			Neutralizing Power of Serum		
		Less than 2 M.C.P. Doses	2 M.C.P. Doses	3 M.C.P. Doses or more	Less than 5 M.C.P. Doses	5-20 M.C.P. Doses	More than 20 M.C.P. Doses
20	Formalized virus suspension 1 or 2 doses of 5 c.c. each	10	4	6	6	9	5
3	Active virus suspension, 2 doses of 5 c.c. each	1	2	0	–	–	3
5	Convalescent from severe attack	0	2	3	–	–	4*
5	Convalescent from mild attack	5	0	0	4*	0	0

* Serum of only 4 animals tested

formalized vaccine, was compared with: (a) that developed by animals receiving similar amounts of active virus, and (b) that of convalescent monkeys. The latter group consisted of 5 animals which had definite paralysis, 4 with considerable residual paralysis and 6 others that showed clinical manifestations of the disease, with or without paresis. The results are summarized in Table IV.

It is evident from this table that the degree of tissue resistance developed by animals given formalized virus compares favorably in at least half the animals with that developed by animals given like amounts of active virus. Moreover, in half of the animals, the immunity compared favorably with animals recovered from a severe attack of the disease. Almost all the monkeys had a better immunity than those recovered from mild attacks of poliomyelitis.

On the other hand, the degree of humoral immunity was much lower in animals treated with formalized than with active virus and also lower than that of monkeys recovered from severe attacks of the disease, but decidedly better than that of animals recovered from a mild attack of the disease.

These experiments have demonstrated conclusively that an appreciable immunity can be developed by formalized virus, as indicated by the resistance of the animals to direct intracerebral inoculation of virus and the presence of antiviral substances in the blood stream.[15] Because of the amount of skin irritation, and even necrosis, resulting from the formalized antigen, it was decided to use lower concentrations of formalin at incubator temperature. Ten per cent cord suspensions were treated with 0.1 per cent and 0.2 per cent formalin, respectively, at 37.5° C. The former concentration of antiseptic inactivated the virus in 12 hours and the latter in 6 hours.

Eleven animals received either 1 or 2 doses each of 5 c.c. of 10 per cent suspension inactivated with 0.2 per

TABLE V

Name Initials only	Age	Weight (lb.)	First Inoculation of Vaccine		Second Inoculation of Vaccine		Neutralizing Power Serum *Before* Immunization M.C.P. Doses	Neutralizing Power Serum *After* Immunization M.C.P. Doses
			Date	Amount and Route Inoculation	Date	Amount and Route Inoculation		
B	1	21	7/18/34	2 Intracutaneously 3 Subcutaneously			20	40 to 80+
C	2	23	7/18/34	3 Intracutaneously 2 Subcutaneously			1 to 4	60 to 80+
W	3	40	7/18/34	1/2 Intracutaneously 4 Subcutaneously			15	120+
C.G.	4	35	7/18/34	1.5 Intracutaneously 3.5 Subcutaneously			40-60	120+
E.S.	4	30	7/20/34	2.5 Intracutaneously 4 Subcutaneously			3 to 5	120+
A	2	31	7/20/34	2.5 Intracutaneously 4 Subcutaneously	7/31/34	1.5 Intracutaneously 3.5 Subcutaneously	20	120+
O	2	27	7/18/34	3 Intracutaneously 2 Subcutaneously	7/31/34	2 Intracutaneously 3 Subcutaneously	less 5	120+
H	3.5	33	7/18/34	1/2 Intracutaneously 4/5 Subcutaneously	7/31/34	1 Intracutaneously 4 Subcutaneously	5 to 15	80 to 120+
O'D.	3.5	30	7/18/34	1/2 Intracutaneously 3.5 Subcutaneously	7/31/34	5 Subcutaneously	10 to 15	120+
T.G.	4	37	7/18/34	5 Subcutaneously	7/31/34	2 Intracutaneously 3 Subcutaneously	60	120+
D.	5	40	7/18/34	1.5 Intracutaneously 3.5 Subcutaneously	7/31/34	2 Intracutaneously 3 Subcutaneously	3 to 5	40+
O.S.	6	43	7/18/34	2.5 Intracutaneously 2.2 Subcutaneously	7/31/34	1.5 Intracutaneously 4 Subcutaneously	10 to 15	80 to 120+

cent formalin. Five received that which had been kept at incubator temperature for 6 hours, the minimum time required to inactivate the virus, the other 6, virus suspension formalized for 24 hours. The animals receiving the formalized cord suspension, treated just long enough to inactivate the virus, developed an immunity quite comparable with that developed by those receiving virus suspension, inactivated at ice box temperature. However, the animals that received the over-treated virus (0–2 per cent formalin for 24 hours) developed less immunity.

Another lot of 6 animals received 2 doses each of 5 c.c. of virus suspension treated with 0.1 per cent formalin for 24 and 72 hours respectively. Five of the 6 resisted direct intracerebral inoculation of 1 or more infective doses of virus, and all showed some humoral immunity. There was no appreciable difference in the degree of immunity produced by 24 or 72 hour treated virus suspension. In both, the virus was over-treated and the degree of immunity was slightly lower than usual.

Although the number of animals is small, this work suggests that the virus should be in contact with the antiseptic for just the amount of time necessary to inactivate it in the incubator. Inactivated at ice box temperature, an additional week of contact between virus and germicide, does not diminish its antigenic power. Whether virus emulsion inactivated at ice box temperature could be stored for long periods, or whether when inactivated at incubator temperature, it can then be stored on ice, must be determined.

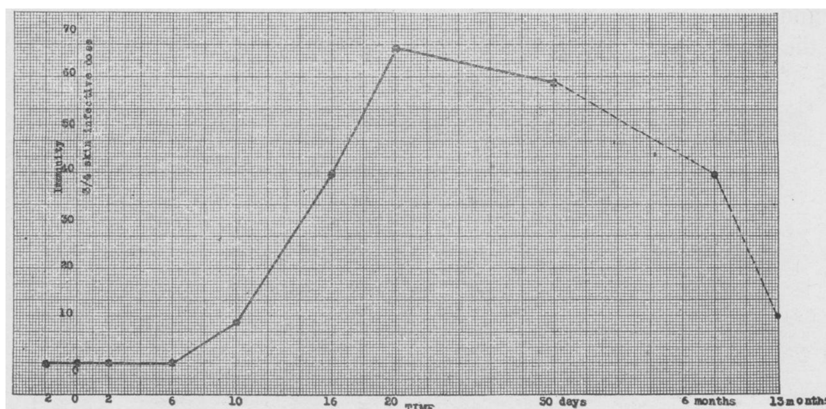
The 0.1 per cent formalized suspension gave but little skin irritation.

In the course of this work certain differences in the immunological response of animals to active and formalized virus were found:

1. The degree of immunity in the case of active virus was proportional to the size of the immunizing dose, the optimum response being obtained with 10 c.c. amounts; while with formalized virus, this relation was not definite, for 5 c.c. seemed as good if not better than 10 c.c. amounts.

2. With active virus, the degree of humoral immunity was decidedly higher than that of tissue immunity, as demonstrated by a comparison between the number of infective doses of virus neutralized by the animal's serum and the number the animal resisted by intracerebral inoculation. Therefore,[8a] humoral immunity is more easily detected than is tissue immunity. With the use of inactivated virus, although the degree of tissue immunity obtained with 5 c.c. amounts often equalled that produced by a like amount of active virus, the neutralizing power of the serum was decidedly lower. Occasionally, in animals immunized with formalized virus, the tissue immunity was greater than that of the serum. In the case of active virus, there was usually

ANTIBODY CURVE 1



a correlation between the humoral and tissue immunity, not evident in the case of the formalized antigen. Normal cord tissue fails to induce the production of neutralizing substances for the virus of poliomyelitis. This tends to rule out the possibility that the higher neutralizing power produced with active virus may be due in part to a nonspecific substance elaborated in response to the injection of normal cord, and which is rendered inert by formalin.

3. Two doses of active virus, when properly spaced, gave decidedly better immunity than a single dose, but, using formalized antigen, 2 doses did not appear to be superior to 1 dose.

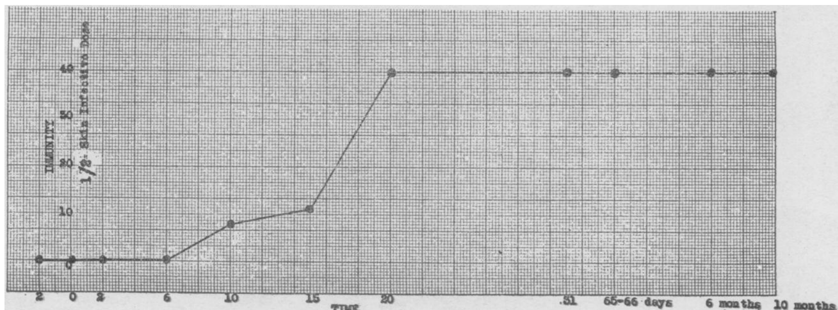
The next step was to determine the first appearance and then the subsequent rate of development of immunity. Inasmuch as the humoral immunity is decidedly higher than tissue immunity in animals receiving active virus, and since they correlate, it was decided to carry this out by following antibody curves in animals receiving active virus. After determining that the blood serum of monkey No. 380 had no neutralizing power for the virus of poliomyelitis, it was given intracutaneously approximately ¾ of a skin infective dose. Serum was collected after 2, 6, 10½, 16, 20, 30 days, 6 and 13 months, respectively, and the neutralizing power tested. The results are shown in Graph 1.

Following a period of at least 6 days, during which no antibody was demonstrable, it became evident between the 6th and 10th days, reaching its height by the 20th day, after which the neutralizing power of the serum diminished so that after 6 months it was approximately ¾ and after 13 months 1/6 of the maximum titre. At the end of 2 years immunity was still demonstrable. To confirm these findings, a second animal, whose serum showed no antibody, received approximately ½ a skin infective dose. The results, given in Graph 2, confirm those of the first animal in showing a lag of about 6 days, with demonstrable antibody between 6 and 10 days, reaching its height about the 20th day. This animal maintained its full immunity for at least 10 months.

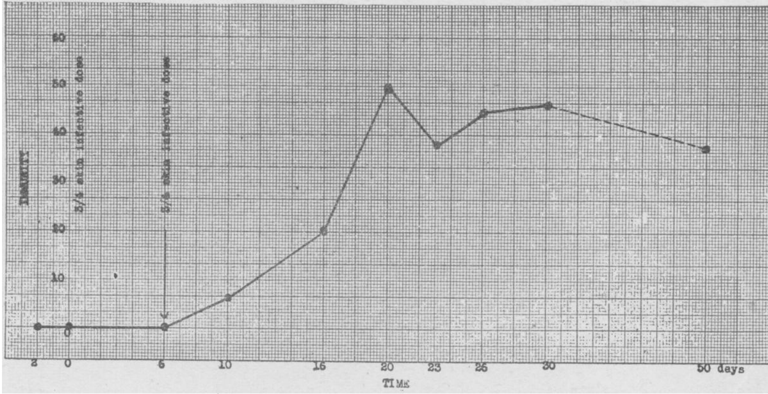
To determine the optimum time for giving the second dose, each of 4 animals, whose serums had no neutralizing power for the virus, received approximately ¾ of a skin infective dose of virus. Each monkey received a second inoculation given 6, 10, 14, or 20 days later. (As in the preceding experiments, the animals were bled at various intervals during the course of immunization and the serums were tested for virus-neutralizing substances.) The results, given in Graphs 3, 4, 5, and 6, show that when a second inoculation is given during the refractory period following the first, it does not produce much additional immunity, but given during the rise or height of antibody response to the first, considerable additional protection follows, especially if given during the rise.

Thus it seems that antibody develops

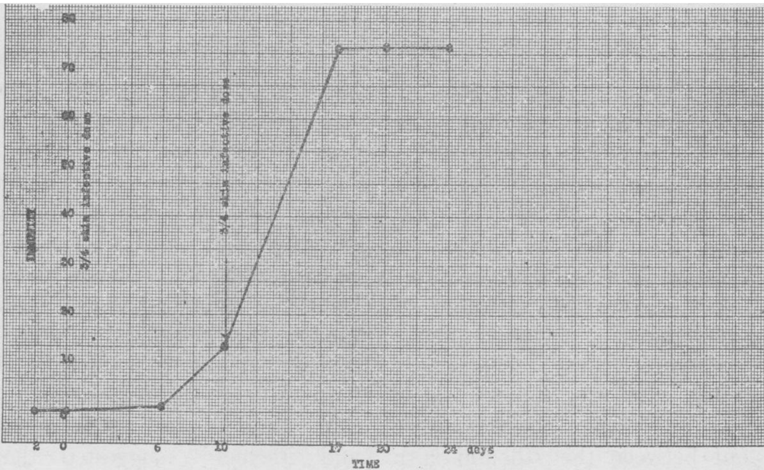
ANTIBODY CURVE 2



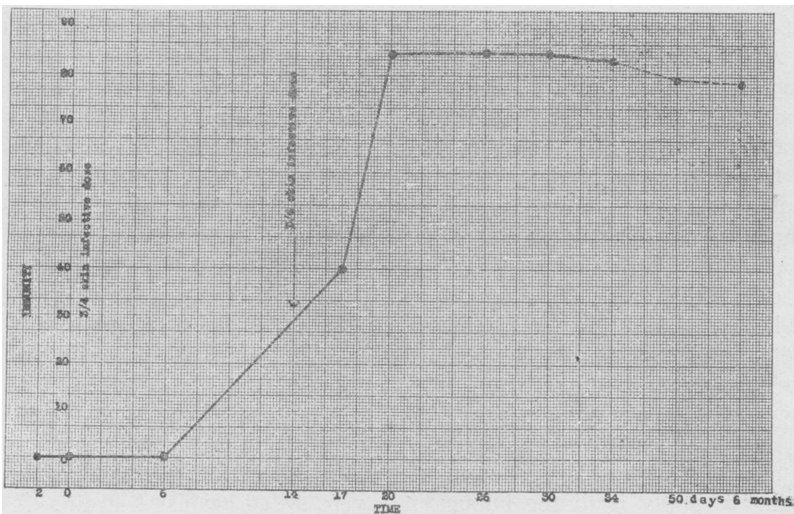
ANTIBODY CURVE 3



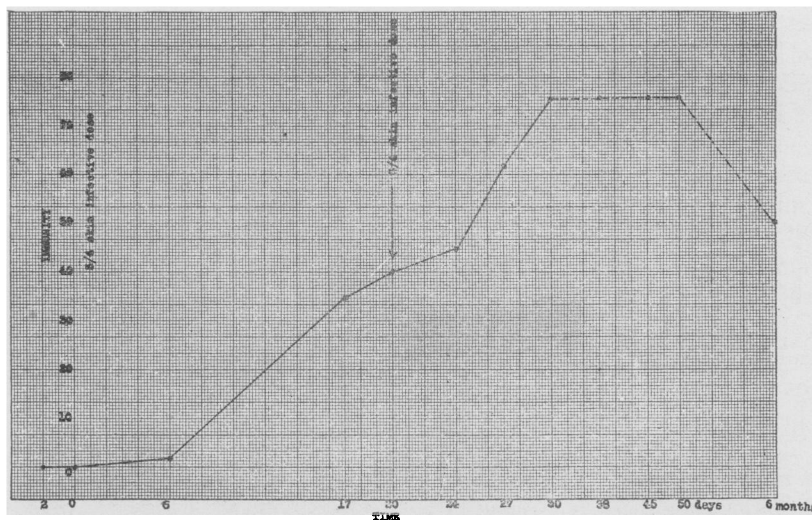
ANTIBODY CURVE 4



ANTIBODY CURVE 5



ANTIBODY CURVE 6



on the 6th day after inoculation, or soon after, and reaches its height in approximately 20 days. The best interval for the 2 inoculations is between 10 and 14 days. At present the only method of determining the success or failure of vaccination is the direct intracerebral test or the demonstration of antiviral substances in the animal's serum. Therefore, the erythrocytic sedimentation rate was followed in a series of 18 monkeys. The tests were carried out between the 7th and 20th days after the administration of antigen, the period during which immunity develops. It was found that of 15 animals which developed resistance to intracerebral inoculation of virus, 13 showed an increased red cell sedimentation rate. None of the 3 animals that failed to develop such immunity showed any change in the sedimentation rate. Inasmuch as the increase in rate might develop at any time between the 7th and 20th days, too many determinations would be required for practical purposes.

The animal experiments had shown that virus suspension inactivated with 0.1 per cent formalin was not only antigenic but also produced no harmful systemic or local reaction. Before attempting to apply it to the human, it was necessary to determine whether the virus fixed for monkeys protected against recently isolated human strains. The literature upon this point is inconclusive. Burnet and Macnamara [16] suggested that the strain isolated from a case in Australia differed antigenically from the animal passage strain they used, while Smith and McKie [17] maintained that only differences in the virulence of the strains existed. On the other hand Weyer,[18] Paul and Trask,[19] and Flexner [20] suggested that there were immunological differences between recently isolated and monkey passage strains. However, Flexner [20] found that although differences existed, the passage virus protected against the recently isolated strains.

Therefore, animals actively immunized and convalescent from attacks with the Fl. monkey passage virus were inoculated with 2 recently isolated strains, 1 of which had undergone 3 and 4 passages through monkeys, and the other 1 passage. The tests were carried out quantitatively in terms of M.C.P. doses of virus suspension.

The sera of 2 animals actively immunized and of 1 monkey convalescent after infection with the passage strain were tested against 1 strain. A definite

quantitative difference was found between this strain and the passage strain, for the serum neutralized approximately only 1/10 as many infective doses of the recent passage virus as of the fixed virus. However, the convalescent animal showed no effects after the intracerebral injection of 5 M.C.P. doses of recent virus. One of 3 animals immunized with the Fl. virus and 3 convalescent animals showed a resistance to direct intracerebral inoculation of multiple infective doses of the second recently isolated strain. These findings are in keeping with those of Flexner [20] and indicate that the monkey passage virus immunizes against recently isolated strains, but that there is a quantitative difference. This appears to be more manifest in the humoral than in the tissue immunity.

It was then decided to test the vaccine on human beings. Before doing so on children, it was deemed advisable to try it upon ourselves, not that we had misgivings about the possibilities of infection, but to determine whether it would produce any disagreeable local or general reactions. Six volunteers from the Bureau of Laboratories were given 5 c.c. doses of 10 per cent virus suspension inactivated with 0.1 per cent formalin for 16 to 48 hours. Three were given 1 dose, 2, 2 doses, and 1, 3 doses. The second inoculation was given 11 days after the first, and the third, 8 days after the second. Those given a single dose were inoculated subcutaneously, while those given more than 1 inoculation received 1 to 2½ c.c. intracutaneously, the remainder subcutaneously. At the time of inoculation there was some soreness lasting but a few minutes and probably due to the formalin. Three of those injected had some induration for a few days, not painful or uncomfortable. No systemic or untoward reaction developed.

The blood sera of all 6 obtained before immunization were tested for antibody and the amount was determined by careful titration. Tests are now in progress to determine whether blood sera obtained between 3 and 4 weeks after immunization showed increased antibody. A preliminary determination on the sera of the 3 receiving more than 1 dose of vaccine suggests an increase. This must be rechecked and the amount of additional antibody must be calculated. The results on the other 3 have still to be obtained and when complete data are on hand, a comparison between the efficacy of 1, 2, and 3 doses will be available.

It was now evident that the vaccine could be administered with perfect safety and so it was given to 12 children, aged 1 to 6 years. Prior to the vaccination, the children were bled in order to determine the amount of antiviral substance in the serum of each.

The virus suspension used for preparation of the vaccine was cultured aerobically and anaerobically before it was treated with 0.1 per cent formalin for 16 hours, 12 hours being the time required to inactivate the virus. Five received a single dose of 5 c.c.; the others were given a second dose, either 11 or 13 days later. One to 2½ c.c. were given intracutaneously, the remainder subcutaneously, in the skin of the abdominal wall.

The children were observed for local and general reactions and temperatures were recorded 4 times daily. There was no apparent general reaction or discomfort and at no time any febrile manifestations that could be attributed to the vaccine. The amount of local reaction was negligible, consisting only of some induration in those receiving the larger amounts intracutaneously. Those receiving a second dose were not rendered sensitive by the first. The bloods were collected at 3 and 4 weeks after the beginning of immunization.

In each instance 0.9 c.c. of blood serum obtained in the control period was

tested against small amounts of virus to determine the level of neutralizing substances before immunization. The sera obtained 3 weeks after vaccination were tested in triplicate, against 40 to 120 infective doses of virus with simultaneous titrations of the virus as controls.

Table IV gives the age and weight of the children, the course of immunization, the antibody content of the sera in the control period and the results of the bleeding after 3 weeks (19 days in the case of K. S. and A.) as far as they have been completed. All 12 children showed the production of appreciable amounts of antibody or antiviral substances. So far, 2 sera have neutralized at least 40, 3 at least 80, and the other 7 at least 100 infective doses of virus.

Immunity was obtained by either 1 or 2 injections. It is too early to state which will give the greater protection. Whether or not the level of neutralizing substance in the control period affects the degree and rapidity of antibody production produced by the vaccine has still to be determined.

The antibody production was decidedly greater in the children than in the monkeys. If the vaccine which produces a relatively high tissue, but low humoral immunity in the monkeys acts similarly for human beings, the degree of protection obtained in these children should be quite appreciable.

Of course we have no index as to the level of immunity required to protect the children against natural infection nor whether the immunity obtained in these is sufficient. The tissue immunity in children cannot be tested, but as the monkeys treated with formalized virus showed a relatively high tissue immunity, sufficient in most cases to withstand intracerebral inoculations of virus, it is quite likely that the children have an appreciable tissue immunity. By analogy with louping-ill,[21] a disease of sheep quite analogous to poliomyelitis, it should be ample to protect against natural exposure to the virus, for formalized louping-ill virus was unable to protect sheep against intracerebral infection, yet did against the natural disease.

SUMMARY AND CONCLUSIONS

Of the various methods tried in *Macacus rhesus* monkeys, for the production of active immunity against the virus of poliomyelitis, virus suspension inactivated with formalin proved most satisfactory. Either 1 or 2 doses gave appreciable immunity and the degree compared favorably with the immunity developed by active virus or after convalescence. Five c.c. appeared to be a satisfactory dose.

A series of adults and children were then given the vaccine without any untoward local or general manifestations. It was shown that the vaccination gave an appreciable antibody response in the humans.

Whether or not the immunity is lifelong has still to be determined. Although longer intervals were not tested in the monkeys, immunity was present after 3 months with formalized virus and 2 years with the active virus.

REFERENCES

1. Netter, A., Gendron and Toraine. *Compt. Rend. Soc. de Biol.*, 70: 625, 707, 739, 1911.
2a. Brodie, M. *J. Immunol.* In press.
2b. Brodie, M., and Elvidge, A. R. *Science*, 79: 235, 1934.
3. Davide, H. Office Internat. d'hyg. pub., *Bull., mens.*, 1928, 20, 74.
4. *Poliomyelitis International Committee, 1932.* Williams & Wilkins, p. 529.
5. Brodie, M. *J. Immunol.*, 25:87, 1933.
6. Brodie, M. *J. Immunol.* In press.
7a. Brodie, M. *J. Immunol.*, 25:71, 1933.
7b. Brodie, M. *Proc. Soc. Exper. Biol. & Med.*, 30:1238, 1933.
8a. Brodie, M., and Goldbloom, A. *J. Exper. Med.*, 53:885, 1931.
8b. Brodie, M. *J. Exper. Med.*, 56:493, 1932.
9a. Aycock, W. L., and Kagan, J. R. *J. Immunol.*, 14:85, 1927.
9b. Deiner, C., and von Wiesner, R. *Wien. Med. Wchnschr.*, 60:2482, 1910.
9c. Levaditi, C. *Ecto dermoses neurotropes monographie de l'Institut Pasteur.* Masson, 1922.
10. Kraus, R. *Ztschr. f. Immunit.*, 69:424, 1931; *Wien. Klin. Wchnschr.*, 23:233, 1910.
11. Erber, B., and Pettit, A. *Comptes Rend. Soc. de Biol.*, 109:819, 1932.

12. Abramson, H. L., and Gerber, H. *J. Immunol.*, 3:435, 1918.
13. Römer. *Epidemic Infantile Paralysis.* Wood, 1913.
14. Jungeblut, C. W., and Engle, E. T. *J. Exper. Med.*, 59:43, 1934.
15. Brodie, M. *Science*, 79:594, 1934; *J. Immunol.* In press.
16. Burnet, F. M., and Macnamara, J. *M. J. Australia*, 162:851, 1929.
17. Smith, R., and McKie, M. *M. J. Australia*, 11, 404, 1933.
18. Weyer, E. R. *Proc. Soc. Exper. Biol. & Med.*, 29:289, 1931.
19. Paul, J. R., and Trask, J. D. *J. Exper. Med.*, 58:513, 1933.
20. Flexnor, S. *J.A.M.A.*, 99:1244, 1932.
21. Gordon, W. S. *Proc. Roy. Soc. Med.*, 27:11, 1934; *Vet. Record*, 14:1, 1934.

Imagination in Public Health

". . . It is inconceivable that such a prolonged and widely spread bodily infection (syphilis) should leave undamaged the vital procreative cells. In what way does this outlook relate itself to practical work? I have personally found it a great stimulus to a better realisation of the reasons for preventive action against the great systemic diseases. The constant thought of death rates and fatal disease is in the end rather depressing. It is like a fresh wind from the hills to conceive that we are, in fact, doing far more—that we are laying the foundations of a work which, in the generations still to come, will largely abolish these chronic indispositions that take so much away from the joy of life. It is true that we are still hampered by lack of knowledge in many avenues, and we wait upon research to show us the lines along which we can usefully apply scientific knowledge. What I do impress, however, is that we as field workers must keep ourselves constantly in touch with all scientific enquiry into such great systemic conditions. Only in that way can we keep our minds living and appreciative of the trend of modern thought. Again, let me lay stress on this fact—that I am not talking eugenic ideals; I am filled with the thoughts of a practical vision; for our prime function is to guide the public in those ways which will deal effectively with the great causes of ill-health, disablement and unhappiness. The field of thought which is opened up by such a generalisation of the effect of individual diseases is almost limitless, and it is obvious that a great correlation of what is ordinarily known as scientific medicine with accurate eugenic experiment would be needed to prove categorically what has been thrown out as a truly imaginative concept for your consideration. But it is such thoughts as these that make our work really interesting and constitute what, to my mind, is an orderly imagination of profound value in keeping us really alive and vigorous in mental outlook and capacity for change. . . ."—Dr. R. Veitch Clark, Imagination in Public Health, *Public Health*, Nov., 1934, p. 62.