EXPERIMENTS ON ACTIVE IMMUNIZATION AGAINST EXPERIMENTAL POLIOMYELITIS

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The results of many efforts to induce resistance in *Macacus rhesus* monkeys against experimental poliomyelitis by means of inoculations of virus in one form or another have been summarized by Stewart and Rhoads (1929) (1) and in the volume published by the Milbank International Committee (1932) (2). What may be derived from these experiments, beginning with the first, undertaken 25 years ago by Flexner and Lewis (3), is that "it is impossible to protect monkeys by the use of killed virus and second, that a definite though inconstant resistance to poliomyelitis can be brought about by the intradermal and subcutaneous introduction of the living virus" (1). The fact also emerges from the numerous trials hitherto reported that resistance is acquired by monkeys when a sufficient amount of active virus is given intra- or subcutaneously in one massive dose (3, 4) or in smaller amounts repeated over a considerable period of time (3–6). Even then protection is not afforded to some animals and the degree of immunity induced varies in others, while now and again a treated monkey succumbs to the disease as a result of the inoculations (1, 5–8).

Two noteworthy series of articles have recently appeared, in one of which was described the immunity obtained through the use of virus completely inactivated by 0.1 per cent formalin (Brodie, 9) and in the other the protection conferred with active but ricinoleated virus (Kolmer and his associates, 10). While the principles underlying both methods had already been employed (2), the recent investigators report results which lead them to believe that immunity can be safely induced with their materials.

Since on the basis of Brodie's and Kolmer's work widespread inoculations of children against poliomyelitis have been undertaken recently,

it was deemed desirable to restudy this problem, following as closely as possible the methods of these investigators. The intention was to determine whether any advance has been made in the experimental immunization of monkeys over that which has been accomplished in the past 25 years, and whether any procedure has been disclosed that might be practical for immunization of man.

For the purpose of comparison we also studied another form of chemically treated virus as immunizing agent, namely, that precipitated by tannin, which will be described first.

Methods

Virus.—The animals selected as source of poliomyelitis virus were extensively paralyzed and moribund as a result of the experimental disease. They were killed by ether inhalation and the spinal cord removed under aseptic conditions. The identification of the particular virus used in the preparation of each immunizing agent was ascertained by (*a*) animal inoculation with production of specific clinical signs and pathological changes, and by (*b*) neutralization with specific homologous strain antiserum. The M.V. and Philadelphia strains (11) of virus were employed.

Method of Testing for Acquired Active Immunity.—Monkeys were tested for induced resistance by the inoculation of homologous strains intracerebrally and intranasally.

The intracerebral test dose[1] consisted of 0.2 cc. of 5 per cent fresh poliomyelitis cord suspension which was filtered through a Berkefeld N filter. Kolmer (10), working with the M.V. strain, states that one infective unit was contained in 0.05 cc. of an unfiltered suspension in some instances and in 0.2 cc. in others. However this may be, the high cost of monkeys makes it impractical to titrate each individual virus sample; hence the test dose for induced resistance should be one that experience has shown to be unequivocally effective. The dosage as given in the following experiments has been consisfently employed in this laboratory for many years with satisfactory results. Normal monkeys receiving it react with the experimental disease within, as a rule, 5 to 11 days; only exceptionally does an animal resist. All the controls of the following series of tests developed the characteristic infection.

The intranasal test for induced immunity consisted of the instillation into each nasal cavity of 1 cc. of 10 per cent glycerolated cord suspension, and after 1 or 2 days' interval the treatment was repeated. The reaction was measured not only by clinical signs but also by cell counts of the spinal fluid withdrawn daily through cisternal puncture. The method is essentially that of Flexner (12) and his associates and suffices satisfactorily to determine the state of immunity in a treated

[1] All such inoculations were made with the aid of full ether anesthesia.

animal. It may be said that the amount as given is not too drastic since in a collateral series of twenty-four monkeys, twenty-one developed poliomyelitis; the three unaffected ones could not be considered immune, only uninfluenced by the treatment, since one of them—the only one retested—was later shown to be susceptible to a similar intranasal instillation of virus. Hence the test dose as practised is in the range of minimal infective dosage. It is of interest that all controls so treated which were employed in the experiments to be reported were successfully infected.

Test for Antiviral Bodies in Serum.—0.8 cc. of undiluted serum is mixed with 0.2 cc. of 5 per cent filtered fresh cord virus, kept at 37°C. for 2 hours and in the cold for 16 to 18 hours, and then injected into the brain of monkeys. For control, the serum is replaced by physiological saline solution. Here again neutralization tests are carried out with the homologous strain of virus. The test can be regarded as a practical one even though the precise titration of antibody content of a serum is not ascertained.

Tannin-Precipitated Virus as Immunizing Agent

In a correlated study on the virus of equine encephalomyelitis, it was found that vegetable-derived tannin (tannic acid) precipitated the proteins of the tissue containing the virus and the latter precipitated with the proteins remained infective although somewhat reduced in potency (13). The virus could not be designated as "attenuated" but merely as present in lesser amounts in the flocculated substance. Under these conditions the infective agent retained its activity for several weeks. As the following will show, similar results were obtained with tannin precipitates of active poliomyelitis tissues.

Preparation of Immunizing Agent.—2.5 gm. of poliomyelitis cord were thoroughly ground with sand and suspended during the grinding in 50 cc. of distilled water. The suspension was spun in an angle centrifuge for 15 minutes at 2,000 R.P.M. The supernatant fluid only was retained and was decanted into a 100 cc. centrifuge flask and 5 cc. of 2 per cent aqueous solution of Mallinckrodt's tannic acid were added. The mixture was energetically shaken and then stored overnight in the cold. After about 18 hours the material was again shaken and centrifuged for 20 minutes at 3,000 R.P.M. The supernatant fluid was discarded and the precipitate washed, with stirring, in 50 cc. of Tyrode's solution. After similar centrifugation, the sediment was collected and resuspended in 50 cc. of hormone broth, pH = 7.6. This suspension was stored in the cold and used as immunizing agent from 3 to 14 days after its preparation.

The tannin-precipitated virus was injected subcutaneously in the amounts to be mentioned and in several instances produced locally small, indurated masses which regressed after 1 or 2 weeks.

TABLE I

Tannin-Precipitated Virus as Immunizing Agent

Monkey No.	Immunization			Tests for active immunity					Tests for passive immunity	
	Antigen	Amount given	Result	Route	Dose	Result	When retested with Phila. virus nasally (2 doses)	Result	Serum procured after last I.D.	Result
									days	
1	M.V. virus 5% fresh cord + tannin	2 cc. S.C. 3 times at 7–11 d. intervals	Died of enteritis 2 d. after 3rd I.D.							
2	" "	" "	Died of Tb. 15 d. after 3rd I.D.							
3	" "	" "	N.S.	I.N.	2 doses M.V. virus 71 d. after 3rd I.D. (3 controls, all P. 6 d.)	P. 5 d. sp.fl. 28–565			10	Pooled serum neutralized (control, P. 8 d.)
4	" "	" "	"	"		P. 6 d. sp.fl. 32–740			10	
5 (control)	" "	0.5 cc. injected I.C.	"							
6	Same with Phila. virus	2 cc. S.C. once	P. 8 d.							
7	" "	" "	P. 10 d.							

8	" "	2 cc. S.C. 3 times at 14–8 d. intervals	N.S.	I.N.	2 doses Phila. virus 41 d. after 3rd I.D. (control, P. 8 d.)	N.S. sp.fl. 21–45	260 d. after last test (2 controls, P. 6 and 9 d.)	N.S. sp.fl. 15–27 cells	15	Serum neutralized (control, P. 8 d.)
9	" "	" "	"	I.C.	Phila. virus 51 d. after 3rd I.D. (control, P. 5 d.)	P. 6 d.			15	" "
10 (control)	" "	1 cc. injected I.C.	P. 6 d.							
11	Phila. virus 1% glycerolated cord + tannin	1,1,2 cc. S.C. at 9–10 d. intervals	N.S.	I.N.	2 doses Phila. virus 22 d. after 3rd I.D. (control, P. 8 d.)	No paralysis. Sp. fl. 14–710 (see text)	260 d. after last test (2 controls, P. 6 and 9 d.)	P. 8 d. sp.fl. 17–310 cells	15	Pooled serum neutralized (control, P. 8 d.)
12	" "	" "	"	"		No paralysis. Sp. fl. 27–105 (see text)	" "	P. 6 d. sp.fl. 25–974 cells	15	
13	" "	" "	"	I.C.	Phila. virus 32 d. after 3rd I.D. (control, P. 5 d.)	P. 6 d.			15	Pooled serum failed to neutralize (control, P. 8 d.)
14	" "	" "	"	"		P. 5 d.			15	
15 (control)	" "	1 cc. injected I.C.	"							

I.D., immunizing dose; S.C., subcutaneous injection; d., day; Tb., generalized tuberculosis; N.S., no symptoms; I.N., intranasal; P., characteristic experimental poliomyelitis, the number following showing the day on which paralysis was first observed; sp.fl., pleocytosis, the first figure representing normal cell count and the second, the reactive count; I.C., intracerebral injection; Phila., Philadelphia 1932 strain of virus.

Results of Preventive Inoculations.—It will be seen from Table I that of eight monkeys injected subcutaneously with the 5 per cent virus suspension (M. V. and Philadelphia strains), two died of non-poliomyelitis affections and of the remaining six, two became moribund after an attack of poliomyelitis following the first subcutaneous dose of 2 cc. In the one instance in which the antigen was Philadelphia virus, 1 cc. inoculated intracranially induced poliomyelitis in a control monkey. When the immunizing agent was reduced in content of virus to 1 per cent of cord by weight and only a total of 4 cc. of it was given subcutaneously to each of four monkeys and 1 cc. intracerebrally to a fifth, none of the five so treated developed disease.

The data in Table I clearly show that material containing active virus can by itself give rise to fatal infection after a single subcutaneous injection. It is significant, however, that of two monkeys receiving three such doses of the same virus sample, one failed to be protected against a subsequent intracerebral test inoculation but the second resisted the intranasal test instillation. Results based on the reactions of only two animals are inconclusive but they serve to bring out one of the difficulties met with in attempting to immunize animals with active virus preparations.

The power of the various tannin-precipitated virus preparations to build up resistance was not great, for it is noted that of three monkeys receiving the Philadelphia virus and which were given the intracerebral test inoculation, all developed poliomyelitis. Of the treated animals injected with the 5 per cent antigen and tested intranasally for immunity, two succumbed and one monkey was found to be resistant to this and a repeated test. Of those receiving the 1 per cent material, both were resistant to the first intranasal test dose but were susceptible to a second test.

Hence only one of the five animals receiving the immunizing agent was found resistant to the intranasal test and that one resisted both of two tests. Even so, one cannot regard this monkey as immune, for, as Flexner (14) shows, monkeys can be refractory to several successive courses of instillations yet respond to a final one of the same virus. In addition, these results confirm Flexner's finding that when virus is placed in contact with the nasal mucosa, pleocytosis may occur, but the increase in the number of cells in the spinal fluid may not be

associated with symptoms of infection or with the development of immunity.

The capacity of tannin-precipitated virus to produce serum antiviral bodies is varied. Of the series injected with 5 per cent virus antigen, the pooled serum of two monkeys and individual sera of two others neutralized virus by the method described; of the animals given the 1 per cent antigen, the pooled serum of two neutralized and that of two others failed to do so. To be noted is that in five instances treated monkeys yielded neutralizing serum but were found, 51, 71, and 301 days after the last immunizing dose was given, to be susceptible in average degree to intracranial or intranasal contact with virus. This is not unusual; it has recurred in the experiments soon to be described with formalin and ricinoleate. Moreover, Stewart and Rhoads (1), Schultz and Gebhardt (15), and recently Aycock[2] and others have reported the lack of correlation existing between serum antiviral bodies and immunity as tested by the cerebral or nasal routes. In other words, the presence in the monkey of serum antiviral bodies, as produced by artificial immunization and determined by the described method, is no definite indicator of the state of active resistance of the animal to the test doses used.

To summarize the results of preventive treatment with tannin-precipitated poliomyelitis virus, it would appear that this product has failed as a satisfactory immunizing agent and that it is restricted by the same uncertainty which living virus as such manifests as a preventive when injected under similar conditions. Too much of the material can induce infection; too little, inconstant and unreliable immunity.

Active Ricinoleate-Treated Virus as Immunizing Agent

Kolmer (10), basing his experiments on those of McKinley and Larson (16), employed 1 per cent sodium ricinoleate to attenuate but not inactivate the poliomyelitis virus in 4 per cent cord suspensions prepared from 1 month old glycerolated tissue. The ricinoleated material was kept in the cold for 1 month before use and then in one series of experiments 0.1 cc. of the agent per kilo body weight was injected subcutaneously five times at 5 day intervals into seven monkeys, and similar dosages were given intracutaneously to three additional animals. They

[2] Personal communication.

showed no symptoms, and 1 month after the last treatment, when subjected to an intracerebral inoculation of 0.2 cc. of 5 per cent virus suspension, one developed poliomyelitis and the others were unaffected. The survivors were again injected intracerebrally with virus up to 17 months later and all but one survived a third cerebral test for resistance.

In repeating the experiment with sodium ricinoleate-treated virus, we used the same virus (M.V. strain) which Kolmer employed and the sodium ricinoleate was sent us through the kindness of the same manufacturers.[3] The methods were those of Kolmer except as regards the intracerebral test dosage: Kolmer employs as a test dose for induced immunity unfiltered and we, filtered suspensions. In addition, we employed nasal instillation, as described, for this purpose, a procedure which he omitted.

Results of Preventive Inoculations with Ricinoleate-Treated Virus.—Reference to Table II shows that six monkeys received the Kolmer vaccine. Of two tested intracerebrally for immunity, both failed to resist and of four instilled intranasally, two developed the disease on the first instillation and a third on a repeated test. Thus only one of the six animals resisted the tests for acquired resistance.

Table II reveals that the pooled serum of two treated animals and the individual sera of the remaining four neutralized virus in each instance. Here again, as occurred with tannin-precipitated virus, the antiviral bodies, as determined by the method given, were present but despite this fact the animals succumbed to the tests for active immunity.

When these experiments were well advanced, a paper was published by Schultz and Gebhardt (15), which stated: "The serums of another series of animals 'immunized' earlier with *living* virus (Kolmer vaccine) neutralized 30 M. I. D. doses of virus per cubic centimeter, but when these animals were subjected to intranasal instillation with active virus, they all developed typical poliomyelitis." We can thus confirm the findings of these investigators.

Formolized Virus as Immunizing Agent

In preparing materials, the methods of Brodie (9) were followed. 0.2 per cent formalin was added to 20 per cent active cord suspensions in equal volumes so that in the end 0.1 per cent formalin was in contact with 10 per cent virus suspension. This was kept at 37°C. for 16 hours, since at the time when this work was

[3] William S. Merrell and Co., Cincinnati.

TABLE II

Ricinoleate-Treated Virus as Immunizing Agent,
Employing 4 Per Cent Glycerolated Poliomyelitis Cord Suspension + 1 Per Cent Sodium Ricinoleate, Subcutaneously

Monkey No.	Immunization		Tests for active immunity					Tests for passive immunity	
	Amount* M. V. virus antigen. 5 doses, 5 d. intervals	Result	Route	Dose given about 30 d. after last I. D.	Result	When retested with M.V. strain nasally (2 doses)	Result	Serum procured after last I.D.	Result
								days	
16	0.3 cc. (total 1.5 cc.)	N.S.	I.C.	M.V. virus	P. 6 d.			20	Pooled serum neutralized; (control, P. 6 d.)
17	0.27 cc. (total 1.35 cc.)	"	"	" "	P. 7 d.			20	
18 (control)	None	—	"	" "	P. 4 d.				
19	0.3 cc. (total 1.5 cc.)	N.S.	I.N.	M.V. virus 2 doses	N.S.	67 d. after last test	P. 11 d. (4 controls P. 7, 9, 10, 11 d.)	20	Serum neutralized; (control, P. 6 d.)
20	0.35 cc. (total 1.75 cc.)	"	"	" "	P. 8 d. sp.fl. 29–644 cells			20	Serum neutralized; (control, P. 6 d.)
21	" "	"	"	" "	P. 8 d. sp.fl. 45–730 cells			17	Serum neutralized; (3 controls, 2 P. 6 d. and 1, 22 d.)
22	0.34 cc. (total 1.7 cc.)	"	"	" "	N.S.	67 d. after last test	N.S. (4 controls same as Monkey 19)	17	Serum neutralized; (2 controls, P. 14 d. and 3 d.)
23 (control)	None	—	"	" "	P. 7 d. sp.fl. 330 cells				
24 (control)	1 cc. injected I.C.	P. 9 d.							

Abbreviations same as in Table I.

*0.1 cc. ricinoleate-treated virus per kilo.

TABLE III

Formolized Virus as Immunizing Agent,
Employing 0.1 Per Cent Formalin Fresh 10 Per Cent Virus (Cord) Suspension, Kept for 16 Hours at 37°C., Intradermally

Monkey No.	Immunization		Tests for active immunity					Tests for passive immunity	
	Amount given (2 x 5 cc. at 13 d. intervals) Strain	Result	Route	Dose	Result	When re-tested intra-cerebrally	Result	Serum procured after last I.D.	Result
								days	
25	Phila.	N.S.*	I.N.	Phila. virus 2 doses 30 d. after last I.D.	P. 8 d. sp. fl. 24–834 cells			21	Pooled serum. Monkey febrile 7 to 11th d. T = 104.8°F.† Excited. Recovery (partial neutralization?); (2 controls, both P. 8 d.)
26	"	"	"	" "	P. 9 d. sp.fl. 17–224 cells			21	
27 (control)	None	—	"	Phila. virus 2 doses	P. 8 d. sp.fl. 22–420 cells				
28	Phila.	N.S.	I.C.	Phila. virus 36 d. after last I.D.	N.S.	57 d. after 1st test dose	N.S.	21	Pooled serum. Monkey febrile 7th to 13th d. T = 105°F., slight ptosis. Recovery (partial neutralization?); (2 controls, P. 8 d.)
29	"	"	"	" "	P. 6 d.			21	
30 (control)	None	—	"	Phila. virus	P. 7 d.				
31 (control)	"	—	"	" "	P. 7 d.				
32 (control)	2 cc. of formolized Phila. virus I.C.	N.S.							

33	M.V.	"	I.N.	M.V. virus 2 doses 30 d. after last I.D.	P. 8 d. sp.fl. 27–460 cells			20	Pooled serum. No neutralization; (control, P. 6 d.)
34	"	"	"	" "	P. 10 d. sp.fl. 33–340 cells			20	
35 (control)	None	—	"	M.V. virus 2 doses	P. 8 d. sp.fl. 25–514 cells				
36	M.V.	N.S.	I.C.	M. V. virus 50 d. after last I.D.	P. 8 d.			20	Pooled serum neutralized; (control, P. 10 d.)
37	"	"	"	" "	P. 5 d.			20	
38 (control)	None	—	"	M.V. virus	P. 8 d.				
39 (control)	"	—	"	" "	P. 7 d.				
40 (control)	2 cc. of formolized M.V. virus I.C.	N.S.							

Abbreviations same as in Table I.

*The monkeys injected with formolized virus showed local skin necrosis with ultimate healing within about 2 weeks.

†T = highest temperature reading during febrile course.

done, Brodie stated that 12 to 16 hours of such contact served to inactivate poliomyelitis virus, and that the 16 hour material was employed by him as immunizing agent.

With respect to dosage for immunization of monkeys, it was first stated by Brodie (17) that one dose of 5 cc. yielded as good results as two doses of 5 cc.; this was later (18) changed so that it was then declared that two injections were more efficacious than a single intradermal one of 5 cc. In the following experiments, however, two doses of 5 cc. each were used throughout.

The intracerebral test for induced resistance as employed by Brodie was made with amounts on the borderline of infectivity, designated as "minimal completely paralyzing doses." In Table III, the intracerebral test was the same as given in the foregoing series of experiments with tannin and sodium ricinoleate, so that a proper comparison could be made of the different methods of immunization. This consisted of 0.2 cc. of filtered 5 per cent fresh cord suspensions. No mention is made by Brodie of determining immunity by means of intranasal instillation of virus; this we have carried out along with the intracerebral test.

Results of Preventive Inoculation with Formolized Virus.—As will be seen in Table III, of eight monkeys injected with formolized virus, only one resisted, and that one was found refractory to two successive intracranial test inoculations. It is common experience among workers in this field to meet with an occasional monkey refractory to poliomyelitis virus, so that it is uncertain whether the animal in question was immunized by the formolized material or not.

Of four sets of pooled serum, as indicated in Table III, one showed neutralization, another, none, and a third and fourth so called incomplete neutralization, due perhaps to low antibody content. The lack of correlation between serum antibodies with active protection has already been commented upon.

It is therefore plain that this method offers, under the experimental conditions employed, an ineffective immunizing material against poliomyelitis in monkeys.

The experience of Schultz and Gebhardt (15) employing the same agent is as follows:

> They injected fifteen monkeys: three subcutaneously, four intramuscularly, and four intradermally, giving 0.1 cc. per kilo of 0.1 per cent formolized 10 per cent virus, and four intravenously with ten times this amount, five times at weekly intervals. 24 days after the last immunizing dose the animals received three M.I.D. of virus. "All developed the disease 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 additional experiments, Schultz and Gebhardt (15) injected the immunizing agent repeatedly in the brain of four monkeys and instilled it repeatedly in the nasal cavities of four others. All eight were proved susceptible to later inoculation with virus, in the brain in the first series and in the nose in the second.

The results we have obtained are corroborative of those of Schultz and Gebhardt, although the latter investigators employed a lesser amount of vaccine, and lead to the conclusion that formolized virus is not an effective preventive against poliomyelitis in the monkey. Other earlier observers (Abramson and Gerber, 19; Römer, 20; and Jungeblut and Engle, 21) also did not succeed in inducing immunity by means of formolized poliomyelitis virus.

DISCUSSION

The object of this study was the investigation of the problem of active immunization of *Macacus rhesus* monkeys by means of chemically treated poliomyelitis virus. The materials employed were tannin-precipitated virus and virus treated with sodium ricinoleate and with formalin. The latter two substances are those with which the vaccines of Kolmer and Brodie respectively are prepared and the tannin material introduced by us was employed for comparative observations.

The virus of poliomyelitis treated with tannin or sodium ricinoleate retains its activity so that intracerebral inoculation of monkeys with the preparations induces characteristic experimental poliomyelitis. Indeed, Kolmer (10) records that 0.2 cc. of his vaccine kept for 5 months when so inoculated induced the disease within 12 days. Further, the tannin-precipitated virus itself brought on infection in two animals after a single subcutaneous injection of 2 cc. It is therefore plain that the chemical treatment in both instances did not act to attenuate the virus.

The results of the experiments can be summarized by stating that if the immunizing agent contains a sufficient amount of virus, the danger arises of infecting an animal with the material itself. Under the experimental conditions employed, these preparations, although active virus was present in them, failed to immunize the inoculated animals regularly. Serum antiviral bodies were, however, produced

by means of the described methods but it was shown that animals in which these antibodies were present did not resist the ordinary tests for active immunity.

From what is here reported, it is apparent that there is no advantage to be derived from the use of the tannin-precipitated, or ricinoleated virus as immunizing agents over unchanged active virus, as employed in the past in this laboratory (Flexner and Lewis, 3; Flexner and Amoss, 22; Stewart and Rhoads, 1; and Rhoads, 23) and elsewhere (Aycock and Kagan, 5, and others).

A study of the recorded experiments of the past 25 years on immunization of monkeys reveals that active poliomyelitis virus itself is not a potent antigen, as are some other viruses; uniform protection is rarely brought about through its use. A greater degree of success in protecting animals can, however, be achieved when large doses over long periods of time are employed—which fact might lead one to suppose that the difficulty with poliomyelitis virus as immunizing agent may be related simply to the amount of antigenic substance present. Some viruses, such as those of equine encephalomyelitis (24) and yellow fever[4] among several others, can be diluted to 10^{-8} and still be infective for the most susceptible host, whereas poliomyelitis virus can be diluted to only a fraction of this amount to reach the limit of infectivity in the monkey. It is still unknown why the antigenic capacity of this virus is relatively less than that of several others. Finally, if amounts of virus sufficing to produce disease in some monkeys but not in others confer no immunity on the unaffected ones, it is to be expected that a lesser amount would be even less effective.

We now come to an estimation of formolized virus. In this instance, the evidence of earlier observers (2), later of Brodie (9, 17), of Schultz and Gebhardt (15), and ourselves points to the inactivation of the virus by the chemical. It is still an open question whether any form of inactive poliomyelitis virus retains the property of immunizing animals (2). An analysis of the results of the present investigation shows that active immunization with formolized virus by the Brodie method does not build up resistance in monkeys to the usual intracerebral or intranasal tests for induced immunity. The amount of antiviral bodies produced in the serum by this vaccine is slight and, as already indi-

[4] Theiler, M., personal communication.

cated, the treated monkeys failed, notwithstanding the presence of antibodies, to resist the tests for active immunity.

There are, therefore, discrepancies in the conclusions of Brodie (9) and ourselves. These may perhaps be ascribed to the fact that Brodie employs borderline dosages in his tests. With such small doses, it is possible that certain monkeys may not receive what for them is an infective dose of virus. At this point we wish to emphasize the fact that the intranasal test dose for immunity employed here was within the range of a minimal infective dose, as we have pointed out before; nevertheless, animals receiving formolized virus (or tannin-precipitated or ricinoleated virus) and among them even those which possessed serum antiviral bodies were found to be susceptible to this test.[5]

There remain for consideration the factors derived from animal experimentation which either Kolmer or Brodie maintains as a basis for the claims that a safe and successful immunizing agent has been made available for use in man.

The first factor which Kolmer (10) stresses as the essential one is the non-infectivity of his preparation. Kolmer admits that the degree of attenuation by sodium ricinoleate is slight or of minor importance but safety is acquired through the use of remote monkey passage virus that has apparently lost its infectivity for man. There is no experimental evidence for this assumption (25, 26).

The second is that ricinoleated and formolized vaccines engender in monkeys serum antiviral bodies and that the same mechanism might apply in man. It has been shown by Schultz and Gebhardt (15) and

[5] As this article goes to press, Brodie states (*J. Am. Med. Assn.*, 1935, **105**, 1089) that virus suspensions should be "just inactivated, for overtreatment or prolonged treatment with solutions of formaldehyde reduced the antigenicity of the vaccine," and therefore recommends the use of virus inactivated for 8 to 12 hours instead of 16. The distinction between "just inactivated" and "overinactivation" is not clear. In view of the still more recent modification (18) of 5 to 6 hours' contact with 0.1 per cent formalin at 37°C., it is apparent that this vaccine contains active virus as shown by Brodie in experiments in which 6 hours' treatment fails to inactivate the virus. The amount of active material may be small since, as Brodie points out, monkeys develop the disease only after repeated inoculation of 6 hour treated suspensions. It is known, however, that such small amounts of active virus do not induce protection in monkeys; still the possibility of infection during the period of immunization with an agent that contains active virus is ever present.

by us that the antibody response in monkeys is slight, although the Kolmer vaccine exceeded the Brodie preparation in this capacity. Despite the presence of acquired antiviral bodies in the serum no active resistance was developed to the recorded test doses for induced immunity.

Finally, the third factor relates to the active protection conferred on monkeys by means of the chemically treated virus. Since unchanged poliomyelitis virus lacks high antigenicity, it is to be expected that vaccines containing a lesser amount of active virus or virus that is inactivated would be still weaker in antigenic power. The results of the present experimental study reveal the ineffective and irregular immunizing capacity of these chemically treated viruses.

CONCLUSION

The results obtained in this investigation indicate that poliomyelitis virus treated with tannin, sodium ricinoleate, or formalin does not constitute a satisfactory immunizing agent in monkeys against the experimental disease.

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