was found to be even much less active than the oil solution just described, under the same conditions, giving an anesthesia of less than 10 minutes or none at all in some cases. This means that "benzocaine", when rendered soluble by the attachment of a diethyl amino group plus HCl (which is procaine HCl) is a less active surface anesthetic than unmodified benzocaine if it is only finely divided, suspended in water.

Conclusions. By a suitably fine dispersion in water benzocaine can be rendered efficacious as a topical anesthetic, to a higher degree than when powdered on the mucous membrane in pure form, or when dissolved in oil, or when rendered water soluble as a diethyl amino compound (=procaine).

11675

Immunological Studies on Intestinal Theiler's Virus, and Its Relation to Poliomyelitic Virus.*

PETER K. OLITSKY.

From the Laboratories of the Rockefeller Institute for Medical Research, New York City.

The properties of Theiler's virus (mouse encephalomyelitis, or so-called "mouse poliomyelitis") as it is found in the intestines of practically all normal albino mice of young adult and mature age, or as it occurs in mice having the spontaneous or experimental disease, have recently been described.[1, 2] The similarity of Theiler's virus to that of human poliomyelitis in many characteristics has been pointed out[1, 2] although Theiler[3] has shown that there is no relationship between them in immunological reactions or in host-susceptibilities. Now that there is at hand the intestinal form of the mouse virus, it was thought desirable to make further attempts to determine the relation of this agent to the virus of poliomyelitis. To this end, studies on the possible existence of active and passive cross immunity were

* The writer thanks Dr. M. Theiler for his cooperation, Dr. E. H. Lennette for a sample of pooled, monkey poliomyelitic antiserum, and Mr. P. Haselbauer for technical assistance.

1 Olitsky, P. K., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 434; 1940, **43**, 296; *J. Exp. Med.*, 1940, **72**, 113.

2 Theiler, M., and Gard, S., *J. Exp. Med.*, 1940, **72**, 49, 79.

3 Theiler, M., *J. Exp. Med.*, 1937, **65**, 705.

undertaken as well as on the pathogenesis of the mouse virus in *rhesus* monkeys.

It is agreed[4] by many investigators that human poliomyelitic virus, passaged for long periods of time in monkeys, is not transmissible to white mice. Our own experiences include observations on more than 275 mice (Rockefeller Institute, albino strain) of ages varying from 15 days to 9 months, and a wide variety of methods of inoculation of the virus. The strains of the latter which were employed, MV and Philadelphia 1932, had been maintained for several years by serial passages in *rhesus* monkeys. The mice were exposed to the virus in the following ways: a) by intranasal instillation (10 to 20% suspension of fresh CNS tissue, given in 3 daily doses; or each dose was preceded by a nasal wash with salt solution, horse serum, phosphate buffer at pH=5, or ether; or the first dose was immediately preceded by intracerebral injection of starch solution or scarification of the nasal mucosa); b) by subcutaneous injection (1 cc simply, or this amount just after starch solution had been given intracerebrally); c) by intraäbdominal inoculation (1 cc once or 3 doses introduced on alternate days); and d) by intracerebral inoculation simply, also followed by 4 brain passages in mice at 24-hour intervals. In addition, the inoculated mouse brain of d) was removed on the 2d day and transferred to monkeys for 2 consecutive monkey-passages. The outcome of all these experiments was negative.

The sera obtained from mice 1 month after the administration of poliomyelitic virus by intranasal, subcutaneous, intraäbdominal, and intracerebral routes were pooled (about 10 sera representing each method of inoculation in a pool) and each respective lot was tested for neutralizing antibodies against MV and Philadelphia virus. 1 part of 5% active filtrate of affected monkey cord and 4 parts of mouse serum were mixed; the serum-virus mixtures were kept for 2 hours at 37° C and overnight in the refrigerator. Then 1 cc was injected intracerebrally in monkeys, of which there were 13 test animals and 8 controls. Virus-neutralization could not be detected by these means. In another experiment, 20 mice 20 to 25 days of age received intraäbdominally 3 times on alternate days 0.5 to 1 cc of 10% MV and Philadelphia strains of poliomyelitic virus; this dose was larger than that which induced antibodies in monkeys.[5] Serum collected 30-40 days after the last dose was pooled and tested for the presence of neutralizing antibodies against intestinal Theiler's virus as it occurs in normal mice; and, as an elaboration of controls

[4] For a recent review of the literature see Toomey, J. A., *J. Pediatrics*, 1940, **16**, 519.

[5] Olitsky, P. K., and Cox, H. R., *J. Exp. Med.*, 1936, **63**, 109.

of the test, also against this virus as present in the CNS of mice acutely ill with encephalomyelitis induced by injection of intestinal contents of normal mice. The neutralization tests were made as just described and the results, as noted in more than 300 mice inoculated intracerebrally with 0.03 cc of serum-virus mixtures (virus in decimal dilutions), disclosed that a) the sera of normal, untreated mice of the same age as that at which the "immunized" mice were bled, *i. e.*, about 2 months, neutralized between 10 and 100 infective cerebral doses of Theiler's virus deriving from intestinal contents or from CNS tissue. b) The sera of mice which had been repeatedly injected with the poliomyelitic viruses showed no greater degree of neutralization than those of normal, uninoculated animals. c) Normal monkey serum had no capacity to inactivate Theiler's virus.

From the results of the foregoing experiments, it can be concluded that the MV and Philadelphia, monkey-passaged strains of poliomyelitic virus are, as determined by the methods here described, not only non-pathogenic in albino mice but also fail to induce antiviral bodies against the homologous strains. Moreover, the serum of uninoculated, normal 2-months-old mice exhibits a certain degree of neutralizing capacity against Theiler's virus as it exists in the intestinal contents of normal mice or in the CNS of such as have experimental encephalomyelitis. Whatever neutralization is observed to occur in sera of animals that have received injections of the poliomyelitic viruses is about equal in degree to that which can be found in untreated mice; hence the poliomyelitic viruses cannot be said to have the power, by themselves, of producing in mice antibodies against Theiler's virus.

The next series of experiments dealt with attempts at detection of neutralizing antibodies in antipoliomyelitic serum, originating from man or *rhesus* monkey, against intestinal Theiler's virus or this virus as found in the CNS of mice having experimental encephalomyelitis. The sera consisted of one sample of pooled, human serum obtained from patients recovered from paralytic poliomyelitis and 7 others collected from monkeys convalescent from paralytic infection induced by MV and Philadelphia viruses. All sera were found to inactivate strains of poliomyelitic virus.† Each sample and Theiler's virus in decimal dilutions (from 10^{-1} to 10^{-4} for intestinal and to 10^{-5} or 10^{-6} for CNS virus) were mixed in equal parts or 4:1 ratios and kept 1 to 2 hours at room temperature or 37° C, and in addition,

† The convalescent monkey sera were found by Dr. Theiler to inactivate also the Lansing strain which C. Armstrong (*Public Health Rep.*, 1939, **54**, 1719) has succeeded in transmitting to cotton rats and mice.

in most instances, overnight (16 hours) in the refrigerator. Normal monkey serum was used as control. The results of injections of the serum-virus mixtures intracerebrally in more than 450 mice showed no definite, demonstrable neutralization of Theiler's virus.

The final tests related to attempts at transmission to *rhesus* monkeys of Theiler's virus deriving from intestinal contents of normal mice (active to 10^{-3} dilution) and from the tissue of the CNS of mice that developed encephalomyelitis after receiving such contents (active to 10^{-6} dilution). Eight monkeys were injected, 1 cc intracerebrally and 4 to 25 cc intraäbdominally, with a 20% suspension of contents or of nervous tissue. None of them exhibited signs of infection nor, 40 days after inoculation, resistance against active MV or Philadelphia strains of poliomyelitic virus. In addition, intestinal Theiler's virus did not apparently survive in the CNS of *rhesus* monkeys that were injected intracerebrally with it: Cerebral tissue from the site of inoculation plus fragments of spinal cord were removed 1, 2, 4 and 6 days after injection and reinoculated intracerebrally into mice (in 10 for each sample) with negative results. Finally, passage was attempted using nervous tissue obtained from monkeys that had received active intestinal Theiler's virus in the brain. A total of 3 consecutive monkey brain-to-brain passages were carried out. The tissue (site of cerebral injection, basal ganglia, pons-medulla, and cord) was removed on the 6th day after inoculation in all instances, made up in saline solution to 20% suspension and 1 to 1.25 cc given intracerebrally along with 25 cc intraäbdominally. All passages were negative. Under the described conditions, intestinal Theiler's virus could not be successfully implanted in the brain of *rhesus* monkeys.

Discussion and Summary. Attempts at transmission of Theiler's virus to *rhesus* monkeys by means of intracerebral inoculation and, further, by consecutive passage in monkeys, failed; the virus, moreover, was not demonstrable in the CNS of monkeys 24 hours after inoculation. Thus, Theiler's virus has its own traits in monkeys, which fact conforms with Theiler's original conclusion[3] that the mouse virus is not identical with that of human poliomyelitis. Further proof of this finding can be deduced from results of immunological studies. In the foregoing experiments two standard strains of poliomyelitic virus were used, the MV and Philadelphia, both monkey-passaged for several years; what would have been the outcome had strains freshly isolated from human cases of poliomyelitis been studied is, of course, unknown. It is apparent, however, that by the methods here used, such monkey-passaged strains are not only nonpathogenic in the type of mouse which is susceptible to Theiler's virus and which carries the latter in its intestines for a good part of

its life, but also fail to produce serum-neutralizing antibodies in such mice against the homologous poliomyelitic viruses and against Theiler's virus. Moreover, antipoliomyelitic sera of human and monkey origin that are capable of neutralizing homologous and other strains of poliomyelitic virus fail to inactivate Theiler's virus. Conversely, serum from mice harboring Theiler's virus does not neutralize the poliomyelitic viruses. On the other hand, serum from normal mice of certain adult age has antiviral bodies against Theiler's virus. In this latter relation, a similarity exists between the virus of Theiler's disease—a natural malady of mice—and that of poliomyelitis of man in that neutralizing antibodies against the latter virus develop with time in man in the absence of clinically apparent disease. It has not as yet been determined, however, whether in man, as in the mouse, there exists the almost universal and prolonged[1, 2] carrier-state—in the intestines or perhaps elsewhere in the host.

11676 P

Isoimmunization in Pregnancy and the Varieties of Isoagglutinins Observed.*

PHILIP LEVINE AND EUGENE M. KATZIN.

From the Division of Laboratories, Newark Beth Israel Hospital, Newark, N. J.

The production of immune isoagglutinins following repeated transfusions, although of common occurrence in some animal species, is very rare in man. The first instance of this sort in man was described by Landsteiner, Levine, and Janes.[1]

Levine and Stetson[2] described a case in which an intra-group agglutinin was responsible for severe post-transfusion symptoms, with anuria after the first transfusion. This occurred in a woman who, after retaining a dead fetus for a period of about 2 months, finally delivered a macerated fetus. In view of the activity of the agglutinin at 37° C and because of its gradual disappearance, it was believed that the antibody developed as a result of immunization, and it was suggested that the products of the retained dead fetus served as the

* This investigation has been aided by a grant from the Blood Transfusion Betterment Association of New York City.

1 Landsteiner, K., Levine, P., and Janes, M. L., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 572.

2 Levine, P., and Stetson, R. E., *J. Am. Med. Assn.*, 1939, **113**, 126.