logical sections of the CNS revealed no pathological changes.

In a subsequent experiment meningoencephalomyelitis was produced in rats substituting spinal cord of guinea pigs for homologous spinal cord.

Summary. The introduction of homologous spinal cord in a water-in-paraffin oil emulsion containing killed tubercle bacilli into the skin of the albino rat produces mononuclear meningo-encephalomyelitis. The symptoms and pathological changes in the CNS and at the site of injection are similar to those found in the guinea pig.

1. Rivers. T. M., Sprunt, D. H., and Berry, G. P., *J. Exp. Med.*, 1933, v58, 39.

2. Rivers. T. M., and Schwentker, F. F., *J. Exp. Med.*, 1935, v61, 689.

3. Kabat, E. A., Wolf, A., and Bezer, A. E., *J. Exp. Med.*, 1947, v85, 117.

4. Morgan, I. M., *J. Exp. Med.*, 1947, v85, 131.

5. Freund, J., Stern, E. R., and Pisani, T. M., *J. Immunol.*, 1947, v57, 179.

6. Freund, J., and Lipton, M. M., PROC. SOC. EXP. BIOL. AND MED., 1948, v68, 373.

7. Morrison, L. R., *Arch. Neur. and Psych.*, 1947, v58, 391.

8. Olitsky, P. K., and Yager, R. H., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 600.

9. Thomas, L., Paterson, P. Y., and Smithwick, B., *J. Exp. Med.*, 1950, v92, 133.

10. Hehre, E., and Freund, J., *Arch. Path.*, 1939, v27, 289.

11. Lumsden, C. E., *Brain*, 1949, v72, 198.

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Recovery of Type I (Brunhilde) Poliomyelitis Virus from Mouse Brain and Spinal Cord.* (19841)

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Dick reported that type 1 (Brunhilde) poliomyelitis virus in the form of monkey central nervous system tissue inoculated into a variety of rodents could be recovered from their brains at least for 10 days from the time of cerebral inoculation(1). No adaptation of this virus to rodents occurred despite his employment of the method of alternating passage between rhesus monkeys and rodents and passage from rodent to rodent. There was neither clinical nor histological evidence of disease in the rodent hosts studied. It was stated "this is highly suggestive that no multiplication had occurred in the brains of these animals."

This interesting phenomenon was explored as part of a study in this laboratory on poliomyelitis virus-host adaptation. The purposes of this report are to confirm Dick's findings in murine brain and also a) to indicate recovery of virus at a site *distant* to the site of inoculation namely the spinal cord, b) to identify the virus recovered as type 1 virus which had not undergone detectable immunological alteration, and c) to indicate that the biological state of the virus was not such as to interfere with the propagation of a mouse adapted type 2 (Lansing) poliomyelitis virus in such mice.

Materials and methods. Type 1 (Brunhilde) poliomyelitis virus was utilized in the form of a 20% suspension of spinal cord enlargements of monkeys sacrificed at the height of paralysis following thalamic inoculation. This virus pool yielded a titer of $10^{-5.4}$ in monkeys. The mouse adapted type 2 (Lansing) poliomyelitis virus was an aliquot of a pool of infected mouse brains. The number of previous mouse passages approximates 250; average titers of $10^{-3.6}$ are observed with intracerebral inoculation of 0.03 ml in young adult mice. Swiss mice of Harlan stock were utilized in all the observations herein reported. Roller-tube tissue-cultures were utilized for the isolation and identification of poliomyelitis virus. Monkey testicle(2) was used as tissue

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source for fibroblasts and the cytopathogenic effect described by Robbins *et al.*(3) utilized as an indicator for the presence of poliomyelitis virus. The type 1 (Brunhilde) antiserum used was obtained from monkeys hyperimmunized with the prototype Brunhilde virus. Such serum has not thus far revealed a common antigen among the 3 immunological types of poliomyelitis virus(4). These tests for presence and neutralization of virus were qualitative. Quantitative studies are in progress and will be reported.

Experimental. A. *Inoculation of mice with unadapted type 1 (Brunhilde) poliomyelitis virus.* Twelve mice were inoculated intracerebrally with 0.03 ml of a 20% suspension of the type 1 virus. Five days later the brains of six mice were removed and frozen; the spinal cords of these animals were then removed and frozen. Ten days following inoculation the remaining animals were sacrificed and their brains and spinal cords also frozen separately. The following day the mouse tissues were thawed and 5% saline suspensions of brains and 20% suspensions of spinal cords prepared and inoculated into tissue-cultures.

B. *Recovery and identification of virus in tissue culture.* The suspensions of brains and of spinal cords of the 5-day and 10-day groups of mice were kept separate. Culture tubes containing actively growing fibroblasts of monkey testicular fragments were inoculated either with 0.4 ml or 0.1 ml of the respective suspensions. No appreciable difference was noted with the size of inoculum. Uninoculated culture tubes were kept as tissue controls and the same type 1 (Brunhilde) poliomyelitis virus in the form of monkey spinal cord tissue used in mice inoculated separately into other roller-tubes. Nine days following inoculation, beginning cytopathogenic effect was noted in the tubes under test as well as in the tubes inoculated with 20% suspension of type 1 (Brunhilde) virus. By 13 days following inoculation the cytopathogenic effect was distinct in all tubes under test and was most pronounced in the tubes inoculated with the suspensions of mouse spinal cords. Individual culture fluids were collected separately. In each of the 4 test situations (brains and spinal cords separately at the 2 stated intervals), it was observed that the virus recovered from the mice had not undergone detectable immunological alteration as indicated by the fact that the cytopathogenic effect produced was specifically inhibited by the type 1 antiserum. In other tissue-culture tests such serum inhibited the cytopathogenic effects of homotypic type 1 (Mahoney) virus.

C. *Failure to demonstrate interference in mice harboring type 1 (Brunhilde) virus in their central nervous system.* Two groups of mice (40 in each) were inoculated intracerebrally with 0.03 ml of the type 1 (Brunhilde) poliomyelitis virus as before. Two similar groups were so inoculated with 20% suspension of normal monkey spinal cord. Five days and 10 days later respective test and control groups were inoculated intracerebrally with 16 LD_{50} of the mouse adapted type 2 (Lansing) poliomyelitis virus. No significant differences as regards time of onset or incidence of paralysis were observed.

Discussion. That type 1 (Brunhilde) poliomyelitis virus which is unadapted for murine brain can be recovered from the site inoculated and from a *distant* site in the mouse spinal cord suggests that the phenomenon described by Dick involves circumstances other than passive persistence of a depot of virus. The sequential fate in mice of intracerebral inocula of living agents whether adapted or not is not entirely clear. Schlesinger(5) has observed that only 3 to 10% of western equine encephalomyelitis virus can be recovered from mouse brain one hour after intracerebral inoculation. This is not due to physical leakage around the inoculation site. Cairns(6) observed a similar situation in studying the fate of intracerebrally inoculated bacteriophage in mice even with inocula as small as 0.005 ml delivered by a micrometer-screw syringe. The recovery from spinal cords of the type 1 poliomyelitis virus inoculated into mouse brains indicated that the virus did not merely persist in a dormant fashion at the inoculation site.

The terms "adaptation" and "host-range" are relative. Formerly it was considered that only type 2 poliomyelitis virus strains could be successfully propagated in mouse brains. Recently type 3 poliomyelitis virus(7) has

been shown capable of propagation in mice. Quantitative studies may indicate whether modified growth cycles of "unadapted" type 1 poliomyelitis virus do in fact occur in the mouse brain. Infectivity for neither monkey nor tissue-culture fibroblasts is lost in the process.

There was failure to demonstrate interference in brains of mice between type 1 virus and mouse-adapted type 2 virus in the dosages and time intervals herein reported. When *incomplete* growth cycles of "unadapted" influenza virus occur in mouse brain(8) a variety of effects including interference with wholly unrelated viruses(9) may be demonstrated. Failure to demonstrate interference in our experiments is in harmony with previous findings of one of us(10) in which it was shown under the conditions therein employed that interference could not be demonstrated between type 2 (Lansing) virus and fecal and spinal cord materials subsequently(4) shown to be immunologically distinct type 1 viruses. It was also found that two immunologically *related* type 2 strains, *i.e.*, M.V. which is "unadapted" for mice and Lansing virus failed to exhibit interference.

Conclusions. Unadapted type 1 (Brunhilde) poliomyelitis virus inoculated intracerebrally in mice can be recovered 5 days and 10 days subsequently from the spinal cord of such mice as well as from their brains. Such virus does not appear to have undergone immunologic alteration and is neutralized by a type 1 (Brunhilde) monkey antiserum. The biological state of this unadapted virus in the central nervous system of the mice is such that it does not interfere with the propagation of mouse adapted type 2 (Lansing) poliomyelitis in such mice. It is suggested that the phenomenon may represent circumstances other than mere passive persistence of virus in the recipient mouse brain.

1. Dick, G. W. A., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 591.

2. Youngner, J. S., Ward, E. N., and Salk, J. E., *Am. J. Hyg.*, 1952, v55, 291.

3. Robbins, F. C., Enders, J. F., and Weller, T. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 370.

4. The Committee on Typing of The National Foundation for Infantile Paralysis, *Am. J. Hyg.*, 1951, v54, 191.

5. Schlesinger, R. W., *J. Exp. Med.*, 1949, v89, 491.

6. Cairns, H. J. F., *Nature*, 1950, v166, 910.

7. Li, C. P., and Habel, K., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 233.

8. Schlesinger, R. W., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 541.

9. Vilches, A., and Hirst, G. K., *J. Immunol.*, 1947, v57, 125.

10. Lepine, P., Steigman, A. J., and Sabin, A. B., *Science*, 1949, v109, 17.

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A Turbidimetric Method for Assay of Vi Antigen. (19842)

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Recent studies(1,6) on the purification of Vi antigen from *Escherichia coli* required a rapid quantitative method for determining the antigen content of extracts and partially purified fractions. Since serological assays were tedious and quantitatively uncertain, a turbidimetric method was devised which took advantage of the propensity of acidic mucopolysaccharides and similar lyophils to form fine flocculent suspensions with albumin at low pH. This is analogous to the turbidimetric assay of hyaluronic acid reported by Kass and Seastone(2) and others(3,4). Although the exact chemical nature of this antigen has not yet been ascertained, its acidic nature, high molecular weight, and good correlation between the specific preciptin titers and the optical densities suggested the use of turbidity measurements as a quantitative measure of the antigen.