

cutaneously in various localities with the balance of the killed virus suspension, *i. e.*, 73 cc. which contained practically the entire 3 brains originally emulsified. Small amounts of the virus suspension were employed for aerobic culture purposes and these tests indicated that no viable bacterial forms (aerobic) were present in the emulsion.

After vaccination with this enormous quantity of killed virus the animal was allowed to remain in his cage for one month, when he was tested intraspinously with 0.5 cc. of fresh herpes brain emulsion. After 3 days the animal died with typical symptoms of herpes encephalitis. The brain was subsequently found to contain potent herpes virus when tested in other animals.

This experiment is an attempt to show conclusively that with killed herpes virus no immunity can be produced in rabbits even when *enormous quantities* of killed virus are employed for this purpose. It is likely that the same pertains to other viruses and gives additional evidence to support the principle, long held, that *living virus is essential* in the production of virus immunity.

7909 P

Immunological Studies with Poliomyelitis and Vaccine Viruses in Monkeys.

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Recent work on attempts to produce active immunity in monkeys with poliomyelitis virus by McKinley and Larson¹ and quite recently by Kramer, Schaeffer and Park,² Brodie,^{3, 4} and Kolmer and Rule^{5, 6} has indicated that it is possible to produce such immunity with virus treated with sodium ricinoleate, immune serum or formalin. In addition Park and his group and Kolmer have em-

¹ McKinley, J. C. and Larson, W. P., *Proc. Soc. Exp. Biol. and Med.*, 1927, **24**, 297.

² Kramer, S. D., Schaeffer, M., and Park, W. H., *J. Immunol.*, 1934, **27**, 199.

³ Brodie, M., *Science*, 1934, **79**, 594.

⁴ Brodie, M., *J. Immunol.*, 1935, **28**, 1.

⁵ Kolmer, J. A. (with the assistance of A. M. Rule), *Am. J. Med. Sci.*, 1934, **188**, 510.

⁶ Kolmer, J. A., and Rule, A. M., *J. Immunol.*, 1934, **26**, 505.

ployed such vaccines without harm in children and adults. Aycock and others have given live virus to monkeys subcutaneously without producing paralysis. Brodie⁷ has demonstrated that a single sub-infective intracutaneous inoculation of active virus gives rise to an immunity which appears at or soon after the sixth day and reaches its height by the twentieth day. The immunity so produced persists for at least one year. A second injection of the antigen gives rise to considerable additional immunity. These various observations give hope that some method may be found to produce immunity against this disease.

We here report briefly a method we have been studying for producing immunity with poliomyelitis virus which involves the use of minute amounts of living and untreated virus given intracutaneously to monkeys. Such a method in order to be of practical use should be harmless and it should produce a degree of immunity sufficient to protect the animals against a subsequent paralyzing dose of poliomyelitis virus given intracerebrally. While the various methods mentioned above depend upon treatment of the virus with some substance such as formalin or a fatty acid salt it may be of practical significance to determine if live, untreated, virus in small doses (similar to vaccine virus) may be employed with efficiency and without danger. Without doubt the chemically treated virus mentioned above is still living for there is no evidence that killed virus of any type will produce immunity. Since viruses are notoriously intracellular parasites it would seem that the chemical treatment of a virus suspension would first destroy extracellular virus and the intracellular virus would then be released in the body tissues very slowly by destructive action upon the virus-containing cells. Herein may be the explanation for the success of such chemically treated virus vaccines.

To approach the problem from another angle we decided to study methods of producing immunity with poliomyelitis virus with live, untreated, virus. We have found that when we employ minute doses, such as 0.5 cc. of a 16% emulsion of our polio infected monkey cord (Aycock strain), and introduce the virus intracutaneously we cannot protect the animal against our virus given, after 6 weeks, intracerebrally. Such animals develop paralysis. However, when we mix such small doses of live, untreated, virus of such potency with the virus of vaccinia and vaccinate the animals by intracutaneous injection we find that such a method may produce a solid immunity in monkeys so that, 6 weeks following vaccination,

⁷ Brodie, M., *J. Immunol.*, 1934, **27**, 395.

they are fully protected against intracerebral injections of paralyzing doses of live virus.

Thomsen⁸ reported briefly on a similar experiment in 1912 but in the experience of this author no immunity was produced in the small number of animals employed in the course of his work. However, this work was reported shortly after the virus of poliomyelitis was discovered. We have not determined the mechanism involved in this reaction but we have postulated the possibility of the gradual entrance of poliomyelitis virus into the tissues of the animal through the aid of the concomitant virus infection, vaccine virus, which may open the pathway for the poliomyelitis virus. In effect we may have a situation similar to chemically treated virus, if our hypothesis is correct, where the living virus is slowly absorbed and in insufficient dosage to produce paralysis. It would be interesting to know if, after long residence in the monkey, the virus of poliomyelitis has undergone some change biologically and, if returned to man, will no longer produce paralysis when given subcutaneously or intracutaneously as we have described.

7910 C

Effect of Age of a Specific Medium on Morphology of Colonies of Certain Pathogenic Fungi.*

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In this work batches of media were allowed to age at laboratory temperature, planted with organisms and morphological differences observed. In aging, the medium loses water and the nutrient concentrates so that while the ratio of ingredients is probably approximately the same the moisture content, as will be noted below, becomes much less. Our routine W medium, consisting of 4% peptone, 1% dextrose, 1½% agar and adjusted to pH 5.6, was used. The age of media which was placed on laboratory bench tops varied by approximately 10-day intervals from fresh medium to 90-day-old medium.

⁸ Thomsen, O., *Z. f. Immunitäts.*, 1912, **14**, 198.

* Contribution No. 50 from the Department of Biology and Public Health, Massachusetts Institute of Technology, Cambridge, Mass.