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Attempts to Produce Immunity with Large Quantities of Killed Herpes Virus.

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It has long been agreed and repeatedly stated that one of the principal differences between viruses and bacteria is the fact that apparently no immunity can be produced with a virus which is not living. So far there has not been a single acceptable experiment which would indicate that immunity may be produced with any virus which has been definitely destroyed by heat, chemicals, or any other means. That quite the opposite is true, with at least many bacterial forms, is well known. However, though the immunology of viruses has been studied a great deal, there have been no experiments reported, so far as we are aware, in which attempts to produce immunity with killed virus in very large quantities have definitely ruled out this possibility. It is obvious that if immunity with any virus depends upon quantity of the antigen employed and not upon the living nature of the virus much might be accomplished, with greater safety, by simply concentrating the virus for immunization purposes.

We have sought to test, by critical experiment, this possibility and have employed the L. F. strain of herpes virus for our experiments. Our herpes strain is one which one of us (E. B. McK.) brought to this country from Brussels, Belgium, in 1925, and for nearly 10 years it has been propagated in the brains of rabbits in this country. It regularly kills rabbits within 3 to 5 days when given in doses of 0.25 cc. of a 10% suspension of infected rabbit brain by the intraspinal route. For our experiment we selected 3 potent, whole, recently herpes-infected rabbit brains and emulsified these brains in 75 cc. of saline. The virus suspension was then heated at between 65 and 68.5°C. in a water bath for one hour. The heated virus suspension was then tested intraspinaly in 2 normal rabbits with 0.5 cc. each. Both of these animals survived showing conclusively, considering the probable amount of virus which would have been present had it not been destroyed, that the herpes virus had been killed at this temperature for the length of time indicated.

After testing for the viability of the virus, and finding that the virus had been destroyed, a large healthy rabbit was injected sub-

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.

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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.