tissues such as the adrenals, ovaries, salivary glands, lymph nodes, bone marrow, subcutaneous tissues, fat, muscle, and periosteum.

Histologically, lesions showed a central area of necrosis with a surrounding zone of edema or hemorrhage and mononuclear cell infiltration. Inclusion bodies were not found.

In conclusion, it should be noted that during the terminal stages of the epidemic, the severity of the infection diminished greatly so that a large proportion of animals of the most susceptible age groups survived infections contracted at this time.

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Experimental Transmission of Rabbit-Pox by a Filterable Virus.

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Experimental transmission of the spontaneous rabbit-pox discussed in the preceding communication[1] was attempted early in the course of the pandemic. Since the result of bacteriological examinations together with the clinical picture of the condition made it appear unlikely that we were dealing with any of the usual bacterial agents, the initial experiments were carried out with Berkefeld "V" filtrates of various organs and tissues from rabbits showing well marked symptoms of the disease. In other experiments, unfiltered suspensions were used. The organs employed included: testicle, liver, spleen, lung, popliteal lymph nodes, brain, spinal cord, blood, and skin. Injections were made into the testicles of rabbits procured from outside sources.

The results of the first experiments were positive in that an orchitis and scrotal edema were pronounced within 48 hours, fever developed (105°-107°), the animals appeared ill, frequently a diarrhea was present, and death occurred in from 4 to 8 days. This condition has been produced successively from rabbit to rabbit by means of filtered material (usually testicle) and the agent is now in the fifteenth serial passage. Other routes of inoculation have also been successfully employed, namely, intracutaneous, subcutaneous, intravenous, intraperitoneal, intramuscular, intracerebral, and nasal and conjunctival instillation. By using small doses and certain

[1] Greene, H. S. N., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 892.

routes of inoculation, it has been possible to reproduce all the clinical and pathological features of the spontaneous disease including the characteristic pock eruption of the skin and mucous membranes. In addition, complete recovery has occurred in these circumstances.

The agent is active in tissue stored in 50% glycerol at ice box temperature for at least 94 days. Unfiltered material is much more potent than that filtered through a Berkefeld "V" candle but an "N" filtrate is also active. Very small doses are sufficient to induce pronounced lesions in various sites and death has followed the intratesticular injection of 1 cc. of filtrate in a dilution of 1:8000.

Rabbits which have recovered from either the spontaneous or the experimental disease have so far been refractory to reinoculation. Furthermore, the serum from recovered animals has the property of neutralizing the virus in a 1:1 serum-virus mixture as shown by intracutaneous tests.

The disease does not resemble Virus III or infectious myxoma of rabbits either clinically or pathologically, and the results of crossed inoculation experiments and of virucidal tests show that there is no relationship between the respective agents. Similar experiments with rabbits immune to the Shope tumor and to the Shope tumor plus infectious myxoma are now in progress.*

With respect to vaccine virus, however, there is evidently some relationship but the two viruses are apparently not identical. Rabbits immune to the N. Y. City Board of Health vaccine virus are refractory to injections of rabbit-pox filtrates variously applied but intradermal injection of unfiltered pox virus results in a positive although highly modified skin reaction. Adult vaccine virus immune rabbits do not develop rabbit pox when exposed to "open" cases but young vaccine virus immunes do. In these instances, the disease was considerably milder than that developed by normal control rabbits (litter mates). The results of virucidal tests with vaccine virus immune serum and rabbit pox virus are in keeping with the above observations in that the serum has a definite but only a partial protective power. We have not been able to produce lesions typical of vaccinia in either the cornea or scarified skin but in several instances cytoplasmic inclusions indistinguishable from Guarnieri bodies were found in corneal epithelial cells. These observations, together with the distinctive clinical features of the spontaneous and experimental rabbit pox disease, tend to the conclusion

* We are indebted to Dr. T. M. Rivers for the infectious myxoma experiments and to Dr. Carl TenBroeck for those with the Shope tumor.

that the present agent is not identical with the vaccine virus with which we have worked.

Information on the host range of the virus is still incomplete. We have not succeeded in inoculating rats but in one instance the virus has been recovered from the testicle of a guinea pig inoculated intratesticularly. The inoculation of mice has also been accomplished by means of the intracerebral route. With this procedure death occurs in 4 to 6 days and the brain contains active virus. It is now in the fifth serial mouse to mouse passage. Other animal species and the calf in particular, are being tested for susceptibility to the virus.

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Cultivation of Vesicular Stomatitis Virus.

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Carrel, Olitsky, and Long[1] reported the successful cultivation of the virus of vesicular stomatitis of horses in 1928. In their cultivation experiments, guinea pig tissues were used throughout. The method they employed consisted in placing embryonic tissue or adult bone marrow in contact with the virus which had been suspended in Tyrode's solution for 1½ to 24 hours and then transferring to Carrel flasks containing coagulated plasma to which serum was added when marrow was used, or embryonic juice when embryonic tissue was used. The flasks were incubated for 7 to 10 days at 39°C.

No further reports of successful *in vitro* cultivation of the virus of vesicular stomatitis have been made.

This paper describes a much simplified method, similar to that employed by Li and Rivers[2, 3] in the cutlivation of vaccine virus, for the cultivation of the virus of vesicular stomatitis. A satisfactory and readily available technique for the titration of the virus is given. Through the kindness of Dr. W. E. Cotton, samples were sent us of Indiana and New Jersey strains of virus which had

[1] Carrel, A., Olitsky, P. K., and Long, P. H., *Compt. rend. Soc. biol.*, 1928, **98**, 827.

[2] Li, C. P., and Rivers, T. M., *J. Exp. Med.*, 1930, **52**, 465.

[3] Rivers, T. M., *J. Exp. Med.*, 1931, **54**, 453.