Preparation of a Collodion Lined Tube—A test tube 1.5 cm in diameter is filled half full of collodion U.S.P. and then emptied slowly while being rotated. The tube is placed inverted in a wire rack and is allowed to stand for at least 18 hours to give time for the ether and alcohol to completely evaporate. The membrane usually adheres firmly to the glass unless separation begins to take place at the rim.

Summary and Conclusions. The coagulation time of normal human blood on a collodion surface is longer than the coagulation time on a paraffin surface even though the force of adhesion between blood and collodion is much greater than between blood and paraffin. There is no clot retraction in collodion lined tubes.

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Attempt to Propagate Poliomyelitis Virus Through Serial Passage in Cotton Rats by "Spreading Factor of Duran-Reynals."

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Numerous attempts have been made to "adapt" poliomyelitis virus to passage in the eastern cotton rat since Armstrong[1] reported that a strain of virus isolated from one of a number of cases occurring during an outbreak of poliomyelitis in Lansing, Michigan, could be propagated in the variety *Sigmodon hispidus hispidus*. These attempts, including those of Armstrong, to obtain takes with other strains of poliomyelitis virus have failed. Toomey[2] has recently reported unsuccessful attempts to propagate 9 other monkey adapted strains of this virus. He inoculated 9 groups of *Sigmodon hispidus littoralis* rats, each with one of 9 strains of virus and later when symptoms failed to develop, these animals were shown to be susceptible to the Lansing strain.

Duran-Reynals[3, 4] has shown that testicular extract ("spreading factor") added to the virus of vaccinia increased the extent of the

1 Armstrong, C., *Pub. Health Rep.*, 1939, **54**, 1719.

2 Toomey, J. A., and Takacs, W. S., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 536.

3 Duran-Reynals, F., *Compt. rend. Soc. de biol.*, 1928, **99**, 6.

4 Duran-Reynals, F., *J. Exp. Med.*, 1929, **50**, 327.

lesions in the rabbit, and Hoffman and Duran-Reynals[5] showed that it had similar enhancing effects on the viruses of herpes, vesicular stomatitis and Borna disease. In this laboratory several experiments have been made recently to further investigate the effects of testicular extract on the pathogenesis of a number of filterable viruses. Our unreported and incomplete experiments, although of a slightly different character, tend to confirm those reported for the virus of herpes and also suggest that a somewhat similar effect is obtained when the spreading factor is combined with minimal doses of the virus of lymphocytic choriomeningitis given to mice intracerebrally.

It was decided to attempt to adapt a strain of poliomyelitis virus to the cotton rat by suspending the virus in testicular extract, in the hope that this factor might enable the virus to propagate in a tissue that presented conditions which possibly were partially though not entirely suitable for its growth. Since others had failed to adapt any but the Lansing strain, by ordinary methods, our only interest was to test the effect of the "spreading factor" in addition to the standard methods. The possibility that the testicular extract might have an inhibitory effect held no significance for the cotton rat itself was to all practical purposes refractory to the virus.

A portion of cord from a monkey infected with the Toomey "T" strain of virus which was obtained from Dr. McKhann of The Children's Hospital in Boston was ground in a sterile mortar with alundum to make a 10% suspension in a fresh 15% saline extract of rabbit's testicle. After centrifugation at low speed the supernatant was inoculated in doses of 0.06 cc intracerebrally, 0.06 cc intranasally, and 1.00 cc intraperitoneally in each of 3 cotton rats *(Sigmodon hispidus littoralis)*. These animals were obtained from central Florida. Two others were inoculated in a similar manner with testicular extract alone. From the tenth to the twelfth day one of the rats inoculated with virus refused food and appeared irritable. It was killed with ether and the brain removed. One half of this (the inoculated hemisphere) was frozen at —76° C and the other half was ground in a mortar with alundum in 0.5 cc of testicular extract and 1.5 cc of infusion broth, centrifuged slowly and inoculated in the dosage and manner previously described to one other cotton rat. Following this, 4 other such serial passages were made, each at an interval of from 12 to 14 days. In each instance after the tenth or twelfth day it was noted, by recording the weight of the daily food consumption, that the intake had decreased 25% to 50% from the normal for that animal. On the last passage the

[5] Hoffman, D. C., and Duran-Reynals, F., *J. Exp. Med.*, 1931, **53**, 43.

cotton rat was weighed daily and a slight weight loss was noted after the tenth day. No paralysis was observed in any of these rats nor after the first passage was any symptom noted suggestive of central nervous system involvement.

One half of the brain from the fifth passage (the inoculated hemisphere) was suspended by grinding as previously in 2.0 cc of infusion broth (without spreading factor) and the supernatant after centrifuging inoculated intracerebrally in 0.5 cc doses to two macacus rhesus monkeys. In a similar manner 2 other monkeys were inoculated with a suspension of the frozen brain from the first passage rat. None of the monkeys developed paralysis or any other signs suggesting poliomyelitis. One of those inoculated with the fifth passage brain died on the 22nd day with severe diarrhea associated with an extensive adhesive peritonitis due to infection with *Strongyloides oesophagostomum.* Fifteen days following the inoculation of the monkeys with the first passage brain and 30 days after inoculating the surviving one with the fifth passage brain, all were inoculated intracerebrally on the contralateral side with a 5% suspension in infusion broth of the same monkey cord used to inoculate the original cotton rats. These monkeys all developed typical poliomyelitis, with the onset of symptoms first noted on the sixth and seventh days and progressing to extensive, complete paralysis.

Discussion. It appears that the cotton rat *Sigmodon hispidus littoralis,* when inoculated under the conditions of this experiment with the Toomey "T" strain of poliomyelitis virus, together with the "spreading factor of Duran-Reynals" does not serve as a host capable of permitting the preservation or propagation of the virus. The infectivity of the original material and the susceptibility of the monkeys used to test for the presence of the virus in the brains of the first and fifth passage rats, were controlled by subsequent inoculation with the same virus. The uniformity of loss of appetite in the cotton rats on the tenth or twelfth day after inoculation was the only symptom suggesting any reaction to this strain of virus. It might be considered that the passage interval was too short or too long (it was shorter than the incubation period noted by Armstrong during the early passages of the Lansing strain and longer than that of the fully adapted virus), but if the virus had retained its activity in the brain of the first passage rat its presence should have been detectable at 12 days by monkey inoculation, since the inoculated portion of the cotton rat brain was used to prepare the suspension.