was, therefore, fixed at 2 mg per day as was mentioned previously. When the dose was increased to 6 mg per day during the latter part of the experiment, the mice showed no ill effects, probably because they had built up a tolerance to choline chloride while receiving the smaller doses.

Summary. A statistical investigation of the estrous cycle graphs showed that there was no significant difference between any of the 3 groups of test animals and that, under the conditions of this experiment, choline chloride did not inhibit the estrous cycle.

10148

Cultivation of Rabies Virus in the Allantois of the Developing Chick Embryo.

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We have attempted to cultivate rabies virus in the allantois of the developing chick embryo following the technic described by Burnet.[1] We have not succeeded in obtaining serial passage beyond the third generation. However, a number of observations have been made on the survival and localization of the virus, which are of some interest, and are briefly summarized in this note.

In our attempt to grow the virus, the allantois of embryos of various ages were infected with brain virus and the eggs left in the incubator for varying lengths of time. At intervals the infected eggs were opened, the region of the allantois around the point of inoculation removed, triturated, passed to other eggs and at the same time the presence and concentration of virus determined by injecting various dilutions intracerebrally into mice.

At the beginning only the allantois was examined and tested for virus. When the egg was not contaminated with bacteria, the allantois appeared normal and the embryo was alive and of normal development. At times there were traumatic lesions on the allantois but these were not specific.

The results may best be grouped in series. In one group embryos 11 to 14 days old were infected with 0.1 cc of 1 :50 dilution of brain virus and the allantois examined 3 to 5 days later by inoculating a

[1] Burnet, F. M., *Special Report Medical Research Council*, 1936, No. 220.

a 1:10 suspension in saline intracerebrally into mice. Of 20 eggs in good condition thus tested 10 contained virus and 10 were negative.

In a second series the allantois were examined 2 hours to 14 days after infection and the concentration of virus determined by titration in mice. In one experiment the virus was present after 2 hours in a dilution of 1:1000 and after 5 days in the same concentration. In another experiment the virus was found in a dilution of 1:10 after 3 hours, 1:100 after 3 days, and 1:1000 after 7 days; in this experiment passages to other eggs were negative after 5 and 8 days and positive in one egg after 10 days in a dilution of 1:10. Further passage failed. In a third experiment, 10-day-old embryos were infected; those opened 2 days later were negative and those after 10 days were positive in a dilution of 1:10,000. Only once has it been possible to carry serial passages from allantois to allantois through 3 generations. The embryos were 7 days old when infected. Nine days after the infection the allantois was positive in a dilution of 1:100 and a passage was made. One egg opened after 10 days was negative; 2 opened after 14 days were positive in dilutions of 1:100 and 1:1000 respectively. A third passage was made and this time only one allantois was positive in a dilution of 1:10. The allantois of the fourth passage were negative.

A summary of the work showed the erratic character of the results. Of 22 embryos tested 10 were positive and 12 negative. It was apparent that the rabies virus persisted in the allantois of the chick embryo for varying lengths of time and at times it was present in greater concentration than at the time of infection. The results, however, were irregular and it is not possible to conclude whether or not actual multiplication took place.

Seeking an answer to this question, another series of cultures was set up in which both the brain of the embryo and the allantois were tested for virus. The embryos were 6 to 7 days old when infected and 20 to 21 days old when tested. In this series the allantois was positive only once in 8 experiments in a dilution of 1:10, whereas the brain was positive in each case in dilutions varying from 1:10 to 1:1000. In the experiment in which the allantois was positive in a dilution of 1:10, the brain was positive in a dilution of 1:1000.

In several instances chicks from infected eggs hatched. One chick was killed when one day old and the virus titer of the brain was 1:100; another chick was killed 3 days after it hatched and the brain contained virus in a dilution of at least 1:1000. A third chick was kept 15 days. During the first week it showed symptoms of excitability and ataxia, which slowly subsided. On the fifteenth day the

chick was killed and the brain tested for virus; it was negative in a dilution of 1:10—presumably the virus had disappeared.

It appears, therefore, that rabies virus does not infect the allantois with regularity but regularly finds its way into the brain of the chick embryo. The development of the embryo is apparently not affected by the presence of the virus and may proceed in the same manner as the uninfected controls. Whether the virus can be adapted to the chick and its virulence for it enhanced by passage is a subject for further investigation.

10149 P

Activation of Renin by Blood Colloids.

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The purpose of these experiments was to learn something of the nature of the pressor action of renin. Renin was prepared from pig's kidneys in such concentration that an amount containing less than 2 mg of nitrogen produced a sharp, sustained rise (30 mm Hg. or more) of blood pressure in an unanesthetized dog. Its vasoconstrictor action was studied on perfused dogs' tails (40 experiments), so eliminating all factors causing vasoconstriction other than peripheral ones.

It was found that undialyzed renin (0.2 cc) caused moderate vasoconstriction, contrariwise dialyzed renin (1 cc) was inactive. Normal heparinized dog's whole blood (dilution of 1 to 3 parts Ringer's solution) was inactive but if dialyzed renin (1 cc) were mixed with dog's blood (1 cc 1:3 dilution) marked vasoconstriction occurred. The undialyzed renin could be potentiated by addition of dog's blood.

Heparinized plasma in doses of 0.5 cc was vaso-inactive but strongly activated dialyzed renin just as whole blood had done. But protein-free ultrafiltrate of plasma no longer exhibited activating capacity while the colloid residue on resuspension was active.

The vasoconstrictor action of undialyzed renin appears to be due in part to non-protein materials contained in it, for the protein-free fluid obtained by boiling the renin produced vasoconstriction, but it was not potentiated by blood as was renin. Dialysis removes these