

10147

Effect of Choline Chloride on Estrous Cycle of Mice.

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Duncan, Gallagher, and Koch¹ stated that choline (a part of the lecithin molecule) has an inhibiting action on the estrous cycle of rats. At the R. B. Jackson Laboratory a similar experiment was tried on mice, but there was no apparent effect on the estrous cycle. The following method was used:

Ten dilute brown virgin female mice (dba stock) about 7 weeks old were injected subcutaneously every day with 2 mg of choline chloride dissolved in water. Injections were given as above for one month; the dose was then increased to 4 mg per day for one week and finally to 6 mg per day for another week.

Vaginal smears were examined every day in order to see if the choline chloride had any inhibiting effect on the estrous cycle. The results were plotted graphically and showed clearly that the estrous cycle had not been affected. The onset of a cycle was recorded as occurring when cornified cells appeared in the vaginal smears.

An equal number of the same stock of mice were injected with equal amounts of ammonium chloride (the inorganic analogue of choline chloride) and again the estrous cycle was not affected.

A third group of 8 mice received no injections at all. Their estrous cycles were plotted graphically and showed no significant difference from the cycles of the mice which were treated with choline chloride and with ammonium chloride.

The average length of the estrous cycles was about 5 to 6 days. The comparison of the mean intervals between cycles in the 3 groups is shown in Table I.

TABLE I.

	Difference	Probable error of difference
Choline chloride vs. controls	.241	$\pm .312$
" " vs. ammonium chloride	.102	$\pm .285$
Ammonium chloride vs. controls	.139	$\pm .314$

Preliminary experiments showed that the lethal dose of choline chloride was about 8 to 10 mg, and that some temporary ill effects occurred when the mice were given only 6 mg. The regular dose

¹ Duncan, Gallagher, and Koch, *Science*, 1937, **85**, 23.

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, tritured, 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.