GROWTH OF DUCK INFLUENZA VIRUS A/DUCK/HOKKAIDO/5/77 (HAV7N2) AND HUMAN INFLUENZA VIRUS A/KUMAMOTO/22/76 (H3N2) IN DUCK AND CHICKEN COLON ORGAN CULTURES

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Recently, duck influenza viruses have been successfully isolated from the duck cloaca and intestines. The isolation of human influenza viruses, however, has not been observable. The present study was attempted to determine if there is any difference in the growth of duck and human influenza viruses in duck colon organ cultures. For comparison, chicken colon organ cultures were also used.

First, the methods of colon organ cultures from duck and chicken were studied, and it was determined that NCTC–DMEM medium, which consisted of one part NCTC–135 medium and nine parts Dulbecco's modified Eagle's medium in 95% O2 and 5% CO2 at 37°C, provided the optimum conditions for maintaining the cultures. Under these conditions, the colons from 1, 7, 14 and 28 day-old ducks and chickens were successfully maintained for 48 and 84 hours respectively.

In the colon of 1 day-old ducks, Hav7N2 grew with a small inoculum such as $10^{2.1}$ EID₅₀/ml, and the virus titer of the medium reached $10^{6.2}$ EID₅₀/ml. On the contrary, H3N2 did not grow in the 1 day-old ducks even with a much larger inoculum such as $10^{7.1}$ EID₅₀/ml.

In the colon of 28 day-old ducks inoculated with an inoculum of more than $10^{3.7}$ EID₅₀/ml, Hav7N2 reached a titer of $10^{5.7}$ EID₅₀/ml, whereas there was no growth of H3N2.

In the colon of 1 day-old chickens, both viruses grew with inoculums of $10^{8.1}$ and $10^{2.7}$ EID₅₀/ml, but the yield of Hav7N2 was approximately 100 times higher than that of H3N2.

In the colon of 28 day-old chickens, Hav7N2 showed considerably good growth with inoculums of more than $10^{3.7}$ EID₅₀/ml, whereas H3N2 inoculated with the same amount of virus demonstrated only slight growth accompanied by a rapid decrease of the virus titers.

Immunofluorescent studies showed that the specific fluorescence was demonstrable in the colon epithelial cells only when the viruses grew well. The fluorescence was observed first in a small number of epithelial cells, and strong fluorescence was observed frequently near the surface of the epithelial cell. Then the fluorescence tended to be demonstrated in the whole cytoplasm of the large numbers of the epithelial cells.
The fact that the duck and not the human influenza viruses showed good growth in the duck colon agrees well with the fact that only duck influenza viruses have frequently been isolated from the duck intestine.

A QUANTITATIVE STUDY ON THE SEMINIFEROUS EPITHELIUM OF THE ADULT MINK IN THE POST-BREEDING SEASON

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Cellular association of the seminiferous epithelium was quantitatively analyzed histologically and kinetically in the testes of five 11-month-old and five 23-month-old Pastel mink immediately after the breeding season. Special attention was paid to the seasonal and aging variation of the spermatogenesis.

1) The cycle of the seminiferous epithelium was classified into 8 stages using the criteria described by Tiba et al. (1968). Discriminant analysis did not show any significant differences between the relative frequencies of each stage in 3 different sites of the testis (the capital pole, equatorial zone and caudal pole). In addition with the exception of one case, there were no significant differences between the left and right testes and 6 different loci in the 11- and 23-month-old cases.

2) The mean vector of stage frequency indicated a significant difference ($P < 0.01$) among 9 mink; however, there were no significant differences between the 2 groups of 11- and 23-month-old mink. The results suggested the importance of great individual variation rather than the aging factor in the seminiferous cycle. Nine mink were classified into 2 groups of more and less than 6.4 of the germinal cell index. Discriminant analysis revealed a significant difference between 2 groups of the index ($P < 0.01$).

3) The mean vector of stage frequency among the 3 groups of 10- (previous data by Tiba et al., 1968), 11- and 23-month-old mink differed greatly.

The findings obtained in this study pointed to the existence of a different arrangement in the kinetics of spermatogenesis between the breeding season and the post-breeding season. In addition, it was also observed that individual variation played a greater role than the aging variation of stage frequency in the cycle of the seminiferous epithelium.