Technical Papers

Distemperoid Virus Interference in Canine Distemper[1]

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Serial passage of the canine distemper virus through ferrets has been shown by Green (*6*) to reduce its virulence for foxes and dogs so that it produces a mild immunizing distemperoid infection when injected into these animals. In 1938, when the distemperoid virus was first used to eliminate distemper from the large Fromm herds of silver foxes (*7*), it was noted that numerous fox pups exposed to fatal infections apparently were cured in the early stages of the infection. Veterinarians trying out the new distemper vaccine in dogs sometimes injected it first into mongrels sick with distemper and observed that the vaccine, instead of exaggerating the infection, seemed to assist in recovery of the dogs. Stader and Slaughenhaupt (*15*), in studying the immunizing effect of distemperoid virus vaccine in dogs, observed that upon injecting the vaccine there was an immediate protection against distemper and referred it to a "cell-block" effect. Schlotthauer (*14*) reported that dogs injected with 15 mg. of distemperoid virus did not contract distemper upon exposure immediately after inoculation, but that dogs injected with smaller doses, such as 7.5 and 2.5 mg., did so.

The interference phenomenon in animals was first observed, independently, by Hoskins (*9*) and by Magrassi (*12*) in 1935. Magrassi reported an interference between nonencephalitic and encephalitic herpes viruses in rabbits, while Hoskins demonstrated interference between neurotropic and pantropic yellow-fever viruses in monkeys. Findlay and MacCallum (*5*) confirmed and extended the work of Hoskins and were able to show that the Rift Valley fever virus protected monkeys against pantropic yellow-fever virus, while the neurotropic strain of yellow-fever virus protected mice against the Rift Valley fever virus. That the virus of lymphocytic choriomeningitis protected monkeys against a virulent monkey strain of poliomyelitis virus was demonstrated by Dalldorf (*3*). Jungeblut and Sanders (*11*) showed that the virus of murine poliomyelitis, also, protected monkeys against virulent monkey strains of poliomyelitis virus, and recently Jungeblut (*10*) has made extensive studies of the factors governing the phenomenon.

Andrewes (*1, 2*) noticed an interference between virus III and the virus of Shope fibroma in rabbits and later used tissue cultures to demonstrate that a strain of influenza A virus interfered with the growth of a neurotropic variant of that virus. Subsequently it was shown by the Henles (*8*) and by Ziegler, Lavin, and Horsfall (*16*) that interference occurs between the viruses of influenza A and influenza B, between these and swine influenza virus, and between inactivated and active influenza viruses; the antagonistic effects of these viruses on one another were observed in embryonated eggs. The interference phenomenon between bacterial viruses has been studied extensively by Delbruck and Luria (*4*), while studies made by workers on similar phenomena between plant viruses have been reviewed by Price (*13*).

We have now obtained experimental data to establish that the ferret-passage virus will, upon intramuscular inoculation, interfere with the course of a distemper infection established by nasal inoculation with a highly virulent distemper virus. In one of our experiments, 40 red fox pups were divided into groups of 10 and each group was inoculated as follows: Group A, as a control group, received virulent distemper virus only. Group B was inoculated with virulent distemper virus and distemperoid virus simultaneously. Group C received virulent distemper virus first and distemperoid virus 3 days later. Group D received virulent distemper virus first and distemperoid virus 12 days later.

The virulent distemper virus was prepared for inoculation from frozen, infected fox spleens. The ground tissue was diluted to 25 per cent in Ringer's solution, and each fox was inoculated intranasally with 0.4 cc. (100 mg.). The distemperoid virus was ferret-passage virus of the sixty-third generation in ferrets, frozen as 25-per cent homogenized ferret spleen in horse serum. In all cases, 200 mg. was inoculated intramuscularly into the legs of the designated foxes. The animals were observed daily for symptoms of distemper. A necropsy was performed on animals that died from the infection, and smears were made of the epithelial cells lining the bladder and the trachea. In every dead animal typical cytoplasmic inclusions characteristic of distemper were seen. Results observed for the different groups of animals are as follows:

Group A. On the sixteenth day following the inoculations of virulent distemper virus, all animals in

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the control group exhibited marked symptoms of distemper. The first death occurred on the twenty-fifth day, and at the end of 36 days all the animals in this group had died of typical distemper.

Group B. In this group, which was inoculated simultaneously with virulent distemper virus and distemperoid virus, 2 foxes showed symptoms on the sixteenth day. By the twenty-third day, 5 animals showed some symptoms of distemper. However, they recovered promptly, and by the thirty-first day all appeared well.

Group C. In the group receiving distemperoid virus 3 days after the inoculation of virulent distemper virus, only 2 foxes showed symptoms. These became sick on the twenty-third day and were normal again on the thirty-first day.

Group D. One of the animals receiving the distemperoid virus 12 days after the virulent distemper virus showed symptoms on the nineteenth day, and 4 of this group showed symptoms by the twenty-third day; all had recovered by the thirty-second day.

The decisive results obtained in this study are further accentuated by the fact that foxes seldom recover from distemper once they have exhibited any symptoms whatsoever. In this case, the distemper virus used was a fox-passage virus of high virulence for that animal. From the results, it seems definitely established that there is a marked antagonistic effect of the distemperoid virus on a virulent distemper infection, and this effect appears sufficiently significant to be utilized practically in the treatment of distemper. In fact, in this experiment the antagonistic effect of the distemperoid vaccine was just as great when the ferret-passage virus was used 12 days after inoculation of virulent virus as when it was used within an hour after such inoculation; and in both cases the distemperoid vaccine prevented any fatalities. Our results corroborate our earlier clinical observations on foxes and those of Stader and Slaughenhaupt on dogs, as well as the experimental observations of Schlotthauer. In addition, a definite therapeutic effect of the modified virus on the virulent infection seems demonstrated for the incubation period and early stage of the infection. Thus, it appears that a modified virus may be useful for both the prevention and the therapeutic treatment of a disease caused by a virulent strain of the same virus.

Summary

A distemper virus modified by ferret passage so as to become a harmless vaccine for foxes and dogs exhibits the interference or cell-blockade phenomenon with respect to a virulent distemper infection in foxes. Ten control foxes receiving virulent distemper virus died, while 30 foxes receiving distemperoid virus in addition lived.

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Availability of Carotene From Kale

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Measurement of the vitamin A value of vegetables presents considerable difficulty. Figures quite disproportionate to known biological values have been reported by various investigators for the chemical determination of the carotene content of certain vegetables. Different vegetables of equal carotene content have been found to vary in their vitamin A value as determined by biological assay. This fact has led to the assumption that when carotene is ingested in the form of vegetable foods, it is less effective than when taken in pure form. It has, at times, been suggested that the discrepancies may be explained by the known inaccuracies of the biological estimations, coupled with the problem of obtaining representative samples. However, it is unlikely that the errors of all workers in this field should be consistently in the same direction. Therefore, the true relationship between the carotene content of vegetables as measured by chemical analysis and bio-assay is of fundamental importance.

As a part of an investigation on the factors influencing the utilization of carotene from vegetable foods, experiments were devised for the study of kale to determine whether or not digestibility of the vegetable tissue is one of the factors causing these differences. These experiments were designed so that chemical chromatographic analyses for carotene were made of the kale parallel with bio-assays on both the kale itself and an extract of the carotenoids from the vegetable.