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Cultivation of the West Nile Virus in the Developing Chick Embryo.

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The developing chick embryo has been employed with considerable success for the cultivation of a number of neurotropic viruses.[1] The present investigation deals with the cultivation in the embryonated egg of another neurotropic virus, namely, the West Nile agent, recently isolated by Smithburn and his coworkers.[2] Mice, Rhesus monkeys[2] and Syrian hamsters[3] are susceptible to this agent but guinea pigs and rabbits are resistant.[2]

Conventional methods were used for the inoculation of developing embryos. The first eggs were infected with a 10% suspension of brain from a hamster moribund with the West Nile disease: this material, when injected intracerebrally in a dilution of 10^{-6}, killed mice. The infected embryos became sluggish within a few days after injection and at this time material was removed aseptically for passage. Various portions of the passage embryos, as well as their membranes and fluids were studied for amount of virus present. Tissues were ground in a mortar and 10% suspensions were prepared with buffered saline containing 1% normal guinea pig serum.

The West Nile virus multiplies well in embryonated egg inoculated by various routes, and can be readily maintained by serial passage of several types of tissue (Table I). Embryos became sluggish 2 to 4 days after inoculation and death usually occurred within the ensuing 24 hours; the disease progressed more rapidly in the eggs inoculated with large quantities of virus. Death occurred with such regularity following inoculation of virus on the chorioallantoic membrane of 10-day embryos or into the yolk sac of 5-day embryos that virus titrations could be satisfactorily carried out by this technic. Comparative titrations done simultaneously in eggs and in mice with dilutions of the 5th passage chorioallantoic membrane showed that eggs were slightly more susceptible than were mice, *i.e.*, a 10^{-6} dilution of the material was lethal for most of the mice inoculated intracerebrally while the 10^{-7} dilution killed most of the infected eggs.

Serial transfers were made on the chorioallantoic membrane of 10-day embryos and in the yolk sac of 5-day embryos for 6 and 7 passages, respectively (Table I). Consecutive passages were discontinued when it became apparent that the West Nile virus could be carried indefinitely in either chorioallantoic membrane or in yolk sac.

The virus became disseminated throughout the embryonated egg following inoculation by all routes investigated. This is evident from the results summarized in Table I, for the embryo contains large amounts of virus following injection of the chorioallantoic membrane, the allantoic sac, or the yolk sac. Furthermore, following introduction of virus into the yolk sac the yolk sac membrane was approximately as infectious as the embryo. Allantoic fluid contained comparatively small amounts of virus, indeed, inoculation into the allantoic sac resulted in the appearance of more virus in the embryo and allantoic membrane than in the allantoic fluid itself.

Pathological changes encountered in embryonated eggs, moribund or dead from infection with the West Nile virus, were not very conspicuous. Marked congestion of the embryo was noted following inoculation by any route. The chorioallantoic membrane was somewhat thickened and edematous following inoculation of virus on this structure, but pock-like lesions were never encountered. Histological examination of a number of the membranes and embryos failed to reveal changes other than those evident on macroscopic inspection.

Summary. The West Nile virus multiplies

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TABLE I.
Comparison of Various Routes of Inoculation and the Distribution of Virus.

Route	Passage	Age of embryo	Infectivity of: Allantoic membrane	Allantoic fluid	Embryo	Embryo head
Allantoic membrane	1st	10 days	10^{-6}	$<10^{-2}$	10^{-5}	
	2nd	"	10^{-5}	10^{-3}		10^{-6}
	4th	"	10^{-5}			10^{-4}
	6th	"				10^{-6}
Yolk sac	1st	5 days	10^{-5}*		10^{-6}	
	2nd	"	10^{-6}*		10^{-6}	
	5th	"			10^{-5}	
	7th	"			10^{-6}	
Allantoic sac	1st	10 days	10^{-4}	10^{-3}		10^{-6}

Infectivity determined by intracerebral titration in mice.
$<$ = less than.
*Yolk sac membrane.

rapidly throughout the embryonated egg following inoculation by any of the usual routes. The embryo contains large amounts of virus while the allantoic fluid contains comparatively small amounts of the active agent. Death of the infected embryos occurs with such regularity that titration of virus can be made as well in eggs as in mice.

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Studies With Dogs Maintained on Diets Low in Fat.*

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The observations and data presented here constitute a part of a general study of the factors which are concerned with the nutrition of the skin. The findings that the degree of unsaturation of the serum lipids tends to be low in patients with eczema[1,2,3] and that the dietary use of fats rich in unsaturated fatty acids exerts a beneficial effect in certain patients with eczematous skin eruptions[1,3,4] together with the observations of Burr and Burr[5,6] regarding the importance of linoleic and arachidonic acids in nutrition, indicate that particular attention must be given to the unsaturated fatty acids in such a study. The dog seems to lend itself as a suitable experimental animal for observing the effects of a fat deficient diet on the general health and condition of the skin of the animal as well as for studying the distribution of the fatty acids in the various lipid fractions of the blood under these conditions.

Material and Methods. At weaning, 8 puppies born to a collie mother were given a diet low in fat composed of skim milk 73.2%, cottage cheese 7.0%, polished rice 5.7% and sucrose 14.1%. To the daily diet of each

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5 Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1929, **82**, 345; *ibid.*, **82**, 366.

6 Burr, G. O., *Federation Proceedings*, 1942, **1**, 224.