Summary. Poliomyelitis developed in all of 8 rhesus monkeys following the injection of freshly passaged "M.V." virus into their sciatic nerves. In 6 of these monkeys roentgenologic examinations of the long bones and determinations of inorganic and organic acid-soluble phosphorus in the blood just before inoculation gave no evidence of vitamin D deficiency. Indications of varying degrees of this deficiency were present in the other two animals.

There was no evidence that the capacity of poliomyelitis virus to invade the central nervous system along peripheral nerves depended on vitamin D deficiency.

13354

Simultaneous Inoculation of Variola and Vaccinia Viruses in Embryonated Eggs.

JOHN B. NELSON. (Introduced by Carl TenBroeck.)

From the Department of Animal and Plant Pathology, The Rockefeller Institute for Medical Research, Princeton, N.J.

In connection with some observations on the stability of variola virus it became of interest to determine the behavior of a small amount of vaccinia virus in the presence of a large amount of variola virus on inoculation in embryonated eggs. The relation of the ensuing findings to the problem of mutability as it pertains to variola virus will be considered elsewhere. The results are reported here simply as one example of the activity of 2 infective agents inoculated simultaneously in fertile eggs, a phase of that method on which there appears to be little data.

Two bacteriologically sterile strains of variola virus (Minnesota and Chinese) which had been carried through 202 and 38 transfers, respectively, in embryonated eggs were used, together with a single strain of vaccinia virus (New York City Board of Health) maintained by occasional egg transfer. The 2 variola strains react identically in 10-day fertile hen's eggs following implantation on the retracted chorioallantoic membrane. By the 3rd day a 10% membrane suspension of either strain produces a thickened confluent area at the site of inoculation but does not affect the embryo. The strain of vaccinia virus behaves very differently, and the 2 virus species may generally be identified by inspection. Eggs inoculated with this strain of vaccinia show a much thinner area of reaction in

the membrane by the 3rd day, the result of rapid cell degeneration, and usually a dead embryo.

Saline suspensions were prepared containing variola virus diluted 10^1 and vaccinia virus diluted 10^6, the dilution figure being based on the wet weight of small membrane sections removed aseptically on the 3rd day from 10-day hen's eggs inoculated with the respective viruses. Prior to dilution the membrane sections were finely ground in glass tissue grinders. Similar dilutions, serving as activity controls, were also set up with each virus alone. Two or 3 embryonated eggs were then inoculated with approximately 0.5 cc of the virus mixtures and the individual suspensions. After incubation at 37°C for 3 days the inoculated eggs were opened for examination, and approximately 10% suspensions prepared from the membranes. Three to 5 successive egg transfers were subsequently made with each series.

The detailed results of one experiment are as follows: Two embryonated eggs opened on the 3rd day after inoculation with a mixture containing Chinese variola virus diluted 10^1 and vaccinia virus diluted 10^6 showed active embryos and the thickened membrane characteristic of variola, an identical reaction being obtained with the 10^1 dilution of variola virus alone. Eggs inoculated with the 10^6 dilution of vaccinia virus alone also showed active embryos with scattered discrete foci in the membrane, indicative of a highly diluted suspension of that virus. A 2nd transfer was made with each series, using 10% membrane suspensions. In the eggs inoculated with the virus mixture the embryos were inactive and the membranes moderately thickened, suggestive of an intermediate reaction. The eggs inoculated with variola virus alone showed the customary thickened membrane and active embryos; whereas in those inoculated with vaccinia virus alone the membrane was thin and the embryos dead, the characteristic reaction of this virus in low dilution. In the 3rd passage the individual virus suspensions behaved in the usual manner, but the mixture resulted in death of the embryos and thinning of the membrane at the site of inoculation, the typical manifestations of vaccinia virus alone.

Essentially the same results were obtained in 2 additional experiments using similar mixtures of vaccinia virus and the Chinese strain of variola, the former being masked in the first transfer but predominating by the 3rd. The procedure was repeated with mixtures of the same vaccinia virus and the Minnesota strain of variola. In the first test there was no apparent development of the vaccinia virus through the 5th transfer although it was demonstrable in the 10^6 dilution

prior to mixing. In 2 subsequent tests, however, the preceding results were duplicated, vaccinia virus multiplying in the presence of a high concentration of variola virus and predominating by the 3rd transfer. It may be added that in the long series of egg inoculations made with the Minnesota strain of variola virus, since it was isolated in 1938, its behavior in embryonated eggs has remained constant with no departure in the direction of vaccinia virus.

Egg membranes from the 3rd transfer of the Chinese variola-vaccinia virus mixture and the 4th transfer of the mixture with the Minnesota strain were tested in dilutions through 10^7 by the inoculation of scarified areas in the skin of susceptible rabbits. A graded response typical of vaccinia virus was obtained through a dilution of 10^6. The corresponding suspensions of the two variola strains alone behaved in the customary way, producing no macroscopic reaction in the skin of inoculated rabbits. Cutaneous inoculation of the monkey to determine the presence of variola virus in membranes inoculated with the virus mixtures was not carried out.

Summary. Mixtures of variola virus diluted 10^1 and vaccinia virus diluted 10^6 were inoculated in embryonated eggs and followed by 3 or more successive transfers. In 5 of the 6 tests the highly diluted vaccinia virus was masked in the 1st transfer but subsequently multiplied and predominated by the 3rd. Two of the transferred mixtures on inoculation in rabbits produced a typical vaccinial reaction in dilutions through 10^6.

13355

Biotin and Prevention of Dermatitis in Turkey Poults.*

H. PATRICK, R. V. BOUCHER, R. ADAMS DUTCHER AND H. C. KNANDEL.

From the Departments of Agricultural and Biological Chemistry and Poultry Husbandry, The Pennsylvania State College, State College, Pa.

During attempts to determine the riboflavin requirement of poults, in which both simplified and commercial type rations were used, dermatitis occurred regardless of the riboflavin intake. Jukes[1]

*Authorized for publication on October 20, 1941, as paper No. 1052 in the Journal Series of the Pennsylvania Agricultural Experiment Station. This research was aided by a grant from the Coöperative G. L. F. Exchange, Inc., Ithaca, N.Y.

[1] Jukes, T. H., *Poultry Sci.*, 1938, **17**, 227.