TABLE II. Utilization of Carotene in Water (20% Tween 40) Administered Subcutaneously to Vit. A-Deficient Rats; 21 Days.

Group	Supplement	No. rats No. survivors	Avg days survival	Avg daily wt gain
I	0	5/1	11	—
II	.1 ml aqueous 20% Tween 40, oral	6/0	14	—
III	9.6 γ carotene in Tween, subcut.	5/5	—	4.7

and the livers and kidneys analyzed for vit. A and carotene. Small amounts of carotene (6-9 γ, total) were found in the livers of the group receiving 4.2 γ carotene by injection, and only traces of the pigment were detected in the group injected wth 1.6 γ carotene. Neither vit. A nor carotene was found in the kidneys of these two groups. The tissues of the other groups contained no detectable vit. A or carotene. Examination of the thigh muscles of the rats receiving injections of aqueous carotene revealed no apparent atrophy. The muscle appeared to be torn, however, presumably due to the repeated insertion of the needle. A light yellow coloration throughout the tissues of the leg indicated that the carotene was not completely absorbed.

A second experiment was performed to determine if aqueous carotene when injected subcutaneously would also be effective in curing vit. A-deficiency. Three groups of vit. A-deficient rats were placed on the vit. A-free ration described above. Group I, the controls, received vit. E in the food, while groups II and III received this vitamin in the supplement. Group II was given daily 0.1 ml of 20% Tween 40 in water, orally, while group III received 9.6 γ of carotene in 0.1 ml of water (20% Tween 40) subcutaneously. The results in Table II show that the Tween solution (group II) had no appreciable effect on the survival of vit. A-deficient rats, and that subcutaneous, aqueous carotene (group III) is well utilized.

Summary. Carotene solubilized in water by the use of Tween 40 will support growth in vit. A-deficient rats when as little as 1.6 γ is injected intramuscularly daily. For maximum growth, approximately 4 to 6 times as much carotene is required parenterally as orally. Aqueous carotene is also well utilized when given subcutaneously. Carotene dissolved in cottonseed oil and administered parenterally is essentially not utilizable.

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Developmental Abnormalities in the Chick Embryo Following Infection with Newcastle Disease Virus. (18867)

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The report of Gregg(1) that maternal rubella may act as a teratogenic agent in human subjects, has focused interest on the possible role of virus infections in the production of congenital abnormalities. In efforts to pursue the study of teratogenicity of viruses, the following laboratory experiments were planned to observe the effects of viral agents on the developing chick embryo. Initial investigations using fowl pox virus were inconclusive. However, Newcastle disease virus produced a high percentage of developmental defects, particularly in the lens of the eye and in the auditory sac.

Methods and materials. The Newcastle disease virus was supplied by Dr. F. R. Beaudette.* Fertile eggs from New Hampshire

1. Gregg, N. McA., Trans. Ophthalmological Soc. of Australia, 1941, v3, 35.

Red chickens were incubated in a forced-draft incubator at 99°F. Virus and control inoculations were made at 45 hours of incubation. By opening all of the eggs and preparing them with cover slips immediately prior to the time of inoculation, all virus and control injections in each experiment were completed within one hour. The virus inoculum consisted of allantoic fluid from a 12-day embryo inoculated 24 hours previously with Newcastle disease virus. Control inoculum consisted of allantoic fluid from a normal 12-day embryo. .01 ml of inoculum was injected beneath the vitelline membrane over the blastoderm near the edge of the area pellucida, the flow from the needle directed toward the embryo. In preliminary experiments virus inoculated embryos uniformly died within 24 to 36 hours. To make valid their study for developmental abnormalities, embryos were harvested alive 20 to 24 hours after inoculation. (This seemed important in light of observations(2) in chick embryos that postmortem changes may simulate developmental defects.) Embryos were fixed in Bouin's solution, stained in dilute alum cochineal, cleared and mounted *in toto* for examination under the dissecting microscope.

Although individual variation in developmental progress of chick embryos of the same incubation setting occurs, the majority of the embryos at the time of inoculation corresponded to developmental Stage 11 as described by Hamburger and Hamilton(3). At this stage the olfactory placode is absent, the auditory vesicle is a wide-open pit, the optic vesicle and optic stalk are present but invagination of the primary optic vesicle and the appearance of the lens placode begins a few hours later. The amnion does not yet cover any of the embryo. At the time of harvest, 20 to 24 hours later, the embryo corresponds approximately to developmental Stage 16 or 17. At these stages the amnion may extend to the 26th somite or may be completely fused over the embryo, the auditory vesicle is an almost closed sac, the double walled optic cup is established, and the mouth of its cavity is practically filled by the lens in the form of a thick walled epithelial sac. The olfactory placodes appear as thickenings of the ectoderm or as shallow pits in the thickened ectoderm.

Results. In the first 150 embryos infected with N.D.V. 42% showed abnormalities of the lens or auditory sac or both. Of the 150 control embryos inoculated with virus-free material, no similar defects were found. In certain subsequent experiments the specific abnormalities were obtained in 80% to 90% of the embryos following inoculation with N.D.V. Virus samples of such known teratogenicity, when stored in hermetically sealed ampules at —50°C, retained these teratogenic properties for at least four weeks. In 75 embryos infected with samples of stored virus 90% showed the defects. More extensive studies are in progress.

General retardation in growth of the embryo and varying degrees of retardation of the amnion were observed in some of the embryos but the outstanding abnormalities were seen in the embryonic lens and the auditory sac (otocyst) ranging from obvious reduction in the size of the two organs to their complete absence. Both defects were found to occur bilaterally or unilaterally. A control embryo showing the normal appearance of the structures concerned is shown in Fig. 1, and embryos showing typical abnormalities are shown in Fig. 2. In embryos in which the lens or

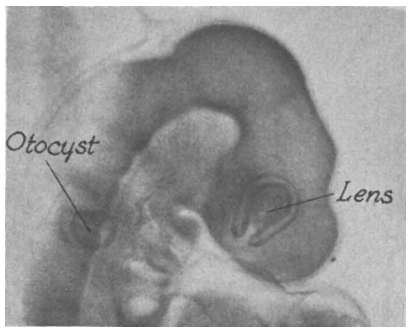

FIG. 1. Normal embryo of 65 hr incubation.

* F. R. Beaudette, Poultry Pathologist, N. J. Agric. Exp. Station, Rutgers University, New Brunswick, N. J.

2. Blattner, R. J., and Williamson, A. P., *Arch. Path.*, 1950, v50, 676.

3. Hamburger, V., and Hamilton, H. L., *Jour. Morph.*, 1951, v88, 49.

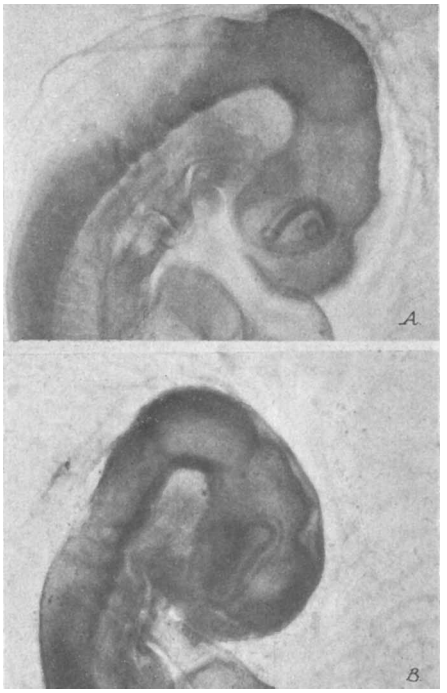

FIG. 2. Newcastle disease infected embryos of 65 hr incubation. Upper photograph demonstrates small lens and small otocyst. Lower photograph shows absence of both lens and otocyst.

otocyst was present but abnormal in structure the defect appeared to consist of significant retardation in morphological differentiation as well as reduction in size of the organ. Although in most instances the optic cup appeared normal, in one or two embryos it appeared slightly distorted. It must be emphasized that although general retardation of growth appeared in some of the embryos, the majority of those showing specific defects of the lens and otocyst were in the same stage of development as the control embryos with respect to visceral arches, limb bud differentiation, and somite count. A typical example of such observations is demonstrated in Fig. 3.

Microscopic examination of serial sections of 4 infected embryos confirmed the conclusions based on observations of the whole mounts. In all embryos studied the otocysts or lens, or both were missing, or severely damaged and markedly retarded. In two embryos the olfactory plates were absent. Detailed study of these microscopic findings is in progress. Preliminary studies of the lens defects suggest findings in autopsy studies of human fetuses from maternal rubella cases in which retardation of the lens was reported by Cordes and Barber(4) and necrosis and degenerative changes were described by Swan(5).

Summary. (1) The lens and auditory sac showed defective development or were completely missing in 42% of 150 chick embryos infected with Newcastle disease virus at 45 hours of incubation. In 150 control embryos, inoculated with normal allantoic fluid, no similar defects were found. (2) In subsequent experiments the specific defects were obtained in 90% of the N.D.V. infected embryos. Virus samples of such known teratogenicity if stored in hermetically sealed ampules at —50°C retained their teratogenic properties for at least 4 weeks.

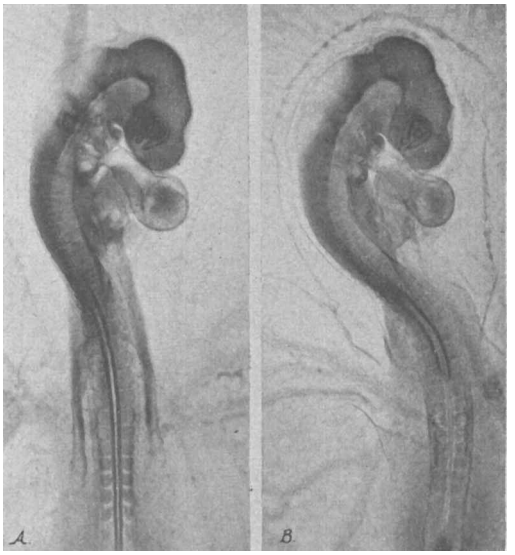

FIG. 3. Embryos of 65 hr incubation. A, normal; B, infected with Newcastle disease virus.

4. Cordes, F. C., and Barber, A., *Arch. Ophth.*, 1946, v36, 135.

5. Swan, C., *J. Path. and Bact.*, 1944, v56, 289.

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