consider the likelihood that the onset of superficial pigment deposition and cessation of calcium secretion are functionally related.

Summary. The eggshell pigments of wild-type and mutant white egg Japanese quail have been identified by spectrophotometric analysis of eggshell solutions, solutions of wild-type superficial pigment, and extracts of uterine tissue. Oöporphyrin and biliverdin both contribute to wild-type eggshell pigmentation but oöporphyrin alone seems responsible for the slight pigmentation of mutant white eggshells. In wild-type quail, deposition of superficial eggshell pigment begins between the second and third hours prior to oviposition and is accompanied by an abrupt reduction of oöporphyrin content in uterine tissue extracts.

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Interferon Inhibition of Cytoplasmic DNA Accumulation in Vaccinia Virus Infection. A Radioautographic Study. (30235)

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While the action of the antiviral protein interferon has been extensively investigated in RNA virus infected cells, few studies on DNA virus infected cells have been published. The growth of a few DNA viruses has been found to be inhibited by interferon but no studies are available on whether virus DNA formation is also inhibited(1). Such studies would contribute to the elucidation of the mechanism of action of interferon.

In the present investigation the effect of interferon on cytoplasmic DNA incorporation in vaccinia virus infected chick embryo fibroblasts was investigated by radioautography (2). Interferon treatment markedly decreased the appearance of cytoplasmic DNA under conditions where virus synthesis was also depressed.

Materials and methods. Chick embryo fibroblasts (CEF) were grown on cover slips set in very lightly seeded glass plates(3). Cells were treated overnight with 10 units of chick interferon diluted in medium or with only medium and then washed. The chick interferon had been prepared by infecting CEF with chikungunya virus for 48 hours. The supernatant fluids were heat inactivated (65°C, 30 minutes) and titrated for interferon content by the method of Lindenmann and Gifford(4). The cells were then infected at a multiplicity of 5 plaque forming units per cell of a chick chorioallantoic membrane grown strain of vaccinia virus. After 2 hours for virus adsorption plates were again washed; 5 hours after infection each plate was pulsed for 1 hour with 3 μc of tritiated thymidine (3 c/mM, Radiochemical Centre, Amersham). The plates were then washed 3 times with phosphate buffered saline, the coverslips removed and the cells were fixed in acetone for 15 minutes at room temperature. The coverslips were then dried for 2 days in a vacuum desiccator and mounted on subbed slides. The slides were dipped in Ilford L-4 emulsion, diluted 1:2 in 1% gelatin, dried,

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TABLE I. Vaccinia Virus Growth in Interferon Treated Chick Embryo Fibroblasts (CEF).

Hours after infection	Titer (plaque forming units/ml)	
	Controls	Interferon treated
2	*18×10^4	12×10^4
4	33×10^4	30×10^4
18	35×10^6	25×10^4
24	17×10^6	15×10^4

* CEF were pretreated with 10 units of interferon or buffer for 14 hours, washed, and infected with a chick chorioallantoic membrane strain of virus at a virus to cell multiplicity of 5:1. After 2 hours the cells were washed, new medium was added, and allowed to incubate at 37°C in a CO_2 incubator for the indicated number of hours. The cells were then forzen and thawed 3 times and supernatant fluids were assayed for vaccinia virus on CEF.

and exposed 6 weeks at 4°C. They were developed for 4 minutes, stopped 10 seconds in 1% acetic acid, and fixed for 5 minutes in Kodak rapid fixer(5). For cell morphology, the coverslips were stained with Giemsa stain.

In the case of virus growth assays, CEF were treated with interferon or buffer and then infected as described previously. At intervals, plates were removed from the 37°, CO_2-incubator, frozen and thawed 3 times, and tritiated in CEF for virus yields.

Results. The marked inhibition of vaccinia virus growth by interferon pretreatment of cells in this system is shown in Table I. Cells were also studied for cytoplasmic incorporation of DNA by radioautography after exposure to tritiated thymidine for one hour. A background of 0-3 single or small groups of cytoplasmic grains per cell was present. Cells containing cytoplasmic aggregates of 10 or more grains were scored as positive, but in positive cells the cytoplasmic accumulations of grains were often so dense that individual grains could not be made out. Cells containing more than one group of cytoplasmic grains were counted as a single positive cell.

The percentage of cells containing cytoplasmic grains 5 hours after infection is shown in Table II. Here it may be seen that 22% or 16% of control cells infected with vaccinia virus contained grains. In control cells which were uninfected and had been pretreated with buffer or interferon only an occasional cell was found to contain cytoplasmic grains probably due to background artifacts. In interferon pretreated and later virus infected cells the percentage of grains was only slightly higher than in uninfected controls.

Discussion. Cytoplasmic DNA incorporation in vaccinia virus infected cells is due to new virus DNA(2). The finding that interferon pretreatment inhibits cytoplasmic DNA incorporation in these experiments therefore indicates that the synthesis of new virus DNA is inhibited. This is probably not the basic mechanism of action of interferon as the growth of RNA viruses which have no DNA dependent step for their replication is also inhibited by interferon. Since RNA synthesis is necessary for the replication of vaccinia virus(6), it is not unlikely that the action of interferon is related to the inhibition of RNA accumulation in virus infected cells.

Consistent with the findings of this study are those of Ohno and Nozima who showed that interferon inhibited the formation of thymidine kinase(7) and cytoplasmic RNA (8) in vaccinia virus infected cells. Both thymidine kinase(9) and early cytoplasmic RNA(10) are formed under the control of the DNA of the infecting virus. Since virus RNA synthesis and synthesis of a specific virus protein were shown to be depressed early in infection it is not unexpected that new virus DNA formation would also be inhibited as shown in this study. Other recent

TABLE II. Effect of Interferon on Cytoplasmic DNA Formation in Vaccinia Virus Infected Chick Embryo Fibroblasts (CEF).

Treatment of cells	% of cells containing cytoplasmic grains	
	Exp 1	Exp 2
Vaccinia virus	*22%	16%
Medium only, no virus	1%	†N.D.
Interferon, no virus	2%	N.D.
Vaccinia virus + interferon	3%	1.5%

* CEF on coverslips were treated with interferon and/or virus as indicated in Table I. After 5 hours of infection the cells were pulsed for 1 hour with 3μC of tritiated thymidine. After washing and fixing L-4 emulsion was put on the coverslips; the coverslips were exposed 6 weeks at 4°, developed, fixed, and stained with Giemsa stain. Each reported result is the average of 2 counts by 2 observers on replicate samples.

† Not studied in this experiment.

studies have shown that the action of interferon is dependent on cellular RNA(3) and protein synthesis(11). A proposed mechanism of action for interferon which would account for most of the facts now known is that interferon acts as an inducer of a new cellular RNA which in turn causes the formation of a new protein(3). The antiviral action of this protein may be due to an inhibition of the accumulation of new viral RNA (3,12).

Summary. Treatment with interferon which markedly depressed vaccinia virus growth also inhibited the production of new virus DNA. This was indicated by the small number of cytoplasmic DNA grains due to tritiated thymidine found in radioautographic studies of vaccinia virus infected cells pretreated with interferon.

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Metabolism of *l*-Alpha-Tocopherol by the Vitamin E-Deficient Rabbit.* (30236)

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The cure of nutritional muscular dystrophy either by oral or by intravenous *l*-alpha-tocopheryl acetate is demonstrated by studies described in this report. The *l*-alpha-tocopheryl acetate was one-fifth as potent as the *d*-epimer and the lower potency was associated with a greater rate of loss of tocopherol from the body.

Methods. Young New Zealand rabbits of both sexes weighing approximately 500 g were fed a purified vitamin E-deficient diet (1) as modified by Diehl(2). All animals received 0.05% sodium sulfaquinoxaline in the drinking water 5 days of each week. The animals that served as controls were given an oral supplement of 8 mg of *dl*-alpha-tocopheryl acetate dissolved in 0.2 ml of corn oil 3 times weekly.

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Recovery experiments were begun when the unsupplemented rabbits showed severe muscular weakness as judged from the ability of the rabbit to right itself when placed on its back(3). The animals weighed between 600 and 900 g at this time, which was 3 to 4 weeks after the purified diet was begun. The oral dose for recovery was 50 mg either of *d*-alpha-tocopheryl acetate or of *l*-alpha-tocopheryl acetate dissolved in one ml of corn oil or olive oil. The intravenous dose was 50 mg of either epimer suspended in 4 ml of 0.15 M NaCl containing 1% polyoxyethylene sorbitan monooleate (Tween 80).

Total tocopherols of serum were measured by the method of Rindi(4) and liver tocopherols were measured by the method of Swick and Baumann(5). Urinary creatine and creatinine were measured by the method of Folin(6) with minor modifications. The ratio of urinary creatine to creatinine was obtained by dividing the μmoles of creatine by