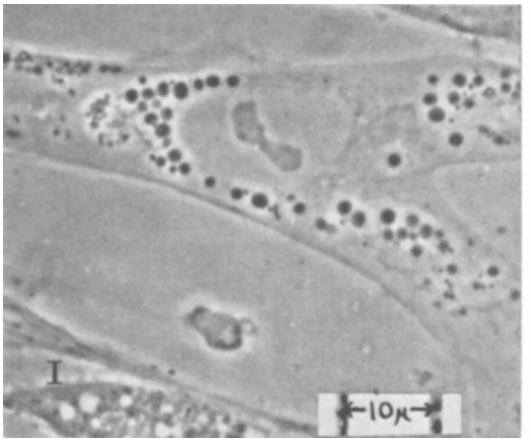

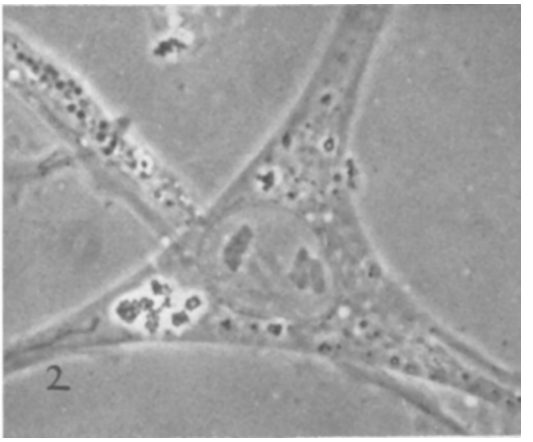

FIG. 1. Normal, untreated chick connective tissue cell in tissue culture *in vitro*. Living; phase microscopy. Distance between two dark bars in rectangular white area is 10 μ.

FIG. 2. Chick connective tissue cell in culture containing 7.5 mg/ml of desoxycorticosterone glycoside. Note reduction of cytoplasmic lipoid material. Living; phase microscopy. Same magnification as in Fig. 1.

trol cultures (based upon about 20 pairs for each concentration) were as follows: 30 μg/ml, –6.2; 15 μg/ml, 2.4; 7.5 μg/ml, 6.3; 3.5 μg/ml, 2.2; 2 μg/ml, 0.0.

Structurally, cells treated with concentrations of desoxycorticosterone glycoside above 30 μg/ml were characterized by enlarged nuclei and an increase in the rate of accumulation of fat droplets in the cytoplasm. At the highest concentrations used, (240 μg/ml) the nuclear membranes appeared to have been ruptured. Below 30 μg/ml photomicrographs show that the fibroblasts accumulated lipids in the cytoplasm at a much reduced rate (Fig. 1 and 2). At these concentrations there were few, if any, enlarged nuclei.

Summary. Concentrations of 15 μg/ml or lower of desoxycorticosterone glycoside stimulate growth of chick embryo fibroblasts and decrease the accumulation of cytoplasmic lipids. Concentrations of 30 μg/ml reduce growth and damage the nuclei of the cells.

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Action of Cortisone on Developing Chick Embryo.* (20255)

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This study was undertaken to determine whether the growth inhibition produced by cortisone is generalized or is limited to certain organs or regions. Previous studies on chick embryos(3,4) and on newly-hatched chicks (2) have emphasized growth-inhibition following injection of cortisone. In the present experiments, effects of cortisone are evidenced by a proportionate weight reduction of all parts tested except for the liver (smaller), eyes (larger), and gonads (larger), when com-

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TABLE I. Relative Body and Organ Weight in Control and Cortisone-Treated Embryos.

	Saline controls ± S.E.*	.5 mg cortisone ± S.E.*	1.0 mg cortisone ± S.E.*
Body wt in g	23.7 ± .9	22.1 ± 1.1	13.6 ± 1.6
Liver†	2322.1 ± 46.5	2057.5 ± 59.8	1598.2 ± 91.2
Heart	730.4 ± 30.5	745.5 ± 26.9	803.5 ± 82.6
Spleen	37.5 ± 2.8	38.4 ± 2.3	35.7 ± 4.9
Ovary	23.8 ± 2.0	27.0 ± 3.2	59.1 ± 9.8
Testes	23.4 ± 2.6	22.2 ± 2.3	35.8 ± 3.5
Tibia	754.6 ± 51.1	741.0 ± 27.5	654.2 ± 34.4
Eye	1879.0 ± 73.1	—	2666.0 ± 163.6

* Stand. error calculated from $\sqrt{\frac{Sd^2}{n(n-1)}}$.

† All organ wt figures expressed as mg/100 g body wt.

parisons are made on the basis of milligrams of organ weight per 100 g of body weight. There is a retention of water in the cortisone-treated chick embryo.

Materials and methods. Chick embryos of 8 days of incubation were treated with a single dose of cortisone acetate (Merck) injected onto the chorioallantoic membrane. The Merck preparation of cortisone was diluted with normal saline so that one-half or one milligram of cortisone acetate was suspended in 0.3 ml of fluid. Embryos similarly injected with 0.3 ml of normal saline were used for controls. Benzyl alcohol is used as a preservative in the cortisone suspension, but controls injected with this alcohol were not considered necessary, since Karnofsky *et al.* (3) found that the amount of benzyl alcohol contained in a milligram dose of cortisone is far below the threshold for producing detectable effects on the chick embryo. To compare the relative sizes of organs, experimental series which had received one-half or one milligram of cortisone and saline-injected controls were sacrificed at 18 days of incubation. The wet weights of the whole embryo, liver, spleen, heart, and tibia were determined. The gonads were fixed *in situ* in Bouin's fluid, washed in 70% alcohol, dissected and weighed as fixed tissues from 70% alcohol. For purposes of comparison, all weights are expressed as milligrams of tissue per 100 g of body weight. Other series were treated with one mg of cortisone and sacrificed at daily intervals from 10 through 18 days of incubation. The lengths of the embryo, neck, forelimb and hindlimb were measured. The embryo was extended by placing it against a straight edge and body length was taken as the distance from the crown to the tail tip. Neck measurements were taken from the base of the skull to the pectoral girdle. Limb measurements were made by straightening the limb and measuring from the body wall to the tip of the longest digit. Wet and dry weights were used in calculating the percent of water in the embryo. To determine dry weight, embryos were dried in a hot-air oven to constant weight.

Results. While cortisone inhibited the growth of all parts of the embryo, not all organs of the dwarfed embryos were affected proportionately. Of the organs weighed, the liver, gonads and eyes showed an effect. The weights of heart, spleen and tibia were not significantly different from the controls when compared on the basis of organ weight per 100 g of body weight. Because of their recessed position, the bird kidneys do not lend themselves to dissection for weighing, but they appeared to be proportionately reduced in size in the dwarfed embryos. Table I summarizes the results of the one-half and one-milligram series.

One-half mg series. Only the liver showed a significant reduction in weight, being reduced to approximately 86.5% of the control weight. The embryos and their membranes were similar to the controls.

One mg series. The entire embryo showed a growth inhibition following treatment with one milligram of cortisone. The liver was reduced to approximately 70% of its control

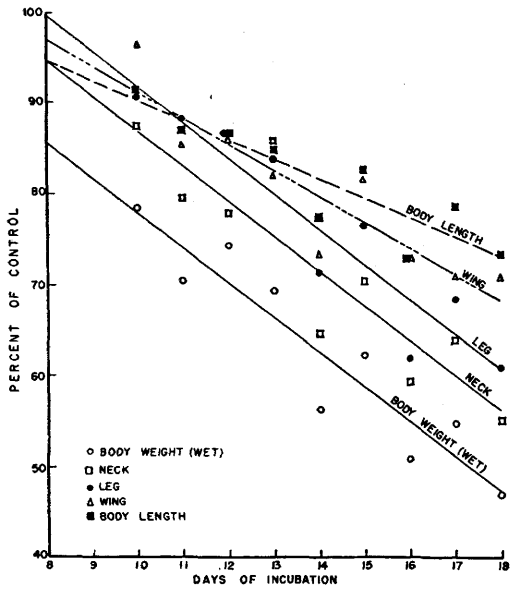

FIG. 1. Graph shows inhibition of growth of the embryo following injection at 8 days with 1 mg cortisone. Lines are extrapolated back to the day of injection. For wing, leg and body wt and body length $p < .001$, and for the neck $p < .01$.

proportional weight. The gonads—both ovaries and testes—and the eyes were relatively heavier. The ovaries of the treated embryos were about 250% of the control weight while the testes represented about 150% of the control weight. The eyes were approximately 142% of their controls.

In embryos showing the most marked cortisone-effect the amnion and chorion formed a tight-fitting double layered sac and the allantois and yolk sac were greatly inhibited. Such yolk sacs had thick convoluted walls which sat on the yolk like a skull cap. Although the yolk inside this cap-like sac was gelatinous, the rest of the yolk was liquid. Feather formation was inhibited over the entire body but most markedly on the head. The degree of feather inhibition is so variable that such an endpoint is not feasible for assaying cortisone activity. The inhibition of feather growth has been reported(3) and might be expected to occur, since it is known that cortisone produces atrophy of the skin and inhibition of hair growth in mammals(1).

The lengths of the body, neck, wings and legs were less in the treated than in control chicks. This is plotted in Fig. 1. The slope of the lines was determined by the method of least squares and the lines are extrapolated back to the eighth day when cortisone was administered. The greatest inhibition seems to be between 8 and 10 days for all parts except the legs.

The body wall of the treated embryos was generally translucent and many embryos were edematous. Comparison of wet and dry weights showed that the treated embryos retained water (Fig. 2). Control embryos began to show a decrease in water content at about 13 days of incubation, but the difference in water content between the treated and control embryos became statistically significant at 15 days of incubation.

Discussion. This study confirms the general results of Karnofsky *et al.*(3) that cortisone acetate inhibits growth and development of the chick embryo beyond 8 to 10 days of incubation. This inhibition is proportional, except that the liver is relatively smaller while the eyes and gonads are relatively larger.

Karnofsky(2) has given cortisone to day-old (40-45 g) chicks at a dose level of 5 mg per day for 10 days. By converting his average weights for several organs to milligrams of organ weight per 100 g of body weight, the following approximate percentages are obtained for immature cortisone-treated chicks: liver, 123% of the control weight; spleen, 76%; adrenals, 113%; heart, 123%. Probably not all of these values are significant. They do indicate a difference both in growth

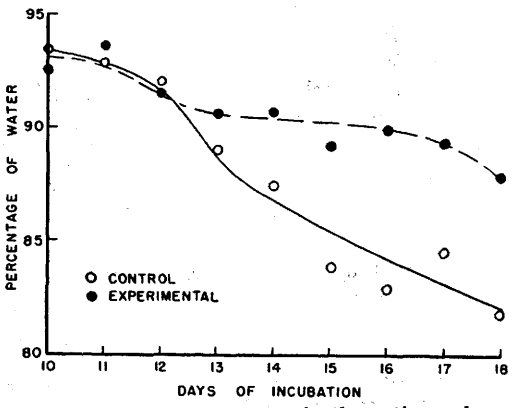

FIG. 2. Percentage of water in the entire embryo. The differences are significant on and after 15 days of incubation.

inhibition of embryonic and newly-hatched chicks and in response to various dosage levels.

Summary. 1. Chick embryos at 8 days of incubation were given one-half or one mg of cortisone acetate by injection onto the chorio-allantoic membrane. 2. The one-half mg dose resulted in a reduction of the liver to approximately 86% of the control weight. 3. In the one mg series, the liver was relatively smaller and the eyes and gonads were relatively larger on the basis of 100 g of body weight. In absolute values, the entire embryo and all organs were greatly reduced in size. 4. Measurements of body length, neck, wings and legs after treatment with one mg cortisone indicated the greatest degree of growth inhibition during 8 to 10 days of incubation for all except the legs. 5. A comparison of wet and dry weights from day 10 through day 18 of incubation showed that cortisone-treated embryos retained water. This retention becomes statistically significant at 15 days of incubation.

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Fibroma Arising from Feather Follicle of Duck Following Local Application of Methylcholanthrene to Skin.* (20256)

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The occurrence of papillomata, squamous cell carcinomata and hemangiomata in the skin of the duck following the local application of methylcholanthrene has been reported (1). In similarly treated ducks a fibroma has been observed to arise from the feather follicles. The pathogenesis and pathologic characteristics of this latter tumor are reported.

Methods and results. The technic used in the local application of a 0.25% acetone solution of methylcholanthrene to the skin of White Pekin ducks has been described(1). Ducks varying in age from 10 to 90 days were used. The birds were observed for a period of 6 months. The feathers were plucked frequently both during and after the local application of methylcholanthrene. This carcinogen was applied daily for 30 days by dropping 0.5 to 2.0 cc of the solution on the skin beneath the right wing and the adjacent portion of the body wall. The neoplasms in some of the ducks were removed for pathological study. Portions of skin showing developing feathers were also removed for histological study. A normal feather follicle is shown in Fig. 1A.

The feather shaft normally is composed of a loose fibrous tissue stroma with few blood vessels. The periphery of the feather as it emerges from the skin is covered by a thin layer of squamous epithelium. In ducks where the feathers are plucked frequently, the new feathers may fail to mature and may appear as a small projection 2-6 mm above the surface of the skin as illustrated in Fig. 2D. These abnormal feathers may become infected, hemorrhagic, gradually become dry, black structures that atrophy and then desquamate; others only atrophy and desquamate. Fig. 1B shows the macroscopic features of a tumor arising from such a follicle. Note the small feather growing from this tumor. Within 48 hours following the time this lesion was photographed, it spontaneously disappeared.

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