peaks, or a hump on the falling phase, were often seen (Fig. 1B), as in crab muscle fibers (*7*); but in other fibers the response was a smoothly rising and falling spike (Fig. 1, C and D). The irregularities resemble those seen in barium-induced spikes of lobster muscle fibers and are due to local inequalities in excitability (*2*).

The spike-generating fibers had characteristics of voltage and current (Fig. 2) that were essentially the same as those of fibers with graded responses (*10*). The effective resistance for small inward currents was about 2×10^5 ohms. For small outward currents it was about 3×10^5 ohms, indicating the occurrence of depolarizing potassium inactivation (*11*). Marked time-variant curvatures of the characteristics that were induced by inward as well as outward currents reflect decreases of membrane resistance that are due to depolarizing potassium activation and hyperpolarizing chloride activation, respectively (*11*), which are characteristic of crayfish muscle fibers.

Muscle fibers that generate spikes appear otherwise to have essentially the same characteristics as those that normally produce graded responses. The length of the sarcomeres is in the same range, they have essentially identical characteristics of voltage and current, and the depolarizing electrogenesis appears to be due to calcium activation in both varieties. The spikes induce twitch contractions, but that may be regarded as a consequence of the brief duration of a larger electrical response.

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Progesterone Antagonism of Estrogen-Induced Cytodifferentiation in Chick Oviduct

Abstract. *Progesterone administered with estrogen to the intact female chick antagonizes the characteristic effects of estrogen to induce cell proliferation and cytodifferentiation of tubular gland cells in the oviduct, events that are accompanied by the appearance of lysozyme. Progesterone appears to exert its effect by preventing proliferation of cells destined to become tubular gland cells, but once such cells do proliferate, subsequent cytodifferentiation and appearance of lysozyme are not prevented.*

Repeated administration of estrogen to immature female chicks markedly increases the weight of the oviduct (*1*) and induces the synthesis of both lysozyme and ovalbumin in the magnum portion of oviduct (*2*). These effects can be influenced by other hormones; this estrogen-stimulated weight increase is augmented by testosterone (*3*) and inhibited somewhat by glucocorticoids (*4*). Although several workers have reported that progesterone antagonizes the increase in oviduct weight induced by estrogen (*5–7*), a synergistic effect has also been described (*3, 8*).

In this investigation we studied the antagonistic effect of progesterone on estrogen-induced growth of the oviduct. This effect can be ascribed largely to an inhibition of proliferation of characteristic tubular gland cells, and, as a result, progesterone prevents the estrogen-induced synthesis of lysozyme. Recently, Kohler *et al.* (*9*) showed that ovalbumin is also localized in tubular gland cells and indicated that cytodifferentiation of these cells was induced by estrogen.

Four-day-old White Leghorn female chicks were given estrogen (17 β estradiol benzoate, 1 mg per chick) with or without progesterone (1 mg per chick). The hormones, in 0.1 ml of sesame oil, were injected daily into left and right thigh muscles. At indicated days after the administration of hormones, chicks were killed and the magnum portion of the oviduct was removed. All studies were limited to this portion. The magnum was weighed and was then homogenized in 9 volumes (weight/volume) of ice-cold distilled water for analysis of lysozyme, nucleic acids, and protein. After nucleic acids were extracted by the method of Schneider (*10*), DNA was assayed by the diphenylamine reaction with calf thymus DNA as standard (*11*), and RNA was assayed by use of the orcinol reagent with yeast RNA as standard (*12*). Protein was estimated by the method of Lowry with bovine serum albumin as standard (*13*), and lysozyme was assayed as described by Litwack (*14*). Activity is expressed as micrograms of lysozyme and is based on the activity of purified egg-white lysozyme (Calbiochem) as standard. For histological examination, parts of the magnum were fixed in 0.1*M* sodium phosphate (*p*H 7.2) containing 4 percent glutaraldehyde (*15*) and then embedded in paraffin. Sections 7 μm thick were stained with Mayer's hematoxylin and eosin.

Figure 1 shows the pattern of increase in wet weight of the magnum resulting from daily administrations of estrogen for 10 days. In the absence of hormone treatment only a twofold increase in wet weight occurred. During the first 2 to 3 days there was a lag in the increase in weight, after which the increase was rapid, amounting to 500-fold by 10 days. Within 3 days of the beginning of daily administration of estrogen there were marked increases in total DNA, as well as in total RNA and in protein

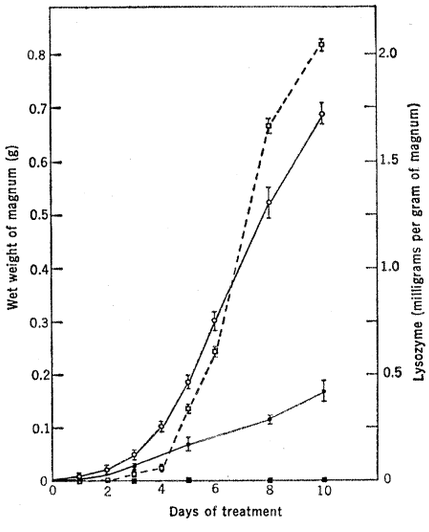

Fig. 1. Changes in the wet weight and lysozyme level in the magnum portion of oviduct during the daily administration of estrogen and its combination with progesterone for 10 days. Each point represents the mean ± standard error of the mean for four to seven chicks. ○, Magnum (wet weight) from estrogen-treated chicks, and ●, from chicks treated with estrogen plus progesterone; □, magnum lysozyme from estrogen-treated chicks, and ■, from chicks treated with estrogen plus progesterone.

Table 1. Changes in DNA, RNA, protein, and lysozyme in the magnum portion of the oviduct during the various hormone treatments. E3, E5, and E10 indicate administration of estrogen for 3, 5, and 10 days, respectively; EP5, 5 days of estrogen plus progesterone; E1 EP4, 1 day of estrogen and 4 days of estrogen plus progesterone; E2 EP3, 2 days of estrogen and 3 days of estrogen plus progesterone; E3 EP2, 3 days of estrogen and 2 days of estrogen plus progesterone; E4 EP1, 4 days of estrogen and 1 day of estrogen plus progesterone; and control, no treatment. Values for total contents (of DNA, RNA, and lysozyme) are expressed in micrograms per magnum and (for protein) in milligrams per magnum. The concentration of lysozyme is expressed in micrograms per gram of magnum (wet weight); values for all other concentrations are in milligrams per gram. Each value represents the mean ± the standard error of the mean for four chicks.

Treatment	DNA		RNA		Ratio of RNA to DNA	Protein		Lysozyme	
	Total	Conc.	Total	Conc.		Total	Conc.	Total	Conc.
Control	35 ± 4	6.4 ± 0.1	36 ± 6	7.0 ± 0.1	1.1	0.4 ± 0.1	60 ± 4	Undetectable	
E3	170 ± 7	3.0 ± .1	513 ± 16	9.3 ± .1	3.1	5.4 ± .5	86 ± 3	2 ± 0.4	33 ± 7
E5	480 ± 9	2.6 ± .1	1730 ± 20	9.6 ± .3	3.6	20.0 ± 1.8	135 ± 5	66 ± 1.0	443 ± 10
E10	1150 ± 12	2.3 ± .1	3880 ± 22	8.2 ± .1	3.6	101.7 ± 2.5	215 ± 7	1500 ± 10.0	2040 ± 50
EP5	110 ± 4	2.0 ± .1	270 ± 7	5.1 ± .1	2.4	5.3 ± 0.2	99 ± 3	Undetectable	
E1 EP4	130 ± 4	2.3 ± .1	330 ± 7	5.7 ± .2	2.5	6.4 ± 1.2	108 ± 4	5 ± 1.0	80 ± 11
E2 EP3	190 ± 3	2.4 ± .2	480 ± 6	6.1 ± .2	2.7	9.3 ± 0.9	107 ± 1	12 ± 2.7	153 ± 38
E3 EP2	250 ± 1	2.5 ± .2	690 ± 4	7.1 ± .2	2.8	13.1 ± .4	133 ± 10	25 ± 3.6	260 ± 30
E4 EP1	310 ± 4	2.6 ± .2	960 ± 4	7.9 ± .1	3.0	16.3 ± 1.4	135 ± 23	45 ± 3.4	373 ± 15

content (Table 1). Thus, during the initial lag period in the increase of total weight, extensive cell proliferation occurred. During this time there was an increase in the concentration of RNA (micrograms per gram of tissue), as well as an increase in the ratio of RNA to DNA. The concentration of DNA, as opposed to total DNA, decreased during this initial 3-day period, which results in large part from an increased uptake of water into the tissue. The change in protein concentration shows yet another pattern. The concentration of protein did not increase markedly until after 3 days of continued treatment with estrogen, at a time when specific oviduct proteins, such as lysozyme, began to appear. No lysozyme was present during the first 2 days of estrogen treatment (Fig. 1); by the 3rd day, activity was just measurable. Thereafter, the content of lysozyme increased so rapidly that the rate of its accumulation exceeded that of the increase in total wet weight between the 6th and 8th days of estrogen treatment.

Histological examination of the magnum 5 days after daily administration of estrogen revealed that the area between epithelial and serosal surfaces was markedly expanded, compared to the untreated tissue, because the tubular gland cells formed a typical acinar pattern. Characteristic of such cells is a basal nucleus and an apical region filled with small granules (Fig. 2, a and b). In the unstimulated gland, this region contains only stromal cells. Immunofluorescent studies by Kohler *et al.* (*9*) showed that tubular gland cells contain ovalbumin. We found that they also contain lysozyme, as indicated by an immunofluorescent technique in which lysozyme antibody is employed (*16*).

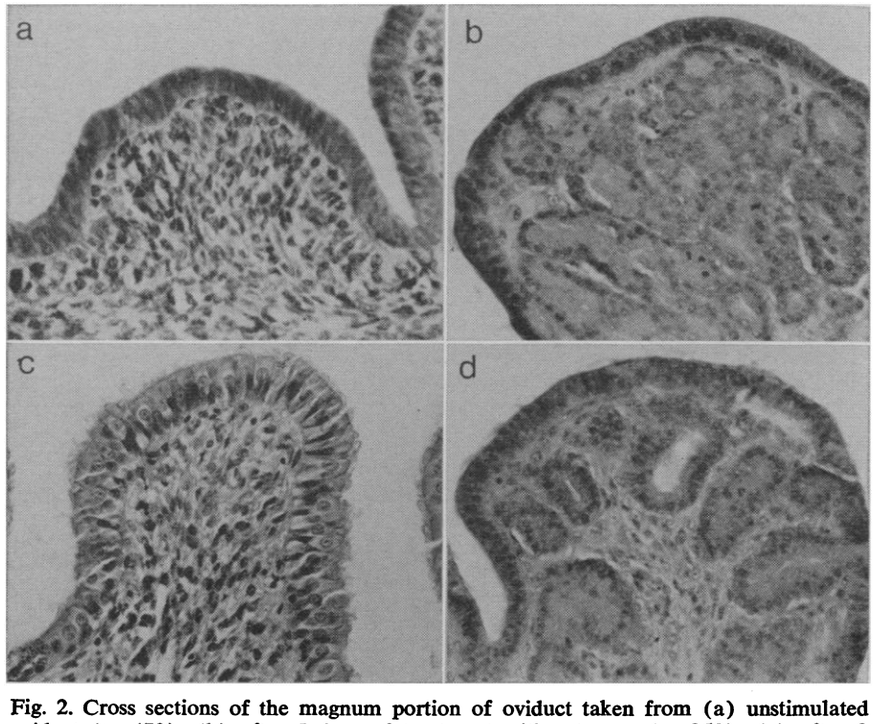

Fig. 2. Cross sections of the magnum portion of oviduct taken from (a) unstimulated oviduct (× 470); (b) after 5 days of treatment with estrogen (× 250); (c) after 2 days of treatment with estrogen and 3 days of treatment with a combination of estrogen and progesterone (× 250); and (d) after 5 days of treatment with estrogen and progesterone (× 300).

The concomitant administration of progesterone with estrogen reduced the estrogen-induced increase in the wet weight of magnum (Fig. 1). After 5 days of combined treatment the total content and the concentrations of DNA, RNA, and protein were lower than those of the estrogen-stimulated oviduct ($P = .01$) (Table 1). It is significant that no lysozyme activity was detectable throughout the 10-day period of combined treatment (Fig. 1). We also found that the effects of estrogen and progesterone on the content of ovalbumin, as measured immunologically, are similar to those described here for lysozyme. The tubular gland cells characteristic of estrogen treatment were absent in the magnum when chicks were treated with both estrogen and progesterone (Fig. 2c). No cytoplasmic granules were found in any region of the magnum. Thus, both the biochemical and histological findings indicate that progesterone antagonized the ability of estrogen to induce proliferation of tubular gland cells and cytodifferentiation associated with the synthesis of lysozyme.

From the difference in the time course of the estrogen-induced cell proliferation and that of the appearance of lysozyme (Fig. 1 and Table 1), it appears that estrogen first stimulates cell

division, followed in time by cytodifferentiation of tubular gland cells, a process that culminates in the appearance of a specific cell product, lysozyme. To determine at which point in time progesterone antagonizes the action of estrogen, administrations of progesterone were begun from 1 to 4 days after the onset of estrogen administration. After 5 days of total treatment, the magnum portion of the oviduct was examined for total content and concentration of DNA, RNA, protein, and lysozyme. Even a 1-day delay in the onset of progesterone treatment resulted in a significant ($P = .01$) increase in the content of DNA by day 5 (compare E1 EP4 with EP5) (Table 1). As the onset of administration of progesterone was delayed for longer periods, the increase in the content of total DNA, as well as in the contents of RNA and protein, was proportionately greater. In addition, the amount of lysozyme also correlated well with the length of the delay in starting the administration of progesterone (Table 1). Thus, even a 1-day delay allowed for the appearance of a detectable amount of lysozyme. A correlation between cell proliferation and subsequent functional differentiation was also evidenced by histological examination of oviduct from chicks treated with estrogen for 2 days and then with combined treatment for 3 days. Nests of normally appearing tubular gland cells with cytoplasmic granules were present among the stromal cells (Fig. 2d). This histological picture, then, is a combination of those patterns characteristic of the magnum after 5 days of either estrogen or combined treatment (Fig. 2, b and c).

The exact site of action of progesterone in antagonizing estrogen-induced cell proliferation and cytodifferentiation of tubular gland cells is unknown. Our results suggest that estrogen-induced proliferation of potential tubular gland cells commences within 24 hours, but that subsequent cytodifferentiation of such cells culminates in the appearance of lysozyme only after 72 hours (Fig. 1). Progesterone prevents occurrence of these processes when given continuously with estrogen, but does not prevent limited cell proliferation, lysozyme synthesis, and formation of tubular gland cells when administrations are begun within 24 hours after the initial estrogen. We therefore suggest as a tentative hypothesis that progesterone acts by inhibiting initial proliferation of potential tubular gland cells, but once this has occurred, cytodifferentiation and lysozyme synthesis are not inhibited.

The progesterone antagonism in this system is relatively tissue-specific, since the estrogen-induced increases in concentrations of calcium, lipid, and phosphoprotein in blood are not affected (*6, 7, 16*). Furthermore, the antagonism appears to be noncompetitive, since the inhibitory effect of progesterone cannot be reversed by increasing the dose of estrogen (*6, 16*). One of the early effects of estrogen in rat uterus is an increased uptake of amino acids and water (*17, 18*). We demonstrated this effect in chick oviduct and found that it is not inhibited by the concomitant administration of progesterone (*16*).

We anticipate that the phenomenon of progesterone inhibition of estrogen-induced tubular gland proliferation may be useful in understanding hormonal regulation of the development and function of this specific cell type.

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Haploid Plants from Pollen Grains

Abstract. *A method is presented by which hundreds of haploid plants of various species of* Nicotiana *can be raised from pollen grains. Stamens should be excised when pollen grains have been individualized, but are still uninucleate and free of starch. When grown in vitro on a relatively simple medium, some pollen grains proliferate into embryo-like structures which develop in stages similar to those of zygotic embryos. The plantlets mature and flower profusely, but do not set seed.*

Haploid organisms are desirable for at least two reasons: mutations induced in them are readily visible, and doubling their chromosomes, with colchicine for example, leads directly to homozygous individuals. This method for obtaining haploid plants in large numbers is based on the stimulation of cell division in immature pollen grains which leads to the production of a plant from the male prothallus alone (androgenesis).

Three to four weeks after stamens of the proper developmental stage have been planted on a suitable medium, embryos and plantlets can be seen emerging from some anthers (Fig. 1). These plantlets can be transplanted to individual tubes on a simplified medium. Once they have formed an adequate root system, they may be transplanted to pots and raised to mature plants which flower profusely. Chromosome counts made on preparations from root tips excised either from plantlets in test tubes or from adult plants have shown the plants to be haploid. A visible sign of the haploid condition was the fact that the flowers did not set seed. In general, haploid plants and flowers were smaller than diploids by about one-third. Large numbers of haploid plants have been raised in this manner from the following species: *Nicotiana tabacum* var. "Wisconsin 38," var. "Red flowered, large leaved" (a mutant originated at Debreczen, Hungary), var. "Corolle double X" (a mutant originated at Bergerac, France), var. "Coulo" (a white-flowered mutant), var. "Maryland Mammoth," and var. "White Burley," *N. sylvestris, N. affinis* (a white-flowered variety), and *N. rustica* (a white-seeded mutant of var. "Zlag").

Procedure is as follows: (i) Select flower buds at stage 2 in the case of *N. tabacum* and stage 3 with *N. sylvestris* (Fig. 2). (ii) Dip the cut end of the pedicel into molten paraffin, then disinfect the whole bud by dipping into 70 percent ethanol and immersing