when tested with the other original strain should yield dikaryons and "diploids" in equal frequency. This expectation was realized: Segregants from the cross of original *A* and *C*, when mated with original *B*, produced 26 dikaryons and 24 "diploids" ($P = .8$ to .9). Segregants from the cross of original *B* with *C*, when mated with original *A*, produced 13 dikaryons and 20 "diploids" ($P = .2$ to .3).

2) All of the progeny of these two outcrosses should yield dikaryons when mated with a "wild-type" strain. This was confirmed in matings with a strain chosen at random from the stock collection.

3) All isolates of both outcrossed progenies should be assignable either as dominant, *dik*$^+$, or as recessive, *dik*, from their interactions with the alternate original strains. When mated among themselves, homozygous dominant and heterozygous pairings should yield dikaryons, whereas homozygous recessive pairings should yield diploids. No exceptions were found to these expectations.

4) Although the two strains in which the recessive allele *dik* was originally found carried various mutations, a series of outcrosses should transfer the recessive allele to strains that are otherwise normal, and in these this allele should elicit the same effects as in the original mutant strains. These expectations have been confirmed: in matings between compatible strains, diploids were readily established whenever each of the two opposed strains carried the recessive allele *dik*.

Whether the recessive alleles of the two strains that were crossed by chance in this instance had a common origin is uncertain; both strains were generated from x-rayed material in laboratory crosses for which no comprehensive pedigrees were maintained. One thing is apparent, however: the recessive allele is not common either in strains collected from nature or in our laboratory strains. In the tens of thousands of different matings that have been made in this laboratory during the past dozen or so years, only this single homozygous recessive pairing was recognized. The dominant allele is overwhelmingly predominant, and thereon depends a unique and most characteristic feature of the higher fungi, the dikaryon.

The *dik*$^+$ gene suppresses karyogamy within the vegetative mycelium to limit the diploid phase to a single nuclear generation in the specialized cells, the basidia, in which meiosis normally occurs. The genetic stabilization of the dikaryon assures for these fungi all of the benefits of the diploid phase while retaining for the dikaryotic components certain prerogatives of the haploid.

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Protein Synthesis: Differential Stimulation of Cell-Specific Proteins in Epithelial Cells of Chick Oviduct

Abstract. *Immunofluorescent and radioautographic studies demonstrate that ovalbumin and avidin are cell-specific proteins synthesized by different epithelial cells in the chick oviduct mucosa. The mechanism of the selective induction of ovalbumin synthesis by estrogen and of avidin synthesis by progesterone may be through stimulation of specific target cells by these hormones.*

The differentiation of specialized epithelial cells that occurs during normal sexual maturation of the hen oviduct mucosa can be reproduced by administration of exogenous hormone (*1*, *2*). Estrogens cause the single layer of primitive stem cells in the immature chick oviduct mucosa to differentiate into three morphologically distinct epithelial cells: (i) tubular gland cells, (ii) ciliated cells, and (iii) goblet cells which are interspersed with the ciliated cells in the surface layer of duct epithelium. Concomitant with the cytodifferentiation, estrogens induce the synthesis of ovalbumin and stimulate general protein synthesis (*3*). The initiation of ovalbumin synthesis coincides with the appearance of discrete secretory granules in the cytoplasm of the tubular gland cells (*2*).

In the chick treated with estrogen, progesterone induces synthesis of the egg-white protein avidin, although general protein synthesis remains unchanged (*4*). This induction by progesterone is accompanied by microscopic evidence of increased secretory activity by the goblet cells (*2*). These observations suggest that ovalbumin and avidin are secreted by different epithelial cells of the oviduct mucosa. We have localized ovalbumin and avidin to different epithelial cell types by means of immunofluorescent and radioautographic techniques.

Five-day-old female Rhode Island Red chicks were divided into two groups. One group received 5 mg of diethylstilbestrol (DES) in sesame oil subcutaneously for 17 days. The other group received 5 mg of DES for 12 days and then 5 mg of progesterone in sesame oil subcutaneously for 5 days. The chicks were killed at regular intervals. The magnum area of the oviduct was excised immediately. A portion was fixed in Zenker-formalin and embedded in paraffin for conventional histology. The remainder was rapidly frozen for special studies.

For immunofluorescent studies, rabbit antiserums to avidin and ovalbumin (*4*) were conjugated to fluorescein isothiocyanate (*5*) and absorbed twice with acetone powder of chicken liver. Frozen sections of oviduct were heat-fixed on glass slides and placed in petri dishes containing moistened filter paper. A dilution (1:2) of the fluorescein-conjugated antiserum in 0.01*M* phosphate-buffered saline was layered on the tissue for 20 minutes before removal by flooding with the same buffer.

Fluorescent staining from antiserum to ovalbumin was localized in the epithelial cells of tubular glands in the oviducts of chicks treated with DES (Fig. 1B). This was present both before and after administration of progesterone. In contrast, oviduct tissue from chicks treated with DES alone showed essentially no localization of antiserum to avidin. However, after treatment with progesterone, fluorescence from labeled antiserum to avidin was clearly localized

to goblet cells in the surface layer of oviduct epithelium (Fig. 1C). Negative control preparations included (i) chicken liver, (ii) unstimulated oviduct, (iii) oviduct previously reacted with unlabeled antiserums, and (iv) oviduct reacted with labeled antiserums which had previously been exposed to excess ovalbumin or avidin.

The ability of ^{3}H-biotin to bind specifically to avidin (*6*) was used for the radioautographic studies. Frozen sections of tissue were placed on glass slides coated with a thin layer of gelatin and air-dried at room temperature or fixed for 10 minutes in formaldehyde vapors. Approximately 60 percent of the capacity to bind biotin is retained after this treatment (*7*). ^{3}H-biotin (2.0 c/mmole) was diluted in 0.2*M* $(NH_4)_2CO_3$ buffer at *p*H 8.4 to a final concentration of 1.0 μc/ml. One drop of this solution was placed on each section for 15 minutes. The sections were washed by immersion of the slides in $(NH_4)_2CO_3$ buffer for 3 minutes; they were then further fixed and dehydrated by sequential 5-minute immersions in 70 percent ethanol, 100 percent ethanol, 95 percent ethanol and 5 percent acetic acid, and 100 percent ethanol before air-drying at room temperature. Kodak AR-10 film was used for stripping, and the slides were developed after 10 weeks.

Although slight background radioactivity was detected over all of the oviduct tissue, the major portion of the radioactivity was well localized over the mucosal surface layer of the oviduct (Fig. 1D). This presumably represented the complex of ^{3}H-biotin and avidin and was in exact agreement with the localization of antiserum to avidin by the immunofluorescent studies. Preparations of oviduct previously reacted with excess unlabeled biotin and chick intestine were used as controls for nonspecific adsorption of ^{3}H-biotin to mucus. These failed to show localized concentration of radioactivity.

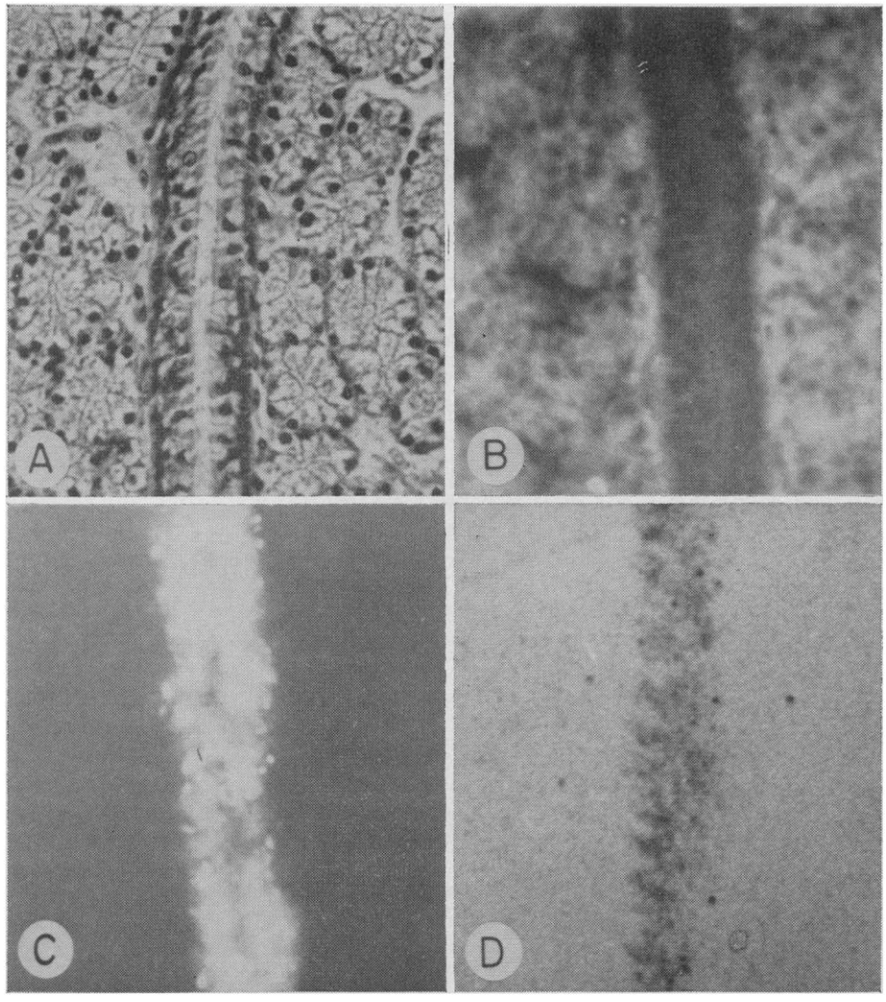

Fig. 1. Localization of ovalbumin and avidin to specific epithelial cell types. (A) Chick oviduct mucosa after treatment with DES and progesterone (hematoxylin and eosin stain, ×250). Apposed surface layers of duct epithelium consist of alternating goblet cells and ciliated cells. Cross sections of tubular glands beneath surface layer produce acinar pattern. (B) Localization of fluorescein-conjugated antiserum to ovalbumin in tubular gland cells. (C) Localization of fluorescein-conjugated antiserum to avidin to the goblet cells of the surface epithelium. (D) Radioautograph of similar area of unstained oviduct after treatment with ^{3}H-biotin. Numerous exposed silver grains correspond to goblet cells in surface epithelium.

The possibilities have existed that (i) the regulatory effects of DES and progesterone on protein synthesis were specific actions on a common target cell, or (ii) DES and progesterone acted on different cell types to induce the synthesis of ovalbumin and avidin. Our study clearly shows a target-cell specificity of DES and progesterone in regulating the synthesis of cell-specific proteins by different cells in a single epithelium. Ovalbumin was localized to the tubular gland cells after treatment with DES; avidin was localized in the goblet cells after treatment with progesterone.

Previous treatment with DES markedly increases the quantity of avidin produced by a given mass of oviduct in response to progesterone (*4*), apparently as a result of causing differentiation of the goblet cells. If estrogen itself has any direct regulatory action on avidin synthesis, it is possibly that of a corepressor since most steroids which inhibit the biologic activity of estrogen will induce avidin synthesis by the estrogen-treated oviduct (*8*).

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