pollen induced 52 percent of the in vitro cultured ovules to remain white and increase in size. Slight fiber elongation was noted. Similar extracts of germinated pollen induced a significant increase in size when furnished in low amounts to in vitro cultured, fertilized ovules, and, as the concentration of pollen extract was increased in the basal medium, fiber elongation decreased. Dihydrozeatin has been tentatively identified as one of the phytohormone components of cotton pollen.

The results presented herein describe an additional step toward being able to culture the component parts of the plant's reproductive system and provide new information on the role of plant growth regulators in embryogenesis and fruit development.

The results are significant from the standpoint that a new research tool is available for studies of (i) the relationships between plant embryogenesis and seed development, (ii) the mechanism whereby pollen hormones induce metaxenia, and (iii) the role of phytohormones in cell elongation and in primary and secondary cell wall biosynthesis.

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16. Research sponsored in part by Cotton Incorporated, 3901 Barrett Drive, Raleigh, North Carolina 27609. I thank Dr. Wasfy Shindy for the gas chromatographic analysis for dihydrozeatin, and Dr. Irwin P. Ting for his support and cooperation in this work.

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Androgen-Concentrating Cells in the Midbrain of a Songbird

Abstract. *Androgen-concentrating cells were found in the midbrain of the chaffinch* Fringilla coelebs *by autoradiography using tritiated testosterone. Labeled cells were localized primarily in the nucleus intercollicularis, an area from which vocalizations can be electrically stimulated in birds. These autoradiographic results suggest that the nucleus intercollicularis is a site in the action of androgens on avian vocal behavior.*

A number of steroid hormones are concentrated by cells in their major target tissues. Estradiol, for instance, is concentrated in accessory reproductive structures, such as the uterus and vagina, but not in other tissues like the lung, adrenals, or skeletal muscle (*1*). Dihydrotestosterone is concentrated in the prostate and seminal vesicles, but not in the lung or thymus (*2*).

Particular regions of the brain also have the ability to retain specific steroid hormones (*3*). For estrogens and androgens the sites of hormone retention in the brain correlate well with the sites at which these hormones are known to act (*3*). This correlation suggests that hormone retention can be used to localize sites of hormone action in the brain. (The validity of such a procedure does not depend on the exact role of this retention in the mechanism of hormonal action.) Light microscopic autoradiography in which tritiated steroids are used provides a powerful tool for localizing cells that concentrate particular hormones.

The present report describes the distribution of androgen-concentrating cells in the midbrain of the chaffinch (*Fringilla coelebs*) and shows their occurrence in an area from which species-typical vocalizations have been stimulated electrically in a number of other avian species. The androgen dependency of several of the vocalizations of the chaffinch has been demonstrated. Two alarm calls restricted to male chaffinches are normally given only during the breeding season but can be elicited during other parts of the year by injections of testosterone (*4*, *5*). Song development in this species and the production of adult song are also dependent on testosterone (*4*, *6–8*). Adult male chaffinches do not sing during the winter months or after castration unless they have been given exogenous androgen (*4*, *7*, *8*). Female chaffinches do not normally sing at all but they also can be induced to sing if given testosterone injections (*4*, *8*). Additional examples of androgen-dependent vocalizations have been found in other species (*9*).

Four male chaffinches, 1 to 4 years

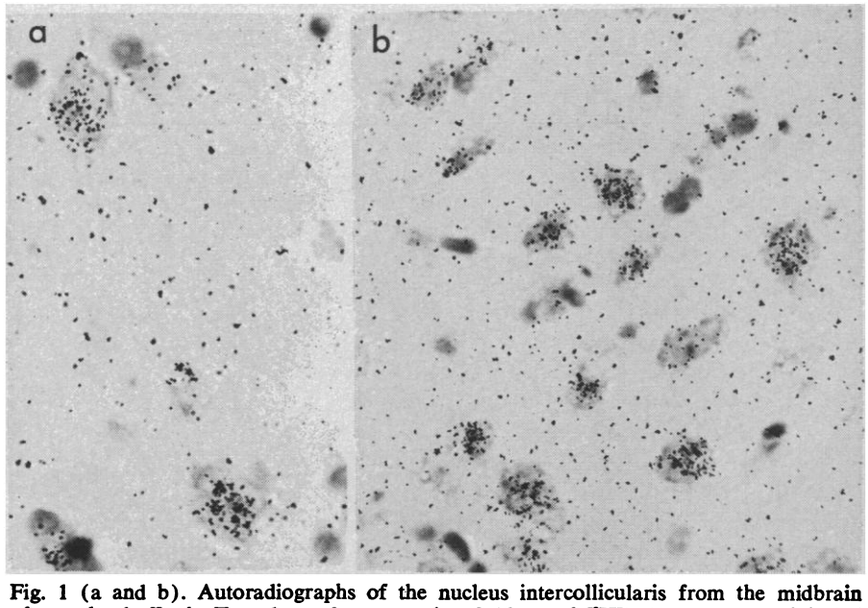

Fig. 1 (a and b). Autoradiographs of the nucleus intercollicularis from the midbrain of a male chaffinch. Two days after castration 0.46 μg of [^{3}H]testosterone was injected into the breast muscle. An hour later, the brain was removed and frozen. Unfixed and unembedded sections 6 μm thick were cut at −19°C. Sections from which these photographs were taken were exposed for 96 days and then developed, fixed, and stained with cresyl violet; (a) × 690; (b) × 525

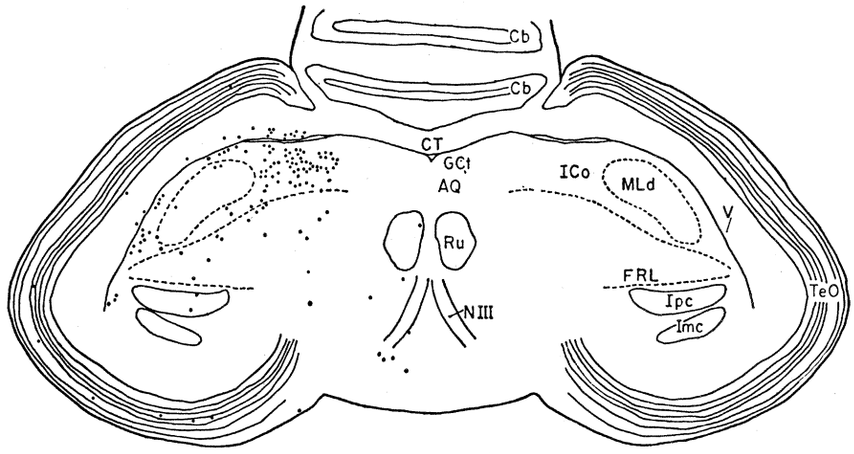

Fig. 2. The distribution of labeled cell bodies in a section through the midbrain of a chaffinch 1 hour after injection of [^{3}H]testosterone. Each dot represents the location of a cell body containing five times the density of grains found betweeen cell bodies. Abbreviations: *AQ*, aqueductus cerebri; *Cb*, cerebellum; *CT*, commissura tectalis; *FRL*, formatio reticularis lateralis mesencephali; *GCT*, substantia grisea centralis; *ICo*, nucleus intercollicularis; *Imc*, nucleus isthmi, pars magnocellularis; *Ipc*, nucleus isthmi, pars parvocellularis; *MLd*, nucleus mesencephalicus lateralis, pars dorsalis; *NIII*, nervus oculomotorius; *Ru*, nucleus ruber; *TeO*, tectum opticum; and *V*, ventriculus.

old, were used in this study (*10*). One to 3 days after castration, 0.3 to 0.5 μg of [^{3}H]testosterone (New England Nuclear; 91 c/mmole) were injected in the breast muscle in 0.1 ml of a mixture of ethanol and saline (1 : 1). After an hour the birds were killed and their brains removed as quickly as possible. The brains were cut into blocks and frozen immediately with Dry Ice. After equilibration in the cryostat at −19°C, sections 6 μm thick, unfixed and unembedded, were cut serially through the brain. The frozen sections were picked up on glass slides precoated with Kodak NTB-3 emulsion by lightly touching the slide to the cryostat knife (*11*). The slides were stored with Drierite in light-tight plastic boxes which were placed in a large lead box, also containing Drierite, and kept at 4°C. After exposures of about 5 months, the autoradiograms were developed with Kodak D-19, fixed, and then stained with cresyl violet. Entire sections were traced by means of a microprojector, and the distribution of labeled cells was plotted under the 40-power objective of a light microscope. A cell body was considered labeled if it contained five times the density of grains found between cell bodies (*12*).

The highest concentration of labeled cell bodies in the midbrain was found in the nucleus intercollicularis (Fig. 1, a and b). In the rostral portion of this nucleus, labeled cells were located primarily medial to the dorsal half of the optic ventricle. More caudally the optic ventricles are continuous with the aqueduct (Fig. 2). At this level the nucleus intercollicularis (ICo) surrounds the nucleus mesencephalicus lateralis, pars dorsalis (MLd), and many of the cells in ICo are labeled (Fig. 2). The large cells of MLd itself are not labeled. Further caudally in the midbrain few labeled cells are found in ICo.

Besides ICo the most heavily labeled areas in the midbrain are the periventricular zone above the optic ventricle (Fig. 2) and the central gray at a level posterior to that shown in Fig. 2. Scattered labeled cells were found elsewhere, for instance in the formatio reticularis lateralis mesencephali (FRL) (Fig. 2). The distribution of labeled cells in the midbrain was similar in all four chaffinches (Fig. 3) (*13*). There was no obvious difference in the occurrence of androgen-concentrating cells between the right and left halves of the brain.

In any autoradiographic study it is important to identify the chemical nature of the labeled compound or compounds. This information is particularly significant when [^{3}H]testosterone is used, since it is known that certain metabolites of this hormone (particularly the Δ^4 reduced steroids) are potent androgens (*2*). Using a procedure for isolating highly purified cell nuclei from the entire chaffinch brain (*14, 15*), we found that about 93 percent of the radioactivity retained by these nuclei consisted of material that chromatographed with testosterone and its Δ^4 reduced metabolites 5α- and 5β-dihydrotestosterone (*16*). We chose to characterize the radioactive compounds in purified brain cell nuclei because earlier studies on the ring dove (*14*) and the rat (*2, 3*) indicated that the nucleus is a major site of androgen binding in target cells.

The topography of labeled cells after the administration of [^{3}H]testosterone has previously been studied only in the rat. In this animal labeled cells were found in certain limbic and hypothalamic areas, but there has been no report of such cells in the midbrain (*17*). Although we have not yet explored the biochemical specificity of the androgen-concentrating cells in the chaffinch, data from the work of Stern and of Rhees *et al.* (*18*), together with the present study, indicate that hormone-binding systems in the avian brain

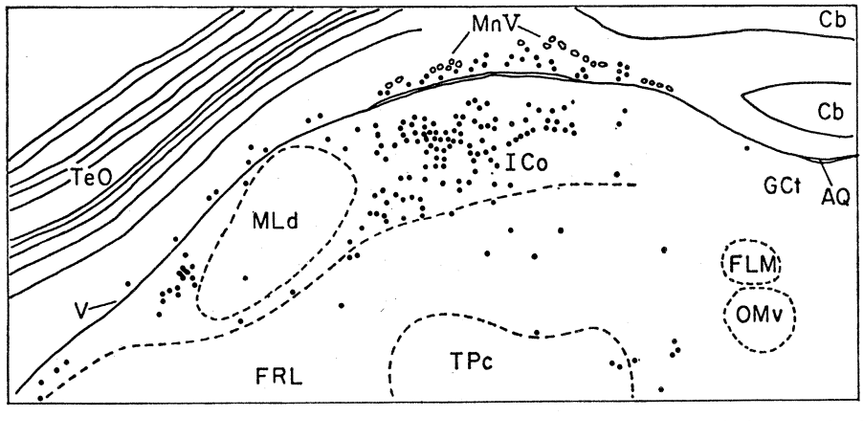

Fig. 3. The distribution of labeled cell bodies in a section through a similar level of the midbrain as that shown in Fig. 2, but taken from a different chaffinch. This figure illustrates the high degree of reliability in the distribution of androgen-concentrating cells between animals. Abbreviations: *FLM*, fasciculus longitudinalis medialis; *MnV*, nucleus mesencephalicus nervi trigemini; *OMv*, nucleus nervi oculomotorii, pars ventralis; and *TPc*, nucleus tegmenti pedunculo-pontinus, pars compacta. Other abbreviations are as in Fig. 2.

show the same high degree of specificity previously seen in the rat brain (*3*).

The presence of androgen-concentrating cells in the midbrain of the chaffinch is particularly interesting in view of the suggestion that the midbrain is involved in the control of vocal behavior in a number of avian species (*19–28*). Popa and Popa (*19*) and Brown (*20*) reported that vocalizations could be electrically stimulated in an area near the optic ventricle. More detailed mapping studies of this region by Newman (*21*) and Potash (*22*) have demonstrated two separate functional areas—the ICo (*23*) from which vocalizations can be elicited with a low threshold and the MLd from which evoked responses can be recorded after auditory stimulation. A variety of vocalizations, including alarm calls, have been stimulated from ICo. Many of these elicited vocalizations have been triggered from several other sites in the brain (for example, hypothalamus and septal area), although normal song has not yet been elicited by stimulation anywhere in the brain. The finding of auditory evoked responses in MLd supports previous neuroanatomical and neurophysiological studies showing auditory sensory input to this nucleus (*29*).

Our autoradiographic results suggest a biochemical division within the midbrain of the chaffinch which correlates well with the two functional areas described by Newman and Potash. Cells in ICo have the ability to retain certain androgenic hormones, while cells in MLd do not have this capacity. In view of the stimulation studies referred to above and the androgen dependency of many avian vocalizations, the concentration of labeled cells in ICo after an injection of [^{3}H]testosterone suggests that this area may be a site of androgen action in the regulation of vocal behavior.

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16. The details of dose of [^{3}H]testosterone, route of injection, and the time the animals were killed were the same as in the autoradiographic experiments. The brain nuclei were extracted with dichloromethane. After the solvent was evaporated, the extracted material was run on silica gel, thin-layer plates in a solvent system containing chloroform and diethyl ether (20 : 1). Testosterone, 5α-dihydrotestosterone, and androstenedione were run as standards. Results are expressed as the percentage of the total radioactivity recovered from the plate. The mean percentages of testosterone and dihydrotestosterone in three experiments were 47 percent and 46 percent, respectively. The possibility that the extent of dihydrotestosterone formation varies in different regions is suggested by a single experiment in which we isolated nuclei from different areas of the brain. The percentages of the radioactivity that migrated with testosterone and dihydrotestosterone, respectively, in the three samples were as follows: optic lobes, 62 and 35 percent; striatum, 47 and 42 percent; and diencephalon, 40 and 53 percent.
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Trilobite Eyes: Calcified Lenses in vivo

Abstract. *The corneal lenses preserved in the eyes of some of the Paleozoic trilobites (Arthropoda) are constructed of calcite that is crystallographically oriented to behave like glass. The calcareous lenses are capable of forming inverted images over a large depth of field and must have been present in the living trilobites.*

The corneal lenses of the eyes in many of the extinct Paleozoic trilobites are commonly preserved as fossil remains along with other parts of the calcitic-chitinous exoskeleton. Although a number of excellent and detailed papers have dealt exclusively with various aspects of trilobite visual organs (*1, 2*) the question of the original composition of the lenses has only briefly been considered. Most workers have assumed that inasmuch as the corneal lenses of modern-day arthropods are modified extensions of the chitinous cuticle (*3*) the same was true for the trilobites. However, the exoskeletons of most trilobites are preserved as fossils not because they were constructed of chitin per se but because this chitin was substantially impregnated with calcium carbonate. Thus, if the lenses of trilobite eyes were unmineralized in life, as is the case with the majority of living organisms, then a problem emerges regarding their fossil preservation. Why should one uncalcified portion of the trilobite cuticle commonly be preserved when other portions,