patients into the normal range (*13*).

While evidence from studies in animals (*3, 6*) suggests that MHPG is a major metabolite of brain norepinephrine, the relation of amine concentration in the brain to metabolite concentration in the CSF is not clear. Significant amounts of labeled MHPG administered intravenously in three patients did not appear to enter the CSF (*14*). Therefore, a source of MHPG in the central nervous system is likely. There is no rostral-caudal CSF gradient for MHPG (*8*), and a probenecid-sensitive transport mechanism does not appear to be a major mechanism for elimination of MHPG (*12*), as is the case for the acid metabolites of dopamine and serotonin. In animals, norepinephrine is abundant in the spinal cord and appears localized to nerve endings of descending tracts, which degenerate after spinal cord transection (*15*). Our study of 13 patients with spinal cord transection, with and without spinal fluid block, suggests that MHPG concentrations in CSF reflect norepinephrine metabolism in spinal cord as well as in brain (*16*). Patients with transected spinal cords had lower MHPG values than did controls, and results were similar in the presence and absence of block in CSF flow.

Although part of the MHPG measured in lumbar CSF may be derived from spinal cord sources, the results reported here suggest that norepinephrine metabolism in the central nervous system may be reduced in depression. The low MHPG values in the CSF of depressed patients support and extend the reports of low MHPG excretion in the urine of depressed patients (*2*).

However, the implication that our data support a primary role of catecholamines in affective illness should be viewed critically. Activity appears to increase MHPG concentration in CSF (*13*), and low levels of MHPG have been found in the CSF of para- and quadraplegics where limited activity may also be a factor (*16*). Thus, the low MHPG concentrations in the CSF of depressed patients may occur secondary to the behavioral inhibition associated with the depression rather than reflect a primary role for brain norepinephrine in the pathogenesis of affective illness. However, the lack of correlation of MHPG with agitation-retardation within the depressed or manic groups appears to indicate that whereas activity may be an important determinant of amine metabolite concentrations in CSF, it clearly is not the only one. In addition, MHPG values in CSF of depressed patients were significantly lower than those in Parkinsonian patients, who also manifest a considerable degree of motor inhibition.

Our results lend support to the idea that central norepinephrine metabolism is altered in depression. Whether this change is etiologically related to depressive illness or is secondarily related to its clinical and biological concomitants remains to be determined.

ROBERT M. POST
EDNA K. GORDON
FREDERICK K. GOODWIN
WILLIAM E. BUNNEY, JR.
Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, Maryland 20014

References and Notes

1. J. J. Schildkraut, *Amer. J. Psychiat.* **122**, 509 (1965); W. E. Bunney, Jr., and J. M. Davis, *Arch. Gen. Psychiat.* **13**, 483 (1965); J. J. Schildkraut and S. S. Kety, *Science* **156**, 21 (1967).
2. J. W. Maas, J. Fawcett, H. Dekirmenjian, *Arch. Gen. Psychiat.* **19**, 129 (1968); K. Greenspan, J. Schildkraut, E. Gordon, L. Baer, M. S. Aronoff, J. Durell, *J. Psychiat. Res.* **7**, 171 (1970); P. A. Bond, F. A. Jenner, G. A. Sampson, *Psychol. Med.* **2**, 81 (1972).
3. J. W. Maas and D. H. Landis, *J. Pharmacol. Exp. Ther.* **163**, 147 (1968).
4. S. E. Gitlow, M. Mendlowitz, L. M. Bertani, S. Wilk, E. K. Wilk, *J. Clin. Invest.* **50**, 859 (1971); J. W. Maas, H. Dekirmenjian, D. Garver, D. E. Redmond, Jr., D. H. Landis, *Brain Res.* **41**, 507 (1972).
5. M. Ebert, R. M. Post, F. K. Goodwin, *Lancet* **1972-II**, 766 (1972).
6. E. Mannarino, N. Kirshner, B. S. Nashold, *J. Neurochem.* **10**, 373 (1963); C. O. Rutledge and J. Jonason, *J. Pharmacol. Exp. Ther.* **157**, 493 (1967); S. M. Schanberg, J. J. Schildkraut, G. R. Breese, I. J. Kopin, *Biochem. Pharmacol.* **17**, 247 (1968).
7. S. M. Schanberg, G. R. Breese, J. J. Schildkraut, E. K. Gordon, I. J. Kopin, *Biochem. Pharmacol.* **17**, 2006 (1968); S. Wilk, K. L. Davis, S. B. Thacker, *Anal. Biochem.* **39**, 398 (1971).
8. E. K. Gordon and J. Oliver, *Clin. Chim. Acta* **35**, 145 (1971).
9. Preliminary results have been reported. W. E. Bunney, Jr., D. L. Murphy, F. K. Goodwin, E. K. Gordon, 124th annual meeting, American Psychiatric Association, Washington, D.C., May 1971; W. E. Bunney, Jr., and E. K. Gordon, 10th annual meeting, American College of Neuropsychopharmacology, Las Vegas, Nevada, January 1972.
10. S. Wilk, B. Shopsin, S. Gershon, M. Suhl, *Nature* **235**, 440 (1972).
11. J. R. Mendell, T. N. Chase, W. K. Engel, *Arch. Neurol.* **25**, 320 (1971); T. N. Chase, E. K. Gordon, L. K. Y. Ng, unpublished manuscript.
12. E. K. Gordon, J. Oliver, F. K. Goodwin, T. N. Chase, R. M. Post, *Neuropharmacology*, in press.
13. R. M. Post, J. Kotin, F. K. Goodwin, E. K. Gordon, *Amer. J. Psychiat.* **130**, 67 (1973).
14. T. N. Chase, personal communication.
15. T. Magnusson and E. Rosengren, *Experientia* **19**, 229 (1963); A. Carlsson, B. Falck, K. Fuxe, N. A. Hillarp, *Acta Physiol. Scand.* **60**, 112 (1964); N. E. Anden, J. Haggendal, T. Magnusson, E. Rosengren, *ibid.* **62**, 115 (1964); A. Dahlstrom and K. Fuxe, *ibid.* **64** (Suppl. 247), 1 (1965).
16. R. M. Post, F. K. Goodwin, E. K. Gordon, D. Watkins, *Science* **179**, 897 (1973).

29 September 1972; revised 3 November 1972 ■

Hormonal Regulation of Growth in Unfertilized Cotton Ovules

Abstract. *Exogenous plant growth regulators can substitute for pollination, fertilization, and subsequent embryo development in cotton. Isolated, unfertilized, immature ovules enlarge in the presence of kinetin, and both enlarge and produce fibers in the presence of indoleacetic acid or gibberellic acid or both. An extract of germinating cotton pollen qualitatively mimics the effect of exogenous hormones.*

Much information concerning the physiology of fruit development and embryogenesis could be obtained if the component organs involved in sexual reproduction could be cultured separately in vitro. Recent literature has described the culture procedures required and hormonal interactions involved in the in vitro development of fertilized cotton ovules and their associated fiber cells. In brief review, the cotton fiber is a single epidermal cell that begins to elongate from the ovule surface at anthesis. If fertilization is accomplished the ovule may be successfully cultured in vitro (*1*), and both fibers (*2*) and embryos (*3*) are produced. This report describes the next basic step, that of the successful in vitro culture of immature cotton seeds, without zygote.

In angiosperms, fusion of the sperm nucleus and the egg nucleus creates the zygote and, for most species, fusion of another sperm nucleus with two polar nuclei of the embryo sac triggers formation of the nutritive endosperm. These creative unions initiate a myriad of biochemical reactions ultimately producing a mature fruit (ovary), containing its seeds (ovules), which in turn house the new plants (embryos).

Examples of the successful culture of excised plant embryos are available from the major groups of seed plants, for example, a gymnosperm (*4*), a dicotyledonous angiosperm (*5*), and a grass (*6*). Isolated plant ovaries, containing fertilized ovules, have been cultured in vitro, giving rise to enlarged fruits and germinable seed (*7, 8*). Seedless fruits from unpollinated flowers have been obtained in vitro by the incorporation into the medium of auxin-

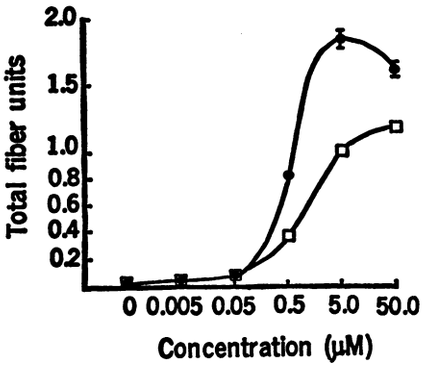

Fig 1. Total Fiber Units produced from unfertilized cotton ovules cultured in vitro for 2 weeks in the presence of varying concentrations of indoleacetic acid (●) or gibberellic acid (□). Twice the standard error limits are indicated if the standard error exceeded 0.05 Total Fiber Unit.

type chemicals (*7, 9*). Similarly, exogenous gibberellins (*10*) and phytokinins (*11*) have been shown to artificially induce parthenocarpy. Viable embryos of an apomictic plant species have been obtained if the entire spike or whole flower was cultured, but not if the excised ovary was cultured (*12*). Isolated fertilized ovules have also been successfully cultured into new plants (*13*). Also, in vitro fertilization and subsequent maturation of whole ovules have been reported (*14*).

Cotton was grown in the greenhouse. On the morning of anthesis, stamens and stigmas were removed from the flowers. Forty-eight hours later, ovules were aseptically removed from each ovary and floated on the surface of 50 ml of liquid medium in a 125-ml culture flask. The medium was as previously reported (*2*) except that 120 m*M* glucose was the sole carbon source for the experiments reported herein. All growth substances were filter-sterilized and added to the previously autoclaved basal medium. Each treatment consisted of at least five flasks with 20 ovules per flask. At the end of 2 weeks the amount of fiber produced from each set of 20 ovules was determined as previously described (*2*): the ovules with associated fibers were shaken to extend the fibers, and placed in a solution of toluidine blue 0; the dye not absorbed was removed by washing, and ovules were destained by placing in a solution of formalin–acetic acid–ethanol (FAA). The intensity of the destaining solution (proportional to the amount of fiber produced) was determined colorimetrically. A standard curve of dilution of a toluidine blue 0 solution was plotted against an arbitrary linear scale called Total Fiber Units. Twenty ovules with fibers of around 7 mm in length yield Total Fiber Units of approximately 2 when destained in 100 ml of FAA.

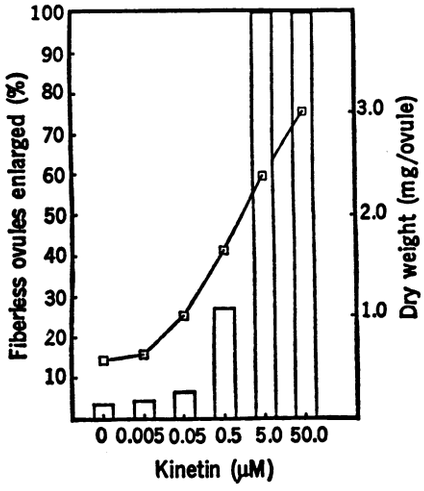

Fig. 2. Percentage of unfertilized ovules (bars), cultured in vitro for 2 weeks, which increased in size and remained white at various concentrations of kinetin. Squares indicate dry weight.

A successful method for germinating cotton pollen has recently been published (*15*) and was used with only minor modifications. Pollen was dusted on the surface of nutrient agar and permitted to develop for 2 hours. Pollen grains and associated tubes were washed into a vessel and boiled for 5 minutes, and the grains and cell debris were removed by filtration and centrifugation. Various portions of the germinated-pollen extract were added to the ovule culture medium, and the medium was autoclaved.

With very few exceptions all unfertilized ovules, transferred to basal culture medium lacking growth substances, shriveled and turned brown by the end of the 2-week culture period. When indoleacetic acid (IAA) or gibberellic acid (GA) was added to the culture medium in the concentration range of 0.5 to 50.0 μM, ovules remained white, increased in size, and produced fibers. The amount of fiber produced by IAA or GA varied slightly from experiment to experiment; however, as a general rule GA produced larger ovules, and supported less fiber but more callus, than did IAA. When IAA and GA were applied simultaneously, the Total Fiber Units recorded showed the effects to be additive. Typical dose-response curves for IAA and GA are shown in Fig. 1. Dry weight of the ovules and associated fibers essentially paralleled the values obtained for fiber production. When kinetin was added to the basal culture medium, ovules remained white and increased in size. The dry weight of ovules also increased as the concentration of kinetin was increased in the culture medium. No elongated fibers were produced, however, at any concentration of kinetin employed. Excessive enlargement (internal callus) with associated browning of the epidermis of the ovule was induced by 50 μM kinetin. These responses due to exogenous kinetin are shown in Fig. 2. Figure 3 shows the visible effects of exogenous kinetin, and GA plus IAA, on cotton ovule development.

In an experiment where more than 99 percent of the unfertilized ovules (controls) shriveled and browned, a water extract of germinated cotton

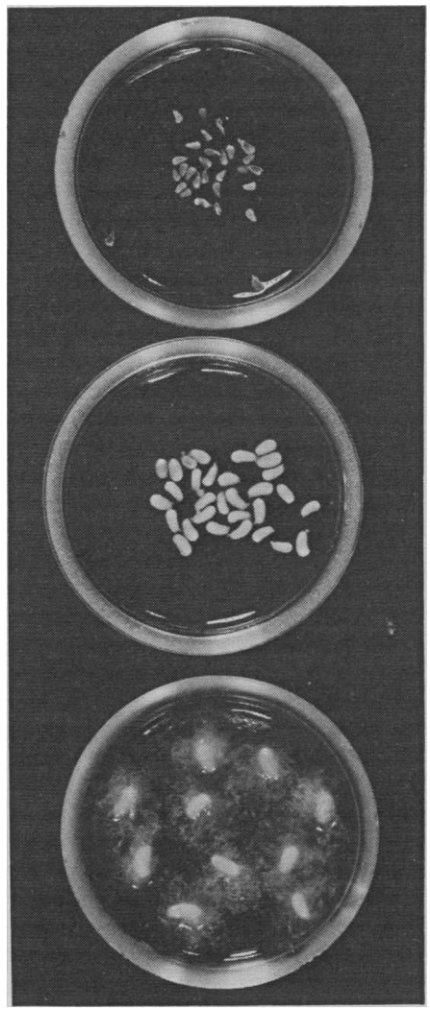

Fig. 3. Unfertilized cotton ovules, cultured for 2 weeks in basal medium (top), basal medium plus 5.0 μM kinetin (middle), and basal medium plus 5.0 μM indoleacetic acid and 5.0 μM gibberellic acid (bottom).

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.

C. A. BEASLEY

Biology Department, University of California, Riverside 92502

References and Notes

1. C. A. Beasley, *BioScience* **21**, 906 (1971).
2. ——— and I. P. Ting, *Amer. J. Bot.*, in press.
3. ———, L. A. Feigen, *Calif. Agr.* **25**, 6 (1971).
4. G. P. Berlyn and J. P. Miksche, *Amer. J. Bot.* **52**, 730 (1965).
5. V. Raghavan and J. G. Torrey, *ibid.* **50**, 540 (1963).
6. S. Narayanaswami, in *Plant Tissue and Organ Culture—A Symposium*, P. Maheshwari and N. S. Ranga Swamy, Eds. (International Soc. of Plant Morphologists, Dept. of Botany, Univ. of Delhi, Delhi, India, 1963), p. 303.
7. J. P. Nitsch, *Amer. J. Bot.* **38**, 566 (1951).
8. N. Maheshwari, *Phytomorphology* **11**, 17 (1961).
9. J. P. Nitsch, in *Plant Tissue and Organ Culture—A Symposium*, P. Maheshwari and N. S. Ranga Swamy, Eds. (International Soc. of Plant Morphologists, Dept. of Botany, Univ. of Delhi, Delhi, India, 1963), p. 198.
10. B. G. Coombe, *Science* **172**, 856 (1971).
11. J. C. Crane and J. van Overbeck, *ibid.* **147**, 1468 (1965).
12. P. P. Née Murgai, in *Plant Tissue and Organ Culture—A Symposium*, P. Maheshwari and N. S. Ranga Swamy, Eds. (International Soc. of Plant Morphologists, Dept. of Botany, Univ. of Delhi, Delhi, India, 1963), p. 281.
13. C. L. Withner, *Amer. Orchid Soc. Bull.* **24**, 380 (1955); M. Kapoor, *Phytomorphology* **9**, 313 (1959); N. Maheshwari and M. Lal, *ibid.* **11**, 307 (1961).
14. K. Kanta, N. S. Ranga Swamy, P. Maheshwari, *Nature* **194**, 1214 (1962); N. S. Ranga Swamy and K. R. Shivanna, *ibid.* **216**, 937 (1967).
15. R. M. Taylor, *Crop Sci.* **12**, 243 (1972).
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.

20 November 1972

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

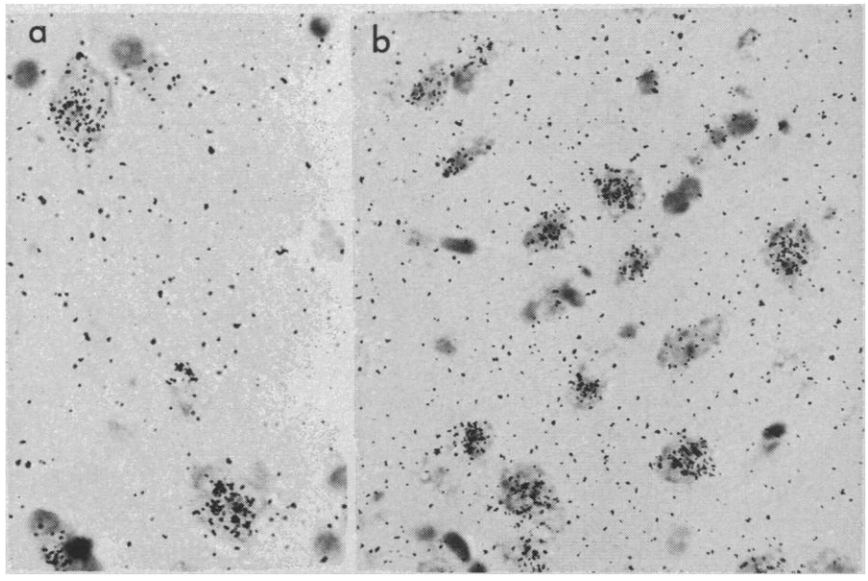

Fig. 1 (a and b). Autoradiographs of the nucleus intercollicularis from the midbrain of a male chaffinch. Two days after castration 0.46 μg of [^{3}H]testosterone was injected into the breast muscle. An hour later, the brain was removed and frozen. Unfixed and unembedded sections 6 μm thick were cut 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