thermal response curves. These were related to the types of responses observed following administration of barbiturate anesthesia (*9*) and of pyrogen (*10*). The present work extends the inventory of these characteristics to include differential responsiveness to the passage of current and to the amines tested. Thermodetectors appear to be relatively insensitive to NE, 5-HT, and ACh, but are sensitive to current flow. Thermosensitive interneurons, by contrast, are amine sensitive but relatively insensitive to current. The insensitivity of the thermodetectors to amines suggests a lack of synaptic input to these unique cells.

The responses of the rat's thermosensitive interneurons to ACh and NE shown in this study are in agreement with what would be predicted from the results of previous microinjection studies. That is, microinjection of ACh into the PO/AH produced coordinated behavioral and physiological heat-dissipation responses (*3*). When applied to single cells, ACh increased the firing rate of warm-sensitive cells, an event which would be expected to activate heat-loss responses. Microinjection of NE into the same hypothalamic region results in the activation of coordinated behavioral and physiological heat-production responses (*2*). As shown here, NE decreased the firing rate of warm-sensitive neurons, an effect which would be expected to initiate increases in heat production and conservation responses.

The responses of thermosensitive neurons in the cat to ACh, 5-HT, and NE were the same as those observed in the rat. These data from the cat are thus in disagreement with the results of microinjection experiments. Norepinephrine has been reported to produce a decrease in body temperature when microinjected into the PO/AH (*5*). However, NE depresses the firing rate of warm-sensitive cells and increases the firing rate of cool-sensitive cells. The responses obtained at the single cell level would require NE to produce a rise, rather than a fall, in body temperature. The factors responsible for the discrepancy between the effects of NE recorded at the single cell level and those at the gross physiological level are not immediately apparent.

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Estrogenic Induction of Ornithine Decarboxylase in vivo and in vitro

Abstract. *Injection of estrogens (17β-estradiol or diethylstilbestrol) into immature chicks results in a marked (30- to 50-fold) increase in the ornithine decarboxylase activity of oviductal homogenates within a 4-hour period. Similar stimulations were obtained when estrogen was injected into hypophysectomized or castrated rats and the uterus was examined for decarboxylase activity. An elevation of decarboxylase activity was obtained in vitro when oviducts from immature chicks were incubated in the presence of estrogen. These data indicate a direct action of estrogen on oviduct tissue to promote a rapid increase in the activity of a specific enzyme and represent the first example of a completely in vitro enzyme response to estrogen.*

Rapid induction of ornithine decarboxylase activity in liver after partial hepatectomy or administration of growth hormones has been reported (*1*). We have previously reported (*2*) that epidermal growth factor (a 6400-molecular-weight polypeptide isolated from the submaxillary gland of the mouse) induces a marked, but transient, increase of ornithine decarboxylase activity. This effect is demonstrable both in cultures of chick embryo epidermis and in the skin of mice after injection of this polypeptide. The similarities in the biochemical response of these cell types to a growth-promoting stimulus suggested the possibility that an increase in ornithine decarboxylase activity and subsequent putrescine and polyamine accumulation may be an early event in tissues in which growth is induced. If this hypothesis was both correct and general, then a tissue growth response mediated by a steroid hormone might also be preceded by an early stimulation of ornithine decarboxylase.

The chick oviduct represents a suitable model in which to test this hypothesis (*3*). Estrogen administration to the immature chick induces a marked growth response (1000-fold increase in wet weight) and results in the differentiation of the immature oviduct epithelium into three completely new cell types (*4*). This hormonal response is accompanied by changes in cell RNA populations, the induction of synthesis of a new complement of cell-specific proteins, and the appearance of new tissue functions (*4*).

In the first series of experiments, estrogen was injected either subcutaneously or intravenously into immature 6-day-old chicks. At intervals thereafter, the animals were killed and homogenates of the oviduct were prepared in 0.05*M* phosphate buffer, *p*H 6.6, containing 2 m*M* ethylenediaminetetraacetate (EDTA). The homogenates were centrifuged for 15 minutes at 10,000*g*, and the supernatant fluid was used for the assay of ornithine decarboxylase activity. The enzyme activity was determined by measurement of the release of $^{14}CO_2$ from DL-[1-^{14}C]ornithine (*2*).

The results (Table 1) of two such experiments show that 4 hours after the subcutaneous injection of diethylstilbestrol the specific activity of the decarboxylase had increased approximately 50-fold (experiment 1). Following this initial peak at 4 hours, the specific activity of the enzyme gradually diminished over the ensuing 6-day period. When a nonlethal dose of cycloheximide (1 mg) was injected 1 hour before estrogen

Table 1. Effect of the injection of estrogen into immature chicks on ornithine decarboxylase activity (expressed as the number of picomoles of $^{14}CO_2$ produced per 30 minutes per 100 μg of protein) of the oviduct. In experiment 1, 5 mg of diethylstilbestrol (dissolved in sesame oil) was injected subcutaneously into immature 6-day-old chicks. At various intervals the chicks were killed, and the oviducts were removed. Duplicate homogenates (three oviducts per homogenate) were prepared and centrifuged (see text). Portions of the 10,000g supernatant fluid were assayed for ornithine decarboxylase activity (*2*) and protein content (*9*). In experiment 2, 10 μg 17β-estradiol dissolved in a solution of ethanol and saline (1:20) was injected intravenously (wing vein) into 6-day-old immature chicks. The assay reaction mixture for determining ornithine decarboxylase activity contained 15 μmole of phosphate buffer (*p*H 6.6), 0.5 μmole of EDTA, 0.06 μmole of pyridoxal phosphate, and 0.06 μmole of DL-[1-^{14}C]ornithine (1.54 mc/mmole), in a total volume of 0.33 ml. The tubes were incubated for 30 minutes at 37°C. Each assay tube contained 0.2 to 2 mg of protein.

Time after estrogen administration	Ornithine decarboxylase activity
Experiment 1	
0	2
4 hours	110
16 hours	84
24 hours	40
72 hours	18
144 hours	4
Experiment 2	
0	3
20 minutes	2
60 minutes	9
120 minutes	66
240 minutes	92

administration, the induction of the decarboxylase activity in the 4-hour period after estrogen administration was inhibited approximately 80 percent. Similarly, a 30-fold increase in enzyme activity was found 4 hours after the intravenous injection of 10 μg of estradiol into these animals (experiment 2). In contrast, an injection of progesterone resulted in a relatively small increase in ornithine decarboxylase activity after a 4-hour period (a two- to fourfold increase over controls in three experiments).

To demonstrate the generality of this response to estrogen, we measured ornithine decarboxylase activity during the stimulation of uterine growth by estrogens. In these experiments, estrogen was injected into rats (Wistar) which had been either castrated or hypophysectomized for 1 week. Four hours later, the uterus was excised, homogenized, and assayed for decarboxylase activity (Table 2). Again there is a manyfold increase in enzyme activity 4 hours after estrogenic stimulation. This stimulation of decarboxylase occurs independently of the type of estrogen (17β-estradiol or diethylstilbestrol), the hormonal state of the animal (castrated or hypophysectomized), or the route of estrogen administration (intraperitoneally or intravenously).

In the final series of experiments, we investigated the ability of estrogens directly to stimulate oviductal ornithine decarboxylase activity in vitro. Chick oviducts were excised and incubated with and without diethylstilbestrol in tissue culture medium at 37°C (Table 3). In the presence of diethylstilbestrol a 15-fold increase in decarboxylase activity was noted. Under these in vitro conditions, cycloheximide virtually abolished all enzyme activity, again suggesting that protein synthesis is required for the estrogen-mediated stimulation of this enzyme. In separate experiments these concentrations of cycloheximide were found to inhibit the incorporation of radioactive amino acids into tissue protein by over 95 percent. To our knowledge, these results represent the first effect of estrogen in vitro on the synthesis of a special protein in any tissue. In fact, estrogen has not been reported to stimulate amino acid incorporation into the total protein when incubated in vitro with target tissues. This stimulation of ornithine decarboxylase may thus represent an early step in estrogen action but the usual in vitro conditions may not be adequate to demonstrate later effects of estrogen action, such as a stimulation of total protein synthesis.

In parallel with the in vivo experiments, progesterone in vitro showed a relatively small capacity to induce ornithine decarboxylase. This result is consistent with the suggestion that the rapid, manyfold induction of ornithine decarboxylase activity may be associated with tissue growth responses, since progesterone acts on the oviduct to stimulate synthesis of a specific protein, avidin, but does not promote oviduct growth. The addition of immature chick serum had no effect, supporting the specificity of the response.

It should be noted (Table 3) that the specific activity of the ornithine decarboxylase in the incubated control tissue (17 to 20 pmole of $^{14}CO_2$ produced per 30 minutes per 100 μg of protein) was distinctly higher than the specific activity of the enzyme in unincubated control tissue (2 to 3 pmole of $^{14}CO_2$ produced per 30 minutes per 100 μg of protein) (Table 1). In the presence of diethylstilbestrol the specific activity was increased to approximately 300. The reasons for the increasing

Table 2. Effect of the injection of estrogens into castrated or hypophysectomized rats on ornithine decarboxylase activity (expressed as the number of picomoles of $^{14}CO_2$ produced per 30 minutes per milligram of protein) of the uterus. In experiment 1, 10 μg of 17β-estradiol was injected intraperitoneally into hypophysectomized rats (175 g). In experiments 2 and 3, the rats were castrated 1 week before the intraperitoneal injection of 2.5 mg of diethylstilbestrol in sesame oil. In experiment 4, 5 μg of 17β-estradiol was injected intravenously into hypophysectomized rats. The uteri were removed 4 hours after estrogen treatment and assayed for ornithine decarboxylase as described in Table 1. The vehicle was injected into the control rats.

Experimental condition	Ornithine decarboxylase activity			
	Exp. 1	Exp. 2	Exp. 3	Exp. 4
Control	3	1	4	4
Estrogen-treated	126	137	265	39

baseline levels are not known, but like the induction by estrogen, it is inhibited by cycloheximide.

Since, in the systems we have studied, inhibitors of protein synthesis also inhibit the induction of ornithine decarboxylase activity, it would appear that de novo synthesis of the enzyme has occurred. However, until the enzyme is isolated or measured in some absolute manner, this conclusion can only be a tentative one. It is not known whether the induction involves the synthesis of new messenger RNA or whether some other mechanism is involved.

Our findings are in accord with previous observations (*5*) that estrogen treatment caused a significant increase

Table 3. Effects of diethylstilbestrol on ornithine decarboxylase activity (expressed as in Table 1) of chick oviducts in vitro. Oviducts from immature 6-day-old chicks were excised and incubated under sterile conditions in 1 ml of Medium 199 tissue culture medium (containing Hanks balanced salts) for 3.5 hours at 37°C. Diethylstilbestrol (DES) or progesterone was added at zero time to a final concentration of 5 μg/ml, and cycloheximide was added at a concentration of 25 μg/ml (experiment 1) or 50 μg/ml (experiment 2). Serum was prepared from immature chicks immediately before incubation and added to experimental flasks to a final concentration of 10 percent. The assay procedure is described in Table 1.

Incubation conditions	Ornithine decarboxylase activity	
	Exp. 1	Exp. 2
Control	20	17
DES	314	344
DES plus cycloheximide	2	2
Control plus cycloheximide	1	1
Progesterone	50	55
Control plus serum	17	21
DES plus serum	285	490

in spermidine concentration in the ovariectomized rat uterus 24 hours after intravenous injection. Testosterone has also been noted to effect a gradual increase in ornithine decarboxylase activity in the prostate of orchiectomized rats (*6*).

Certain steroids and polypeptide hormones which induce growth also induce the rapid appearance of ornithine decarboxylase activity in their target organs and an accumulation of amines derived from ornithine. Although an unambiguous biological role for putrescine and the polyamines has yet to be defined, they have been implicated in a large and diverse number of biological processes. For example, polyamines have been reported to stimulate protein synthesis, to stabilize polysomes, to stabilize membranes, to stimulate RNA and DNA synthesis, and to stimulate aminoacyl–transfer RNA formation (*7*). In many of these reactions the polyamines may replace, at least partially, a Mg^{2+} requirement, and it has been suggested that polyamines may be more important for protein synthesis in vivo than is Mg^{2+} (*8*).

The fact that both steroids and polypeptide hormones can act as inducers of ornithine decarboxylase suggests that the induction of the decarboxylase is an early, but not necessarily primary, event in the action of these hormones. It is conceivable that the rapid induction of ornithine decarboxylase and the subsequent accumulation of putrescine or polyamines provide a mechanism by which the cell may rapidly alter its internal environment to optimize conditions for a variety of biosynthetic reactions.

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Apospory in Sorghum bicolor (L.) Moench

Abstract. *A line of* Sorghum bicolor *(L.) Moench was discovered to reproduce by apospory, a type of apomixis. The formation of an embryo by a nucellar cell without fertilization was establshed by cytological observations of ovaries and by progeny tests.*

Apomixis, seed production without fertilization, has tremendous potential in the production of cereal grain hybrids. The F_1 hybrids are generally superior and are widely used, but they must be produced by repeated controlled crossing of parental lines. An apomictic hybrid would fix heterosis, since it perpetuates itself through identical offspring for successive generations.

We now report a form of apomixis in a polygynaceous line (*1*) of *Sorghum bicolor* (L.) Moench in which the embryo is formed by apospory from a somatic cell in the nucellus (*2*). This type of embryo development and its significance has been reported in several species (*2–4*), but we know of no documented report on apospory in a grain crop.

Method of reproduction was determined by cytological study of ovules and was confirmed by evaluation of progeny. Serial sections of ovaries were made at various stages of development and were stained in safranine–fast green in order to observe megasporogenesis and development of the embryo sac. The apomictic line was crossed with a pollen parent that had a dominant genetic marker.

Some ovules of this line had aposporous development, whereas those in other florets had sexual development. In sexually reproduced sorghum the megaspore mother cell differentiates and divides to form a linear tetrad of megaspores (*5*). The functional chalazal megaspore (Fig. 1A) continues to enlarge, the nucleus divides, and an embryo sac is formed with an egg, two polar nuclei, two synergids, and three antipodals (Fig. 1B).

Megasporogenesis in ovules with aposporous development proceeds identically to that in ovules which develop sexually, up to the time of formation of the functional chalazal megaspore. At this time the nucellar cells become active (Fig. 1C). These nucellar cells enlarge and the nuclei divide to form aposporous embryo sacs, each with two polar nuclei and an egg (Fig. 1, D to F). At this stage the chalazal megaspore apparently degenerates. There are usually two to five well-differentiated aposporous embryo sacs within each ovule.

Rao and Narayana (*6*) reported "a suspicion of possible apomictic development" in *S. bicolor*. A single fully differentiated eight-nucleate embryo sac arose from a cell at the chalazal end of the tetrad of megaspores. They assumed that the embryo sac originated from a nucellar cell, but the figures they presented may be interpreted as normal development of the functional megaspore. Apparently there was no evidence of additional nucellar activity in the ovule or formation of multiple aposporous embryo sacs as noted in our material, and their studies of progeny were inconclusive.

Apospory in this line of sorghum actually contributes to the formation of seed and progeny. Self-sterile plants which produced no pollen, possessing the polygynaceous character, were crossed with a pollen parent that had a dominant marker gene *Pc*, which causes susceptibility to toxin of the fungus *Periconia circinata* (Mang.) Sacc. The aposporous stock was recessive *pc* and resistant. The seedlings resulting from these crosses were screened for resistance to toxin (*7*). Up to 25 percent of one progeny of 16 and 20 percent of another progeny of 25 were resistant. We concluded that these resistant progeny arose from aposporic embryo sacs. Pollination was necessary for stimulation, for the development of endosperm, or for both.

To be ideal for plant breeding (*8*), apomixis should be obligate and trans-