stabilize the population of synapses. When a nerve terminal is destroyed, the regulator would no longer be present and adjacent terminals could then proliferate and occupy the vacated synaptic sites. Diamond and his colleagues have presented some experimental evidence for such an interpretation (*12*). The results of our studies suggest that propagated action potentials do not play the only role in controlling the release of putative chemotropic or regulator substances and in maintaining normal synaptic connections since blocking conducted activity in one vagus nerve with TTX neither caused a remodeling of functional ganglionic innervation nor did it prevent sprouting in the TTX-treated nerve after the opposite vagus was destroyed.

S. ROPER
C.-P. KO

Department of Anatomy and
Physiology, University of Colorado Medical Center, Denver 80262

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7. Tetrodotoxin-impregnated cuffs contained 1.5 percent TTX by weight. Commercially prepared TTX samples, in addition to 1 mg of the drug contained 5 mg of dry citrate buffer, *p*H 4.8. Hence, a 1.5 percent TTX cuff will also contain 7.5 percent dry buffer. Our citrate buffer control cuffs contained 9 percent buffer by weight, and our NaCl control cuffs contained 20 percent NaCl by weight.
8. In some experiments, vagal stimulation proximal to an implanted TTX cuff blocked the heart rate at low (4 to 5 V) or intermediate (for example, 10 to 40 V) intensities. Data from these experiments were not included in the final analysis. Our criterion for accepting the results of any one experiment was that the stimulus intensity applied central to the TTX cuff had to be at least one order of magnitude greater than that which was just effective when applied distal to the cuff.
9. M. C. Brown and R. Ironton, *Nature (London)* **265**, 459 (1977). These investigators showed that TTX cuffs applied to motor nerves in mice cause some degree of morphological sprouting at the treated end plates, but without accompanying functional changes. We have examined zinc iodide–stained cardiac ganglia from frogs with TTX cuffs implanted around one vagus nerve. To date, our material has not revealed any morphological changes comparable to those seen by Brown and Ironton. However, if a small number of fine nerve terminal sprouts occurred in TTX-treated cardiac ganglia, they might not be revealed by the somewhat capricious nature of zinc iodide staining.
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Induction of Ovalbumin Synthesis in Immature Chicks by Actinomycin D and Thioacetamide

Abstract. *Actinomycin D and thioacetamide induced ovalbumin synthesis and increased serum progesterone concentrations in immature chicks. The increase in progesterone induced by the carcinogens actinomycin D and thioacetamide may account for the induction of ovalbumin synthesis.*

We recently reported a novel effect of the administration of the carcinogen ethionine in immature chicks (*1, 2*). The effects of ethionine on the oviducts of immature chicks that have previously been stimulated with estrogen and subsequently withdrawn from estrogen administration for 3 to 4 weeks are similar to those caused by the administration of progesterone. Ethionine induces synthesis of the egg white proteins ovalbumin and conalbumin and also produces cellular changes in the oviduct characteristic of hormone administration. The effect of ethionine on the oviduct appears to be an indirect consequence of its influence on steroid hormone metabolism: it produces tenfold increase in serum progesterone concentrations (*2*). We show here that two other carcinogens, thioacetamide and actinomycin D, induce ovalbumin synthesis in estrogen-treated chicks and increase serum progesterone concentrations.

Estrogen administration to immature chicks results in cytodifferentiation of tubular gland cells which synthesize the egg white proteins ovalbumin, conalbumin, ovomucoid, and lysozyme. The continuous presence of estrogen is required for sustained synthesis of these proteins in immature chicks. Discontinuation of estrogen administration results in gradual decline in cell-specific protein synthesis. Ovalbumin synthesis is not detected after 3 to 4 weeks of estrogen withdrawal. Either estrogen or progesterone can induce the synthesis of major egg white proteins when given as a secondary stimulation to chicks withdrawn from estrogen stimulation (*3–5*).

Four-day-old Calhorn chicks (from Meyer Brothers' Hatchery) were injected intramuscularly below the knee daily with 1 mg of estradiol benzoate in sesame oil (*3*) for 10 days (primary estrogen stimulation). After 3 to 4 weeks of estrogen withdrawal the chicks were used in the experiments; at this stage, ovalbumin synthesis in the oviducts is not detected. The chicks were decapitated and their oviducts removed. The magnum portion of the oviduct was freed of adjoining tissue and was cut into small pieces. The magnum explants were incubated for 3 hours in vitro (*3*), 100 to 200 mg of tissue being placed in 2 ml of medium in 25-ml rubber-stoppered Erlenmeyer flasks. The temperature was maintained at 37°C, and the flasks were subjected to constant shaking and were gassed with 95 percent O_2, 5 percent CO_2, at hourly intervals. At the end of the incubation period the pieces of tissue were blotted on filter paper, homogenized in a Potter-Elvehjem homogenizer with 2 ml of 15 m*M* sodium chloride and 10 m*M* sodium phosphate, *p*H 7.5, and centrifuged at 100,000*g* for 1 hour. The supernatant obtained at high speed was used for subsequent analyses.

Administration of thioacetamide or actinomycin D to immature chicks 4 weeks after primary estrogen stimulation induced ovalbumin synthesis and increased progesterone concentrations (Table 1). Labeled ovalbumin was determined by specific immunoprecipitation with monospecific antiserum against purified ovalbumin (*1, 3*). The identity of the ovalbumin precipitated by antiserum was confirmed by electrophoresis on sodium dodecyl sulfate–polyacrylamide gel with authentic markers (Fig. 1). An increase in serum progesterone brought about by actinomycin D in immature chicks has also been observed by Elo *et al.* (*6*). Progesterone is known to induce ovalbumin synthesis in chicks when it is given as a secondary stimulation to chicks withdrawn from estrogen stimulation (*4, 5*). The increase in serum progesterone brought about by actinomycin D and thioacetamide may account for the induction of ovalbumin synthesis in chicks. The lag period for the induction of ovalbumin synthesis is much longer with actinomycin D (1 day), thioacetamide (2 days, data not shown), or ethio-

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nine [3 days (*1*)] than it is with progesterone or estradiol (2 or 3 hours, respectively) (*7*).

Thioacetamide is hepatotoxic and carcinogenic (*8*), and it produces enlargement of the nucleus and nucleolus (*8*), alterations in RNA metabolism (*9*), interruptions in microsomal electron transport (*10*), acute thymic atrophy, and tubular renal necrosis (*11*). Actinomycin D binds to DNA, is an inhibitor of DNA-dependent RNA synthesis, and produces nuclear segregation (*12*). It is cytotoxic primarily to proliferating cells (*13*) and is, therefore, used as an antitumor agent (*14*). Actinomycin D produces tumors in mice and rats (*15*) and impairs the immune response (*16*) and pinocytosis (*17*).

Actinomycin D has a paradoxical effect on protein synthesis in chicks. When it is administered at the same time as estrogen, at a dose of 5 mg per kilogram of body weight, it prevents the estrogen-mediated induction of ovalbumin synthesis in chick oviduct measured after 9 hours (*3*). But when actinomycin D (5 mg/kg, body weight) is administered at a time when oviduct magnum is actively synthesizing ovalbumin, it increases the synthesis of ovalbumin relative to total protein synthesis (*18*). The stimulatory effect of actinomycin D on protein synthesis (superinduction) is well recognized in a variety of other systems (*19*).

The increase in serum progesterone and the induction of ovalbumin synthesis in chicks (which do not normally synthesize ovalbumin) brought about by a lower dose of actinomycin D (400 μg/kg) represent still another effect of actinomycin D.

Progesterone is a common precursor of estrogens, androgens, and corticosteroids. Increases in progesterone brought about by carcinogens may affect the concentrations of other hormones. How carcinogens of such different structures as ethionine, thioacetamide, and actinomycin D increase serum progesterone remains to be elucidated. It may be that they increase the synthesis of progesterone or decrease its excretion or conversion to estrogens, androgens, and corticosteroids. Administration of estrogens or progestins is known to produce tumors in animals (*20*, *21*). Bogden *et al.* (*22*) have observed an increase in serum estradiol and a decrease in progesterone in rats treated with several antineoplastic agents. In rats, 3-methylcholanthrene has been reported to affect the release of progestin by the ovary (*23*). Progesterone is immunosuppressive (*24*). Ethionine, thioacetamide, and actinomycin D are not detected as mutagens in the Ames mutagenicity test (*25*). The phenomenon of hormone imbalance produced by different carcinogens described here may have implications in the mechanisms of carcinogenesis.

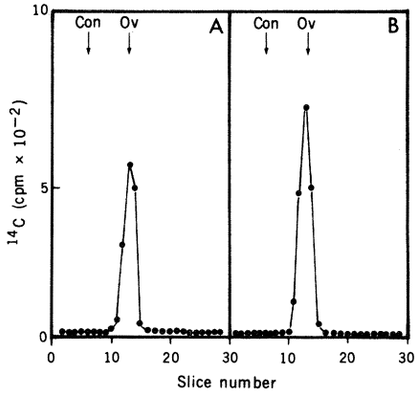

Fig. 1. Sodium dodecyl sulfate–polyacrylamide disk gel electrophoresis of labeled proteins precipitated with antiserum to ovalbumin. Immunoprecipitation was carried out with 50-μl portions of supernatant obtained by high-speed centrifugation of oviduct magnum explants (see Table 1 for details). The washed antibody precipitates, which were mixed with internal markers (^{3}H-labeled ovalbumin and conalbumin that had been synthesized in estrogen-stimulated oviduct explants) were subjected to coelectrophoresis (*3*). The mobility of conalbumin (*Con*) and of ovalbumin (*Ov*) is indicated by arrows. (A) Data from chicks injected with thioacetamide 3 to 4 weeks after estrogen withdrawal. (B) Data from chicks injected with actinomycin D 3 to 4 weeks after estrogen withdrawal.

Table 1. Induction of ovalbumin synthesis in immature chicks by thioacetamide and actinomycin D. Chicks withdrawn from estrogen stimulation for 4 weeks following primary stimulation were injected intraperitoneally in groups of three with thioacetamide (0.25 mg per gram of body weight in water) for 3 days or with a single injection of actinomycin D (Sigma; 0.4 μg per gram of body weight in saline). The thioacetamide-injected chicks were killed after 3 days and the actinomycin D–injected chicks were killed after 24 hours. Ovalbumin synthesis in the oviduct magnum explants was determined as described (*1*). Values for ovalbumin synthesis are the averages of three chicks. Analyses of pooled serums from three chicks for progesterone (*26*) by radioimmunoassay were performed by Laboratory Procedures, Upjohn Co. [see (*2*)]. Serum samples were submitted for analyses under different code numbers. Values for duplicate progesterone determinations are given. These experiments were repeated with other batches of chicks with the same results.

Treatment	Ovalbumin synthesis in oviduct explants (% of total protein synthesis)	Serum progesterone (ng/100 ml)
	Experiment 1	
None	Not detected	89 40
Thioacetamide	9.1 ± 3.9	296 371
	Experiment 2	
None	Not detected	100 81
Actinomycin D	8.7 ± 1.9	351 496

OPENDRA K. SHARMA
Department of Microbiology, University of Colorado Medical Center, Denver 80262, and *Department of Basic Oncology, AMC Cancer Research Center and Hospital, Lakewood, Colorado 80214*

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