Therefore, the synthesis of the binding protein is correlated with an increase in cell number and is not directly related to estrogen induction. If the function of the receptor (EBS) is to interact with estrogen and then to act as an effector unit, either by derepression or activation of gene sites, then it seems necessary that estrogen receptors should develop prior to their need and independently of estrogen induction. Receptor numbers, after the initial developmental period, would only be a function of cell numbers and cell proliferation even after the cells are exposed to estrogen.

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Cardiac Glycosides and Distastefulness: Some Observations on the Palatability Spectrum of Butterflies

Abstract. *The monarch butterfly* Danaus plexippus *sequesters cardiac gylcosides from northern species of* Asclepias *formerly reported to lack these noxious compounds. Thus, a new explanation must be sought as to why the monarchs reared on these northern asclepiads are palatable to avian predators.*

Monarch butterflies *Danaus plexippus*, when reared on tropical asclepiads and apocynads, contain cardiac glycosides which make them unpalatable to avian predators (*1*, *2*). In addition, Brower (*1*) has noted that when this butterfly is raised on three of the northern species of *Asclepias*—*A. syriaca*, *A. incarnata*, and *A. tuberosa*—it is acceptable to avian predators which could not eat the tropical cohort. His conclusion, based on gustatory evidence, was that the three northern species of *Asclepias* do not possess cardiac glycosides. However, the European *Asclepias syriaca* contains at least nine cardiac glycosides (*3*). This apparent disparity has prompted me to determine whether five of the many species of *Asclepias* in Canada elaborate cardenolides, and further, if they do, to determine whether these substances are then found in the monarch and other insects that feed upon these plants.

Glycosides were extracted from seeds (by refluxing with a methanol-ethanol mixture, 1 : 1), leaves (with 50 percent aqueous ethanol), and insect tissue (with a chloroform-methanol mixture, 2 : 1). The extracts were purified on a Florisil column (*4*) (with increasing

Table 1. Materials tested for presence of cardiac glycosides. +, Positive response; blank, not determined; + in last four columns, indicates a positive response in the cardenolide R_F range defined by ouabain and digitoxin (Nutritional Biochemicals Co.) on silica gel G with an ethylene dichloride : methanol : formamide (80 : 25 : 1) solvent system. INO, inotropic response in rat heart by means of carotid cannulation; ALDO, antagonism of inotropic response; DNBoic, 3,5-dinitrobenzoic acid + NaOH spray reagent; NAPH, 1,2-naphthoquinone-4-sulfonic acid + NaOH spray reagent; DNBzene, 3,5-dinitrobenzene + NaOH spray reagent; XANTH, xanthydrol in glacial acetic acid + HCl spray reagent.

Materials	INO	ALDO	DNBoic	NAPH	DNBzene	XANTH
			Chemical controls			
Ouabain ($3 \times 10^{-7}M$)	+	+	+	+	+	+
Aldosterone ($3 \times 10^{-7}M$)	+					
			Plants			
Asclepias syriaca (Ontario)						
Seeds	+	+	+	+	+	+
Pods			+	+	+	+
Leaves			+	+	+	+
Asclepias incarnata (Ontario)						
Seeds	+	+	+	+	+	+
Pods			+	+	+	+
Leaves			+	+	+	+
Asclepias speciosa (British Columbia)						
Seeds	+	+	+	+	+	+
Pods			+	+	+	+
Leaves			+	+	+	+
Asclepias ovalifolia (Manitoba)						
Leaves			+	+		+
Asclepias tuberosa (Ontario)						
Leaves			+	+		+
Asclepias spp. (Jamaica)						
Leaves	+		+	+	+	+
			Plant controls			
Digitalis purpurea						
Leaves	+		+	+	+	+
Nerium oleander						
Leaves	+		+	+	+	+
			Insects			
Danaus plexippus						
Ontario, indigene	+		+	+		+
Ontario, migrant	+		+	+		+
Manitoba	+		+	+		+

amounts of methanol in diethyl ether), and their identity was verified by thin-layer chromatography on silica gel G, by spectrophotometry (*5*), and by biological assay (inotropic response in rat heart, and antagonism of this by aldosterone). The glycosidic compounds obtained from the plant and insect extracts showed no loss of identity or inotropic activity on acid and base hydrolysis.

Cardiac glycosides are present in all northern species of *Asclepias* said by Brower (*1*) to lack these compounds, and in two other northern species of *Asclepias* studied (Table 1); these compounds are thus available to insects feeding on various portions of the plant. The seeds are the richest source of these noxious compounds in three of the species examined. The indigenous monarchs (*6*), which are leaf feeders in the larval stage, seem to have sequestered cardiac glycosides from these northern species of *Asclepias*. It has also been shown that *Danaus plexippus* is not atypical. *Oncopeltus fasciatus* (*7*), *Lygaeus kalmii kalmii, Lygaeus kalmii angustomarginatus, Tetraopes tetraophthalmus*, and *Tetraopes oregonensis*, all of which feed upon northern *Asclepias*, contain cardiac glycosides (*7*). Also, fifteen other species of *Asclepias* (from diverse parts of North America) possess cardiac glycosides (*8*). Thus, Brower is incorrect in assuming that the northern species of *Asclepias* lack cardiac glycosides. The geographical range of *Asclepias* possessing cardiac glycosides is indeed much more extensive than indicated by Brower (*1*).

However, since Brower (*1*) reports that the northern species of *Danaus* feeding on *Asclepias* is not unpalatable, then some alternative explanation must be sought to explain his birds' behavior. Several possibilities seem to exist. It is possible that the northern species of *Asclepias* lack specific glycosides that are present in the southern species of *Asclepias*. In the five northern species examined (at least 15 compounds in seeds, and at least six in leaves and pods) and one tropical species (at least 12 compounds) tested there are a large number of compounds that react positively to *m*-dinitrobenzoic acid plus NaOH. Further, in the 15 additional asclepiads studied some three to ten cardiac glycosides have been detected and Kupchan *et al.* (*9*) report at least nine cardiac glycosides (positive to *m*-dinitrobenzene plus NaOH) in *A. curassavica*. Until the specific identity of all these glycosides is established, qualitative chemical differences in the host plants cannot be ruled out.

It is also possible that the various species of *Asclepias* differ in the concentration of component glycosides. Brower (*1*) mentions three specific glycosides in *A. curassavica* and in *Danaus* fed on this plant, but it is not certain that these are either the most important or the sole glycosides involved in effecting impalatability. Indeed, the present study on limited material has detected at least eight compounds positive to *m*-dinitrobenzoic acid plus NaOH in indigenous northern *Danaus*.

The blue jay *Cyanocitta cristata bromia* is the major avian predator employed in studies of palatability (*1, 2, 10, 11*). One would expect interspecific variation in the responses of (avian) predators to foods containing cardiac glycosides, a factor which could complicate the palatability spectrum. Further, it is also possible that there is a threshold concentration of glycosides that the predator must ingest to induce emesis: such a threshold may again involve specific glycosides or total concentration of cardenolides in the insects. We do not know whether *Danaus* feeding on *Asclepias* simply accumulates glycosides according to the type and concentration in the plant, or if it has specific selective or concentration mechanisms.

Not until the chemical composition of the various asclepiad hosts is accurately determined and the insect physiology thoroughly known, will it be possible to deduce the true basis of the palatability spectrum observed by Brower. The present results suggest that it will be more complex than originally postulated.

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7. *Oncopeltus fasciatus* concentrates cardiac glycosides in its ventral metathoracic stink glands, and in previously undescribed dorsolateral meso- and metathoracic and dorsolateral abdominal glands. I thank Dr. D. Feir and Mr. R. Cannings for specimens of insects from Missouri and Ontario.
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Ozone Uptake by Bean Leaves

Abstract. *The removal of ozone from the air by bean leaves is regulated by the same factors that control the exchange of water vapor between leaves and the atmosphere.*

Ozone is one of the principal air pollutants causing damage to plants in the United States (*1*). The uptake of ozone by leaves is essentially an exchange of a gas between leaves and the atmosphere. As such, this uptake must be regulated by the same factors that control the exchange of other gases, such as water vapor. The loss of water vapor from leaves by transpiration is commonly analyzed in terms of resistance, seconds per centimeter (*2*). Thus, we measured ozone uptake by leaves, converted these data to resistance to ozone uptake, and compared this resistance to water-vapor loss.

The rate of removal of ozone was measured as the time t (in seconds) for the concentration C to decline from 200 to 150 parts per billion (ppb) within a closed, illuminated, 75-liter chamber equipped with a fan. Temperature within the chamber was 15°C ± 1.5°. The concentration of ozone in the chamber was increased by pumping in air irradiated by ultraviolet lamps in a separate box. When the desired concentration was reached, the ozone-rich air