

ABSTRACT

Demetrice L. Ward. THE EFFECTS OF PROLACTIN ON EPIDERMAL GLYCOCONJUGATES IN THE RED-SPOTTED NEWT Notophthalmus viridescens viridescens USING LECTIN HISTOCHEMISTRY. (Under the direction of Dr. Charles Singhas). Department of Biology, July 1992.

The present study uses lectin histochemistry to examine the extent to which prolactin (PRL)-induced integumental changes in red-spotted newts are due to alterations in the expression and distribution of glycoconjugates within the epidermis and epidermal glands. A panel of eight horseradish peroxidase labelled lectins (LTA, UEA-I, DBA, SBA, GSAI-B4, WGA, PNA, CON A), which bind specifically to terminal carbohydrate moieties of glycoproteins and glycolipids was chosen. The epidermis of PRL-injected newts showed a marked decrease in lectin binding for LTA, DBA, SBA, and PNA as compared to control saline-injected newts. The binding of WGA to sialic acid increased with PRL injection, while GSAI-B4 and CON A showed little to no change in binding. Temporal studies revealed that differential lectin binding occurred by day 6 in PRL-injected newts as well as the epidermis of freshly collected newts undergoing conditioning. Seasonal examination revealed similarities in the expression and distribution of epidermal glycoconjugates between freshly collected winter animals and laboratory conditioned animals that had received PRL. Together, these findings demonstrate that prolactin may bring about integumental changes in the red-spotted newt by affecting the expression and distribution of both secretory and structural glycoconjugates.

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ON EPIDERMAL GLYCOCONJUGATES
IN THE RED-SPOTTED NEWT,
Notophthalmus viridescens viridescens,
USING LECTIN HISTOCHEMISTRY

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Presented to
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East Carolina University

In Partial Fullfilment
of the Requirement for the Degree
Master of Science in Biology

by
Demetrice Ward
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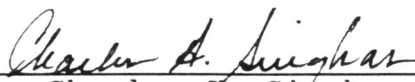
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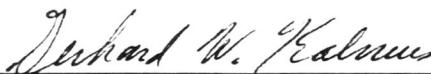
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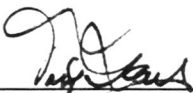
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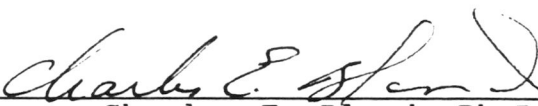
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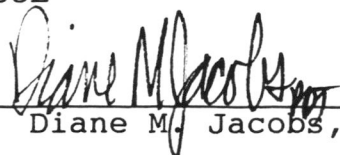
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INTRODUCTION

The vertebrate integument represents the primary interface between an organism and its external environment. Its constituents are an outer epidermis of ectodermal origin and a subjacent, connective tissue dermis of mesodermal origin. The surface layer of the epidermis may be one or two cell layers in thickness and is composed of flattened, partially or fully keratinized cells termed keratinocytes. Keratinization results from the degradation of cell contents and concurrent synthesis and deposition of keratin proteins (Lauker, 1974; Krejsa, 1979; Mogilnoya and Shubich, 1982; Navas et al, 1987). Transitional cells, which represent developing keratinocytes, are present in two or three layers, are located just below the stratum corneum, and are characterized ultrastructurally by the presence of tonofilaments consisting of prekeratin (DeRobertis and DeRobertis, 1987). Below the transitional layer lies the stratum basale, the only layer that is mitotically active and therefore the source from which other epidermal cell layers are derived. The stratum basale is one cell layer in thickness and rests upon a basement membrane.

It has been reported that the basement membrane originates from basal epithelial cells and, perhaps either directly or indirectly, from the dermis (Krejsa, 1979; Leeson et al., 1988). The basement membrane has been described as a

membrane complex that structurally separates epidermis from dermis and is composed of two primary layers: a juxtaepidermal basal lamina and a subjacent basal lamella (Krejja, 1979). The basement membrane provides a connection between the basal cells of the epidermis and the connective tissue of the underlying dermis (Leeson et al., 1988). Krejja (1979) states that the basement membrane has a pivotal role in an ontogenic phenomenon referred to as "functional epithelial extinction" (FEE). In FEE, epithelial cells originating in the basal epithelial layer give rise to cells or cell generations that move upward, away from the basement membrane to serve specific functions in the epidermis. Krejja suggested that the basement membrane is crucial to basal cell-mesenchymal (dermis) interactions, and that such interactions may be correlated with the complexity of the vertebrate integument.

The integument has been implicated in a variety of biological functions including protection, chemoreception, osmoregulation, and respiration. The chemoreceptive properties of the integument possibly aid in exterior detection of chemicals that could harm or disturb internal structures (Romer and Parsons, 1986), whereas osmoregulative activities have been shown to control water loss in marine and terrestrial vertebrates and water uptake in fresh water species (Krejja, 1979). Respiration is accomplished by the exchange of carbon dioxide and oxygen across the integument in many amphibians (Noble, 1931; Romer and Parsons, 1986).

Cutaneous respiration can only occur across surfaces that are moist, thin, and readily permeable, and are close to a vascular supply (Hoar, 1983).

The versatility of the integument is enhanced by the many structural modifications that have evolved from it. Structures arising from keratinized epidermis include horny scales, nails, claws, and hoofs. Other modifications include feathers, horny epidermal structures believed to have evolved from reptilian scales; hair, formed of keratinized epidermis and evolutionarily considered to be a new structural element of the skin; and epidermal glands having both mucus and serous secretory products (Romer and Parson, 1986).

The typical schema for integumental morphology is similar in both anuran and urodele amphibians (Kent, 1987) and is composed of an epidermis and an underlying dermis. The outermost layer of the epidermis may consist of a single-layer of keratinized cells, as in many aquatic and land salamanders, or may be devoid of keratin as in neotenic salamanders such as Necturus (Duellman and Trueb, 1986). Transitional cells are typically present in two layers whereas the basal layer is one cell in thickness. In many aquatic amphibians the structure of the integument, i.e. thin, well vascularized etc., is essential for successful cutaneous respiration, especially when the lungs are greatly reduced or absent, as in the lungless salamanders of the family Plethodontidae (Noble, 1931; Moore, 1964; Hickman et al., 1988).

In larval amphibians, the integument consists of a single layer of cells and unicellular glands (Krejsa, 1979). Following metamorphosis, epidermal glands become multicellular and are present as two types: mucous and granular (Noble, 1931; Krejsa, 1979;). Mucous glands are small, numerous, and widely spread over the body and secrete a transparent substance thought to serve as a lubricant in water and as an antidesiccant on land (Noble, 1931).

Granular glands, so named due to the presence of granules, produce a protective apocrine secretion (Noble, 1931; Krejsa, 1979; Kent, 1987;). Excessive excitement or violent stimulation will cause granular glands to discharge their secretory product onto the surface of the skin. The subsequent effect of this irritant or toxin on potential predators ranges from skin inflammation to paralysis or even death (Noble, 1931; Krejsa, 1979). It has been suggested that the low rate of adult mortality observed in the California newt may be attributed to protective skin toxins that discourage would be predators (Krejsa, 1979). Granular glands are large, few in number, and appear in various areas on the body depending on the species. In some anurans such as true toads, family Bufonidae, granular glands appear as enlarged paratoid glands behind the eyes. Others, such as Notoden bennetti (Catholic frog) demonstrate these glands on the entire dorsal surface, while species of Rana have ridges along the back (Noble, 1931; Krejsa, 1979). Among urodeles,

salamanders such as Tylototriton may have granular glands in the form of warts, whereas Plethodon exhibits thickened portions in the integument of the back and tail (Noble, 1931).

The red-spotted newt, Notophthalmus viridescens viridescens (Figure 1) is an aquatic urodele whose unique lifecycle is reflected by integumental changes. During the aquatic larval phase, the epidermis consists of a single cell layer containing unicellular glands. After the first metamorphosis, a terrestrial stage, ensues that lasts between 3 and 5 years. During this "red eft" stage, the skin has a rough, "sand papery" appearance that results from the presence of keratinized epidermal tubercles. Following a second metamorphosis, the adult newt becomes permanently aquatic and is covered with smooth skin that shows increased cutaneous secretory activity (Dent et al., 1973; Singhas and Dent, 1975; Spearman, 1977; Hoffman and Dent, 1978).

Events associated with metamorphosis, such as the sojourn on land and the eventual return to water, are regulated by the endocrine system. It has been demonstrated in tiger salamanders (Ambystoma tigrinum) that thyroxine administration elicits a preference for, and induces migration to, dry substrate (Duvall and Norris, 1980). Metamorphosed salamanders also exhibit higher circulating thyroxine levels than do those having an aquatic preference (Duvall and Norris, 1980). Thus, thyroxine is believed to control urodele migration to land (Norris, 1985). Alternatively, several

studies have reported that prolactin (PRL) causes juvenile red-spotted newts to migrate to water (Adams, 1932; Chadwick, 1941; Grant and Grant, 1958;), and this activity has been used as a bioassay for PRL (Norris, 1985).

Dent et al. (1973) reported that 1.2 - 120 units of exogenous PRL caused the skin of intact, laboratory conditioned newts to become smooth and slimy due to increased cutaneous secretion. Pituitary autografts with the apparent subsequent release of endogenous PRL into the blood stream causes the integument of the newt to increase in smoothness and cutaneous secretion (Adams, 1932; Chadwick, 1941; Grant and Grant, 1958; Dent, 1966).

Molting activity in the newt involves an interaction between prolactin and thyroxine. As reported by Dent and co-workers (1973), PRL alone is not responsible for the induction of molting but probably serves a facilitative role when introduced with thyroxine. Thyroidectomized newts exposed to 10ug/liter of l-thyroxine and injected with 12 units of PRL were observed to molt normally, and their skin became smooth and cutaneous secretion increased. It was concluded that changes in the integument caused by PRL were to some extent mediated by thyroid hormone.

Studies have shown that secondary sexual characteristics of newts, i.e., increased tailfin height and the presence of nuptial pads, are also under the influence of hormones. Singhas and Dent (1975) report that in combination,

testosterone and PRL maintained nuptial pads in a well-formed, mature state in the male red-spotted newt. They also reported that exogenous PRL administered alone or in combination with testosterone counteracted, almost completely, the loss of secondary sexual characteristics that is seen when newts are laboratory conditioned. Additionally, they reported that the cyclic expression of nuptial pads and increased tailfin height is seasonally correlated.

Cutaneous secretion in the red-spotted newt, as in other amphibians, originates from mucous and granular glands (Figure 2). These multicellular glands are present in the skin of adult and juvenile animals and are PAS positive, indicating the presence of carbohydrates. Granular glands are distributed uniformly over the trunk and limbs, and are more numerous than mucous glands. Through muscarinic receptors, granular glands are stimulated by cholinergic agents such as acetylcholine (Hoffman and Dent, 1978). Similar reports have been published concerning granular gland response to acetylcholine in the crested newt, Triturus cristatus (Hoffman and Dent, 1978); however, it has also been noted that in anurans (e.g., Xenopus laevis) granular glands are under adrenergic stimulation (Bastian and Zarrow, 1954; Watlington, 1968; Benson and Hadley, 1969; Hoffman and Dent, 1978). The secretory product of granular glands, tetrodotoxin, is a neurotoxin which serves a protective function (Fuhrman, 1967; Hoffman and Dent, 1978). In contrast, mucous producing glands

have an innervated myoepithelium, which does not respond to stimulation by cholinergic neurotransmitters (Hoffman and Dent, 1978).

Seasonal variations in the integument of the red-spotted newt have been reported by several investigators. In the winter months, skin texture is smooth and cutaneous secretory activity is maximal; whereas in the summer cutaneous secretion decreases and the skin becomes rough. Seasonal variation in hormonal activity has been reported by Scalenghe and coworkers, (1968) in the crested newt, in which PRL levels were shown to be higher during winter months when compared to animals collected during the summer. In the red-spotted newt, it has been postulated that seasonal variation in cutaneous secretion and texture of the integument results from cyclic changes in levels of PRL and/ or thyroid hormone.

Glycoconjugates consist of glycoproteins and glycolipids and are products of association between a carbohydrate (a "glycan") and either a protein or lipid (Montreuil, 1980). Glycoconjugates have been implicated in biological processes such as transport, differentiation, and cell-to-cell interaction (Rauvala et al., 1981). Alterations in the composition and/or distribution of glycoconjugates may indicate changes in the functional state of cells and tissues. The present study was undertaken to evaluate the effects of PRL on the expression and distribution of secretory and structural glycoconjugates in the epidermis of the red-spotted

newt. Additionally, temporal changes in epidermal glycoconjugates during conditioning and PRL injection are evaluated, and seasonal variations in epidermal glycoconjugates are examined.

In order to evaluate in situ changes in these glycoconjugates, the present study uses lectin histochemistry. Lectins are di- and multivalent proteins which have a high affinity for carbohydrate residues of specific structure and configuration (Goldstein et al., 1980; Barondes, 1981; Navas et al., 1987). While carbohydrate stains such as PAS and alcian blue differentiate broad categories of complex carbohydrates, lectins provide information relative to carbohydrate structure and orientation (Spicer and Schulte, 1992). By using lectin histochemistry to probe glycoconjugates that may be involved in specialized biological activities, this study attempts to elucidate PRL's effect on glycoconjugates in the epidermis.

FIGURE 1.

The red-spotted newt, Notophthalmus viridescens
viridescens:

1. Following 14 days of prolactin injection, the integument becomes smooth and slimy and cutaneous secretion is increased.

2. Laboratory conditioned newt injected with saline; the integument remains rough in appearance and cutaneous secretory activity is decreased.



FIGURE 2

1. Mallory's triple stain of control, saline-injected newt; larger granular gland (gg) stains magenta, mucous gland (mg) stains blue; dermal melanophores (dm), rough keratinized s. corneum (sc) stains magenta. Original magnification X50. Bar = 103 um.

2. Mallory's triple stain of PRL-injected (X 14 days) newt; mucous (mg) and granular (gg) glands present; dermal melanophores; s. corneum (sc) has smooth appearance; dermis (d). Original magnification X50. Bar = 103 um.

3. Illustration of red-spotted newt integument; s. corneum (sc); transitional layers (tl); stratum basale (sb); basement membrane (bm); dermal melanophores (dm); dermis (d); granular gland (gg), mucous gland (mg).

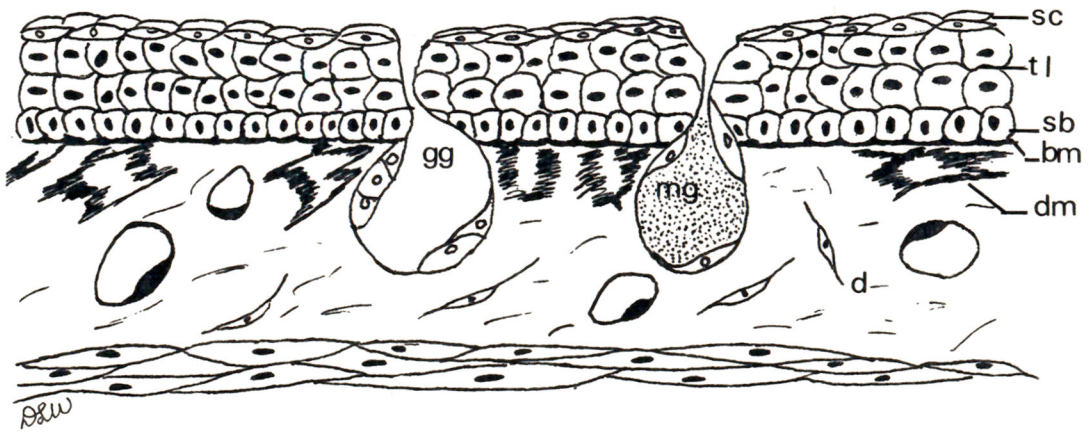
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METHODS

Collection of Animals

Adult red-spotted newts were collected from artificial ponds during January, 1991 near Springfield, West Virginia and transported to the laboratory in oxygenated pond water. Animals were maintained at 10°C prior to laboratory conditioning.

Laboratory Conditioning

Animals were maintained on a diet of lean ground beef fortified with dibasic calcium phosphate and cod liver oil and were fed twice weekly. The newts were housed under ambient lighting and temperature conditions for a minimum period of two weeks before being included in experimental protocols. At the conclusion of this project, newts were returned to the ponds and released.

Effects of Prolactin on Epidermal Glycoconjugates

The effects of exogenous prolactin (PRL) on epidermal glycoconjugates were examined using laboratory conditioned animals that were randomly assigned to either experimental or control groups (n=6). Experimental animals received intraperitoneal injections of ovine PRL (1.5 U in a total volume of 0.1 ml amphibian saline) on alternate days over a two week period. Control animals were injected with an equal

volume of 0.85% amphibian saline alone over the same treatment period. On day 15, samples of the integument were obtained for analysis as described below.

The prolactin (PRL) used for this investigation (ovine prolactin 250 IU, Activity 20-50 IU per mg) was obtained as a gift from the National Hormone and Pituitary Distribution Program of the National Institutes of Health. It was prepared for injection as described by Singhas and Dent (1975). A dose of 1.5 U was used in the present study because it had been previously shown to adequately promote changes in texture and cutaneous secretion in the newt (Dent et al., 1973; Hoffman and Dent, 1978).

PRL-Induced Changes in Temporal Expression of Epidermal Glycoconjugates

The effects of PRL on temporal changes in the expression of epidermal glycoconjugates were examined by injecting twenty-five laboratory conditioned animals with ovine prolactin (1.5U/0.1ml, IP) on alternate days for two weeks. On days 3, 6, 9, 12, and 15 five animals were randomly selected and a sample of the integument obtained for analysis. Integument texture was evaluated for each animal on each injection day by the following scale: "rough", "smooth", "slimy and smooth". After use, animals were allowed to recover and were released.

Effects of Conditioning on Temporal Expression of Epidermal Glycoconjugates

When freshly collected newts are maintained at 5 to 10°C, they retain their "winter" characteristics including nuptial pads, elevated tailfin height, and increased cutaneous secretion for up to 6 months. However, these characteristics are lost within two weeks when these animals undergo laboratory conditioning. To evaluate glycoconjugate changes during this conditioning process, newts that had been stored at 5 to 10°C, were allowed to condition to the laboratory environment for 14 days. During this period, two animals were arbitrarily chosen every third day for sampling.

Seasonal Changes in Glycoconjugate Distribution

Several investigators have reported seasonal changes in the epidermis of the newt that are similar to the reported effects of prolactin. During the late summer, the skin is rough and shows little evidence of cutaneous activity. Laboratory conditioning results in integumental changes that resemble what is seen in summer animals. In order to evaluate the role that glycoconjugates may have in these seasonal changes, freshly collected winter animals (n=10) were compared with laboratory conditioned newts that had a skin pattern comparable to the summer condition.

Tissue Preparation

All newts were anesthetized in 0.1% MS-222 (Sigma, St. Louis, MO) and 4 x 6mm skin biopsies were removed from the proximal tail region. For histochemical analysis, the tissues were fixed in B4G (6% HgCl_2 , in sodium acetate containing 0.1% glutaraldehyde, pH 6.0) as described by Schulte and Spicer (1983), or in buffered formalin, pH 7.2. The tissue was then dehydrated in a graded series of ethanol, cleared in toluene, and embedded in paraffin. Sections were cut at 5 μm on a rotary microtome and affixed to glass slides. After rehydration through graded ethanol, slides were rinsed in distilled water prior to application of lectins. Tissues were initially characterized using hematoxylin, Mallory's Triple stain, Periodic Acid-Schiff (PAS), and Alcian blue techniques (Humason, 1967).

Lectin Staining

A panel of horseradish peroxidase conjugated (HRP) lectins (see Table 1) were used to probe epidermal glycoconjugates for specific carbohydrate moieties. Conjugated lectins purchased from Sigma were diluted 1:50 in phosphate buffered saline (PBS), pH 7.2, containing 0.1mM CaCl_2 , MgCl_2 , and MnCl_2 such that the final concentration of each was approximately 10-50 $\mu\text{g}/\text{ml}$.

Lectin staining procedures followed the methods of Schulte and Spicer (1983). After sections were deparaffinized and rehydrated, slides were immersed for 3

minutes in Lugol's solution, followed by a 3 minute treatment in sodium thiosulfate and 5 minute wash in distilled water to remove mercuric chloride. Sections were then incubated for 10 minutes in 3% H_2O_2 to block endogenous peroxidase activity and rinsed in 0.1M PBS. Sections were flooded with each lectin and placed in a humidified chamber for 30 minutes at room temperature. Following incubation, the lectin conjugates were developed by exposing sections to 0.05% diaminobenzidine - 3% H_2O_2 , pH 7.0 for 10 minutes.

Specificity of lectin binding was evaluated using control sections that were incubated with PBS alone or with a 10X molar excess of specific inhibitory sugar. Slides were examined visually for the amount and intensity of reaction product using a Nikon Microphot-Fx equipped for differential interference contrast microscopy.

Evaluation of lectin staining

Lectin stain intensity was evaluated using the following scale:

- 0 - unstained
- 1 - light stain
- 2 - moderate stain
- 3 - heavy stain
- 4 - very heavy stain

Previous studies have also used subjective scales in order to describe lectin binding (Mariassy et al., 1988; Suter et al, 1991).

Table 1. HRP-lectins and Carbohydrate Specificity

<u>Lectin</u>	<u>Carbohydrate</u>	<u>Source</u>
LTA	L-Fucose	<u>Lotus tetragonolobus</u>
UEA-I	L-Fucose	<u>Ulex europaeus</u>
DBA	D-GalNAc	<u>Dolichos biflorus</u>
SBA	D-GalNAc	<u>Glycine max</u>
GSAI-B4	D-Gal	<u>Bandeiraea simplicifolia</u>
CON A	D-Mannose, D-Glucose	<u>Canavalia ensiformis</u>
WGA	Sialic acid, GluNAc	<u>Triticum vulgare</u>
PNA	D-Gal(1,3)- D-GalNAc	<u>Arachis hypogaea</u>

RESULTS

Effects of Prolactin Injection on Epidermal Glycoconjugates

During the 14-day conditioning period, all newts demonstrated substantial reductions in cutaneous secretory activity that was accompanied by increased roughness of the epidermis. These integumental changes were reversed after treatment with 1.5 U of ovine-prolactin (PRL) on alternate days for a 2 week period. In contrast, no change was found in the skin of saline-injected control newts following the same treatment period. Lectin binding to sections of PRL-treated and control skin is summarized in Table 2.

LTA is specific for fucose residues and results using this lectin are presented in Figure 3A-B. In control animals the corneum stained heavily while transitional and basal layers of the epidermis exhibited light staining of both cytoplasm and surfaces of cells. Granular glands stained heavily in control animals. PRL-injected newts demonstrated moderate lectin reactivity extending throughout the epidermis. In contrast to the heavy staining seen in control sections, granular glands stained only lightly following PRL-treatment. Mucous glands were not observed in either experimental group. Neither the epidermis nor epidermal glands showed evidence of lectin binding after incubation with the fucose specific lectin, UEA-I in either treatment groups; therefore UEA-I was omitted from the tables.

Both soy bean agglutinin (SBA) (Figure 3C-D) and *Dolichos biflorus* (DBA) (Figure 4A-B) specifically bind N-acetyl-galactosamine residues. SBA heavily stained corneum and transitional cells in control animals as well as the surface of basal cells. Moderate to heavy intercellular staining was also present noted within these cells. Less staining was evident in epidermal glands of control sections: granular glands stained lightly whereas mucous glands stained moderately. SBA reactivity was substantially decreased throughout the epidermis and within epidermal glands following PRL treatment. An exception to this pattern was the surface of transitional cells which were moderately stained. GalNAc residues bound by DBA were also heavily stained in the corneum and upper transitional cells of the epidermis in control newts and were only slightly reactive in basal cells. This pattern was significantly different from that seen with SBA even though both lectins were specific for GalNAc. Heavy staining was present in granular glands and very intense reactivity was seen in mucous glands of control animals. Prolactin treatment reduced DBA staining in the epidermis. Only moderate staining was observed in the corneum and surfaces of transitional cells.

The cytoplasm of transitional and basal cells failed to stain as did the surfaces of basal cells. Interestingly, epidermal glands uniformly failed to react with DBA after PRL treatment even though they remained slightly reactive with SBA.

Galactose residues identified by GSAI-B4 were heavily stained in all layers of the epidermis of control animals. Epidermal glands stained lightly. After PRL injection, the corneum showed decreased binding of reaction product, i.e., light stain, whereas the surfaces of transitional and basal cells stained similarly in intensity to the controls. An intracellular decrease from heavy to moderate reactivity was evident in transitional and basal cells following PRL-treatment. Staining in glandular epithelial cells did not differ from controls.

CON A recognizes glucose and mannose residues. The corneum of control newts stained heavily, and this pattern did not change after PRL-treatment. The cytoplasm of cells in the transitional and basal layers stained moderately in control epidermis, but following PRL treatment no reactivity was evident in these areas. Similarly, the basement membrane stained heavily in control newts but binding was absent after treatment with PRL. CON A stained granular glands moderately in both groups.

Sialic acid and N-acetylglucosamine residues bound by wheat germ (WGA) were heavily stained stain throughout the epidermis of control newts, which included corneum, surfaces and cytoplasm of transitional and basal cells (Figure 5A-B).

Mucous glands also stained heavily and granular glands stained moderately. The corneum of PRL treated newts showed very heavy stain. No differences were observed in lectin

reactivity between PRL and control groups within the transitional and basal layers, although columns of these cells exhibited cytoplasmic staining. Granular glands stained moderately; mucous glands heavily.

PNA is specific for the conjugated sugar galactose-(1,3)-N-acetyl-galactosamine (Figure 6A-B). It is unique in that it was the only lectin that frequently bound to the basement membrane. In the epidermis of control newts, the corneum stained very heavily as did the surfaces of transitional and basal cells, and the basement membrane. Intracellularly, transitional cells stained heavily whereas basal cells showed no stain. Granular glands stained lightly and mucous glands stained heavily. Prolactin treatment decreased reactivity of this lectin in the epidermis. Surfaces of transitional cells stained moderately as did the cytoplasm. No stain was observed in basal cells either on surfaces or within the cytoplasm. The basement membrane showed no stain. Mucous glands stained moderately, whereas some areas of granular glands stained heavily.

Table 2. Lectin Reactivity in the Integument of the Red-Spotted Newt:
Effects of Prolactin Injections x 14 days

	Lectin						
	<u>LTA</u>	<u>SBA</u>	<u>DBA</u>	<u>GSA1-B4</u>	<u>ConA</u>	<u>WGA</u>	<u>PNA</u>
Epidermis:							
Stratum corneum	3/2	3/1	4/2	3/1	3/3	3/4	4/2
Transitional cells							
-surface	1/2	3/2	3/2	3/3	3/3	3/3	4/2
-cytoplasm	1/2	3/1	2/1	3/2	2/0	3/3	3/2
Stratum basale							
-surface	1/2	3/1	1/0	3/3	3/3	3/3	4/0
-cytoplasm	1/1	2/1	1/0	3/2	2/0	3/3	0/0
Basement membrane	0/0	0/0	0/0	0/0	3/0	0/0	4/3
Epidermal Glands:							
Granular	3/1	1/1	3/0	1/1	2/2	2/2	1/1
Mucous	*/*	2/1	4/0	1/1	2/3	3/3	3/2

Numbers represent lectin stain intensity ranging from 0 (no stain) to 4 (heavy stain) for control animals / prolactin treated animals. N=6 in each treatment group. * not observed.

Effects of Laboratory Conditioning on Temporal Expression of Epidermal Glycoconjugates

Changes in integumental glycoconjugates associated with laboratory conditioning are summarized in Table 3A and presented in the Appendix.

During the 14-day conditioning period LTA binding was confined to the corneum and transitional cell layers. Only slight staining was observed on day 3 (Figure 7A) throughout the epidermis, whereas the heaviest stain was evident in the corneum by day 6 (Figure 7B). Comparatively, day 6 also stained more intensely than days 3, 9, and 12 in the transitional layer (Appendix, Figure 1A). Epidermal glands showed no evidence of reactivity throughout the conditioning period.

N-acetylgalactosamine residues bound by SBA gradually increased in reactivity from day 3 of conditioning through day 15 (Appendix, Figure 1B). Day 3 (Figure 8A) and 6 showed moderate binding in the corneum and transitional cells. Basal cells demonstrated light stain at day 3; moderate stain at day 6, which continued until day 15. Staining in both the corneum and transitional layers increased progressively from moderate to very heavy stain by day 12. Days 12 and 15 revealed no change in stain intensity in the transitional cells as compared to day 9 (Figure 8B). The corneum exhibited very heavy stain by days 12 and 15. Mucous glands demonstrated more binding of this lectin (moderate to heavy) than did

granular glands (light to moderate) (Table 3a).

DBA also showed a progressive increase in binding GalNAc residues from day 3 to day 15 in the corneum (Appendix, Figure 2A). A fluctuation in DBA binding was observed in transitional cells where moderate reactivity was recorded on days 3 (Figure 8C), 9, and 12, but heavy staining occurred on day 6 (Figure 8D) and 15. Basal cells, were slightly stained by day 3, but this staining was absent by day 9. Interestingly, intense staining of basal cells with this lectin occurred by day 15.

GSAI-B4 (Appendix, Figure 2B), bound galactose residues and showed no change in stain intensity during the conditioning period, except for a slight decrease in intensity at day 9 in the corneum and transitional layers. Cell surfaces were considerably more reactive than cytoplasm in these layers. An exception was found on day 9, where a decrease from heavy to moderate staining was noted in the corneum and the transitional layers. Light or no stain was present in granular glands: mucous glands were not observed (Table 3A).

CON A binding to mannose and glucose residues demonstrated little variation in reactivity during conditioning (Appendix, Figure 3A). At days 3 and 6 the epidermis stained moderately. Heavy staining as noted by days 9 and 12. Granular glands stained moderately and mucous glands heavily (Table 3A).

Sialic acid and N-acetylglucosamine bound by WGA, did not change during conditioning (Appendix, Figure 3B). Except for very heavy binding on day 3, the corneum remained heavily stained during conditioning. Moderate to heavy stain was seen in transitional and basal cell layers. Moderate staining occurred in the granular and mucous glands. (Table 3A).

During conditioning, PNA showed an intense binding of galactose (1-3)-N-acetylgalactosamine residues (Appendix, Figure 4). The corneum stained heavily on days 3, 12, and 15 whereas days 6 (Figure 9A) and 9 stained heavy to moderate. By day 9 the transitional cells and basal cells stained heavy to light (3 to 2). Day 12 (Figure 9B) and 15 both show very heavy stain throughout the epidermis. The basement membrane and mucous glands stained heavy (3) to very heavy (4), whereas granular glands stained only light (1) to moderate (2) (Table 3A).

Table 3A. Lectin Binding of Glycoconjugates in epidermal glands:
Conditioning

	SBA		DBA		GSAI-B4		CON A		WGA		PNA	
	<u>M</u>	<u>G</u>	<u>M</u>	<u>G</u>	<u>M</u>	<u>G</u>	<u>M</u>	<u>G</u>	<u>M</u>	<u>G</u>	<u>M</u>	<u>G</u>
Day 3	*	1	2	1	*	1	3	2	3	2	4	1
Day 6	2	1	3	2	*	1	3	*	2	2	4	2
Day 9	3	2	3	2	*	0	3	2	2	1	*	2
Day 12	3	2	2	1	*	2	2	2	3	2	3	1
Day 15	*	*	4	3	*	1	*	*	3	2	*	*

Table 3B. Lectin binding of glycoconjugates in epidermal glands:
PRL-injected (14 x days)

	SBA		DBA		GSAI-B4		CON A		WGA		PNA	
	<u>M</u>	<u>G</u>	<u>M</u>	<u>G</u>	<u>M</u>	<u>G</u>	<u>M</u>	<u>G</u>	<u>M</u>	<u>G</u>	<u>M</u>	<u>G</u>
Day 3	*	2	2	2	*	*	3	2	2	3	*	*
Day 6	*	*	2	1	*	*	4	3	2	2	4	3
Day 9	2	2	*	1	*	0	*	3	*	2	1	2
Day 12	0	1	*	*	0	0	3	2	2	3	3	1
Day 15	*	1	*	2	*	1	*	2	3	2	2	1

M - Mucous glands
G - Granular glands

* - not observed

PRL-induced Changes in the Temporal Expression of Epidermal Glycoconjugates

Temporal changes in integumental glycoconjugate expression following administration of exogenous PRL are presented in Table 3B and the Appendix.

LTA showed an overall decrease in binding intensity throughout the corneum (Appendix, Figure 5A). Lectin binding in the corneum was maximal by day 3 and decreased progressively to light stain by day 12. Other areas of the epidermis were only slightly reactive.

SBA binding of N-acetylgalactosamine residues resulted in identical stain intensity in the epidermis of PRL-injected newts at days 3 (Figures 10A-C) and 6 (Appendix, Figure 5B). By day 9, transitional and basal cells remained unchanged but binding in the corneum sharply decreased and this pattern confined through day 15. The basal layer stained moderately and showed no change in stain intensity during PRL-treatment. When present, epidermal glands varied in stain intensity from no stain (0) to moderate stain (2).

N-acetylgalactosamine residues bound by DBA gradually decreased in intensity in the corneum from day 3 to 15 of PRL-injection. Intense stain is observed on days 3 and 6 in the corneum and transitional layers of the epidermis but (Appendix, Figure 6A) decreases by day 9 (Figure 11A). Transitional cells (Figure 11B) stain prominently, but other epidermal cell layers show little or no stain by day 12.

Basal cells show evidence of stain only on day 3, when heavy staining was present. Epidermal glands ranged from light to moderate in stain intensity during PRL treatment.

The binding of GSAI-B4 to galactose residues (Appendix, Figure 6B) remained constant in stain intensity in PRL-injected animals. Identical staining was seen in the epidermis for days 3 and 6. Days 9 through 15 stained with similar intensity, showing decreased reactivity in the corneum and an increase in the basal cell and transitional cells. Epidermal glands were infrequently observed, and/or did not show evidence of stain.

Mannose and glucose specific CON A showed little change in staining following PRL injection (Appendix, Figure 7A). The epidermis stained intensely with this lectin. Exceptions include the corneum's decrease in stain (heavy to moderate) at day 6 and its increase (moderate to very heavy) on day 9. Basal cells remained heavy stained from days 3 to 15. Mucous glands stain more intensely (heavy to very heavy), than granular glands (moderate to heavy).

Wheat germ agglutinin increased binding intensity (heavy (3) to very heavy(4)) from day 3 to day 15 in the corneum (Appendix, Figure 7B). An exception was seen on day 9, where a decrease to moderate binding was noted (Figure 12A). Moderate stain was seen in the other epidermal areas, i.e., basal and transitional cells. By day 12 (Figure 12B), a marked increase in binding occurred in the corneum.

Epidermal glands stained moderate to heavy.

PNA showed decreased binding of galactose-(1,3)-N-acetylgalactosamine residues from day 3 to day 15 of PRL-injection throughout the epidermis and epidermal glands (Appendix, Figure 8). During PRL-injection, the heaviest stain was evident over the corneum on day 3, (Figure 13A-B) while the basal layer exhibited the lightest stain on days 6 and 9. Decreased stain intensity was also evident in the basement membrane. Mucous and granular glands showed an overall decrease in stain intensity.

Seasonal Effects on Epidermal Glycoconjugates

Epidermal glycoconjugates of freshly collected winter newts having increased cutaneous secretion and bearing secondary sexual characteristics, i.e., nuptial pads and increased tailfin height, were compared to conditioned and PRL-injected animals. Laboratory conditioned newts resemble summer animals, with decreased cutaneous secretion and absence of secondary sexual characteristics. On the other hand, PRL-injected newts exhibit increased cutaneous secretion and tailfin height. The results are listed in Table 4.

LTA was not very reactive in the epidermis in any of the groups, however, the corneum of the laboratory conditioned and PRL-injected animals was more reactive (moderate) than the corneum of freshly collected winter group (light).

SBA (Figure 14A-C) revealed similar stain intensity for

each group in the transitional cells and the basal cells. The corneum of freshly collected winter animals stained lighter than the laboratory conditioned, and in PRL-treated animals the corneum stained lightly.

With DBA (Figure 15A-C), laboratory conditioned animals showed increased stain intensity in the upper epidermis in comparison to winter freshly collected and PRL-treated animals. The corneum revealed the most prominent difference: staining was absent in winter freshly collected and PRL-treated animals, while in laboratory conditioned animals stain was intense.

GSAI-B4 (galactose), demonstrates similar stain intensity in transitional cells for each group, though the intercellular area stains intensely in laboratory conditioned newts. Winter freshly collected animals show heavy staining of the corneum, laboratory conditioned animals exhibit very heavy stain and PRL-treated animals show light stain.

CON A (mannose) stain intensity is moderate in the epidermis of both winter freshly collected and laboratory conditioned animals. In PRL-treated animals increased stain intensity is obvious throughout the epidermis.

Sialic acid, bound by WGA, showed increased stain intensity in the corneum of winter freshly collected and PRL-injected animals as compared to laboratory conditioned animals. Other epidermal cell layers and glands stained similarly for each group.

In the epidermis of laboratory conditioned animals, PNA was very reactive as intense stain was seen from the corneum through the basal cell layer. The basement membrane and granular glands stain lightly in this group while the mucous glands stained heavily. Freshly collected winter animals also showed intense stain in the corneum, however, the transitional cells and basal cells showed no stain. The basement membrane and mucous glands stained intensely, but granular glands stained identical to those of laboratory conditioned animals. PRL-injected animals showed heavy stain in the upper epidermal cell layers, no stain was seen in basal cells. The basement membrane also showed no evidence of stain, mucous glands stained moderately and granular glands stained lightly.

Conditioned animals showed minimal or no difference in lectin staining intensity between cytoplasmic and surface areas of transitional and basal cell layers. Following prolactin treatment, cell surfaces stained more intensely than cytoplasmic cell areas. Similarly, freshly collected animals revealed increased staining of the surface as compared to the cytoplasm of transitional and basal cell layers.

Mallory's Triple Stain

Mallory's Triple stain demonstrated magenta staining in the corneum of conditioned newts, however PRL-injected newts did not show comparable staining of the corneum. Other epidermal cell layers stained mostly basophilic. Mucous glands also stained basophilic, whereas granular glands were eosinophilic in both conditioned and PRL-injected animals.

Table 4. Lectin Reactivity in the Integument of the Red-Spotted Newt:
Effects of Seasonal Variation

	Lectin						
	<u>LTA</u>	<u>SBA</u>	<u>DBA</u>	<u>GSA1-B4</u>	<u>ConA</u>	<u>WGA</u>	<u>PNA</u>
Epidermis:							
Stratum corneum	1/3/2	2/3/1	0/4/2	3/3/1	2/3/3	4/3/4	4/4/2
Transitional cells							
-surface	1/1/2	2/3/2	2/3/2	3/3/3	2/3/3	3/3/3	0/4/2
-cytoplasm	1/1/2	1/3/1	1/2/1	2/3/2	1/2/0	2/3/3	0/3/2
Stratum basale							
-surface	0/1/2	1/3/1	1/1/0	3/3/3	2/3/3	3/3/3	0/4/0
-cytoplasm	0/1/1	1/2/1	0/1/0	2/3/2	1/2/0	2/3/3	0/0/0
Basement membrane	0/0/0	0/0/0	0/0/0	0/0/0	0/3/0	0/0/0	4/4/3
Epidermal Glands:							
Granular	0/3/1	1/1/1	1/3/0	1/1/1	2/2/2	2/2/2	1/1/1
Mucous	0/*/*	*/2/1	2/4/0	*/1/1	3/2/3	3/3/3	4/3/2

Numbers represent lectin stain intensity ranging from 0 (no stain) to 4 (heavy stain) for freshly collected winter animals / control animals / prolactin treated animals. N=6 in each treatment group. * not observed.

FIGURE 3

A. LTA (fucose) showing heavy stain of the corneum (sc) of control (saline-injected) newt; granular gland (gg) also stains heavily; s.corneum has rough appearance.

Original magnification X100. Bar = 51 um.

B. LTA (fucose) following 14 days of prolactin treatment; s.corneum (sc) does not stain as intensely; dermal melanophores (dm) evident; granular glands show sparse, light stain.

Original magnification X100. Bar = 51 um.

C. SBA (GalNAc); s. corneum of control newt stains heavily; mucous gland (mg) stains moderately, granular gland (gg) present.

Original magnification X100. Bar = 51 um.

D. SBA (GalNAc) following prolactin injection shows a marked decrease in stain intensity; s. corneum stain is dramatically decreased. Heaviest stain is evident on the cell membrane of transitional layer (tl).

Original magnification X100. Bar = 51 um.

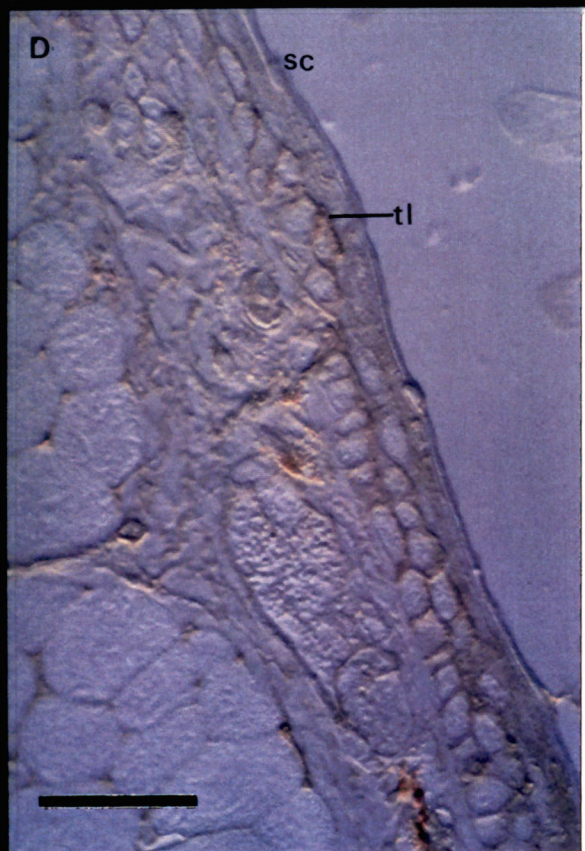
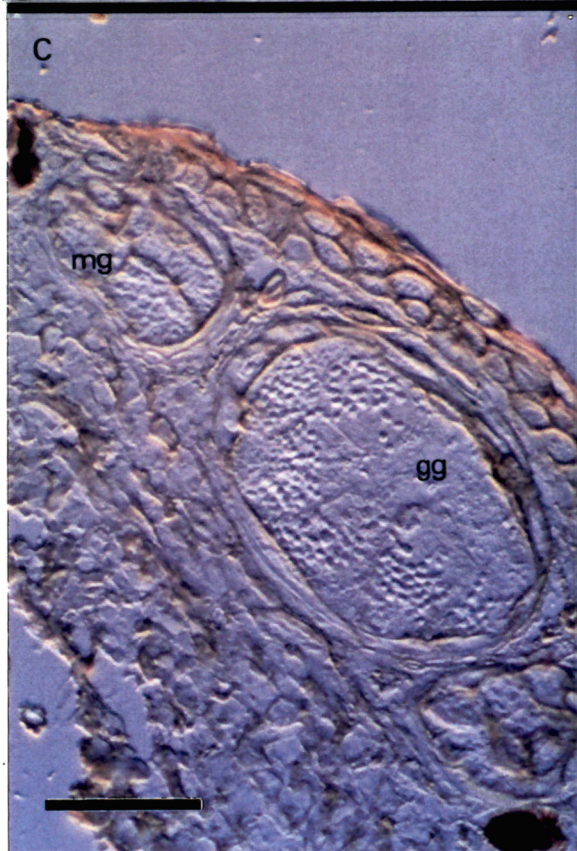
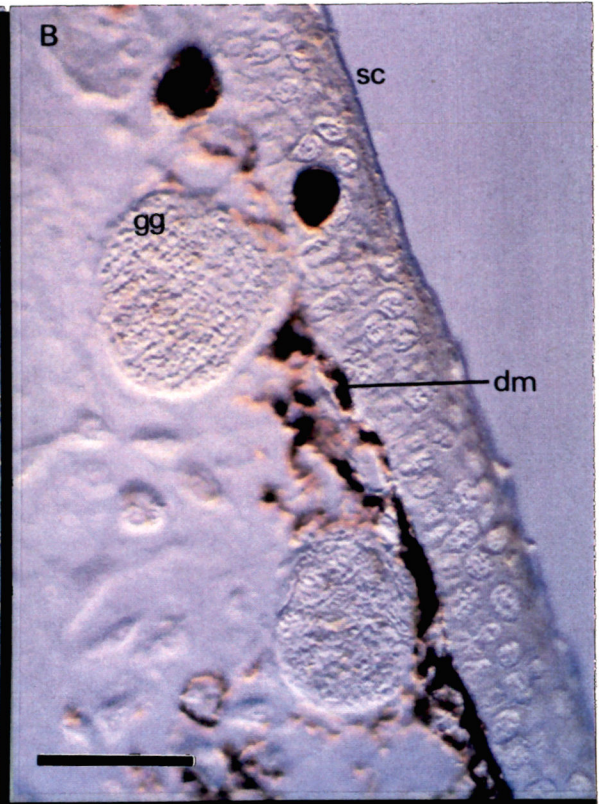


FIGURE 4

A. DBA, specific for galactose residues stained the s. corneum (sc) and granular glands (gg) heavily; developing mucous gland (dg) stained heavily; basal cells showed slight stain in control animals.
Original magnification X100. Bar = 51 um.

B. DBA following prolactin injection showed a decrease in lectin binding of GalNAc residues; moderate stain persisted throughout the tissue; stratum corneum (sc); granular gland (gg).
Original magnification X100. Bar = 51 um.

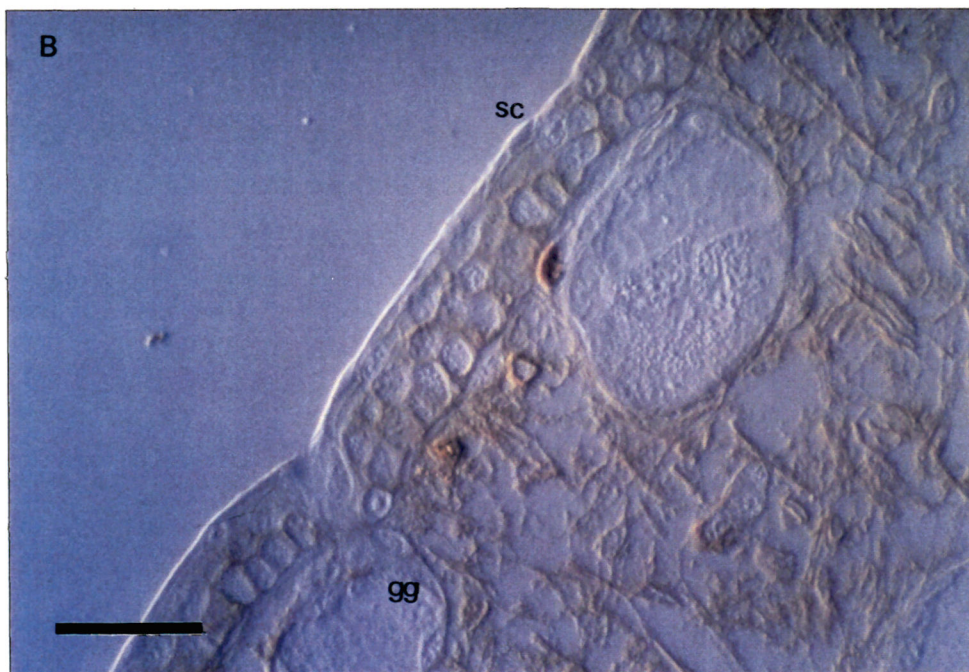
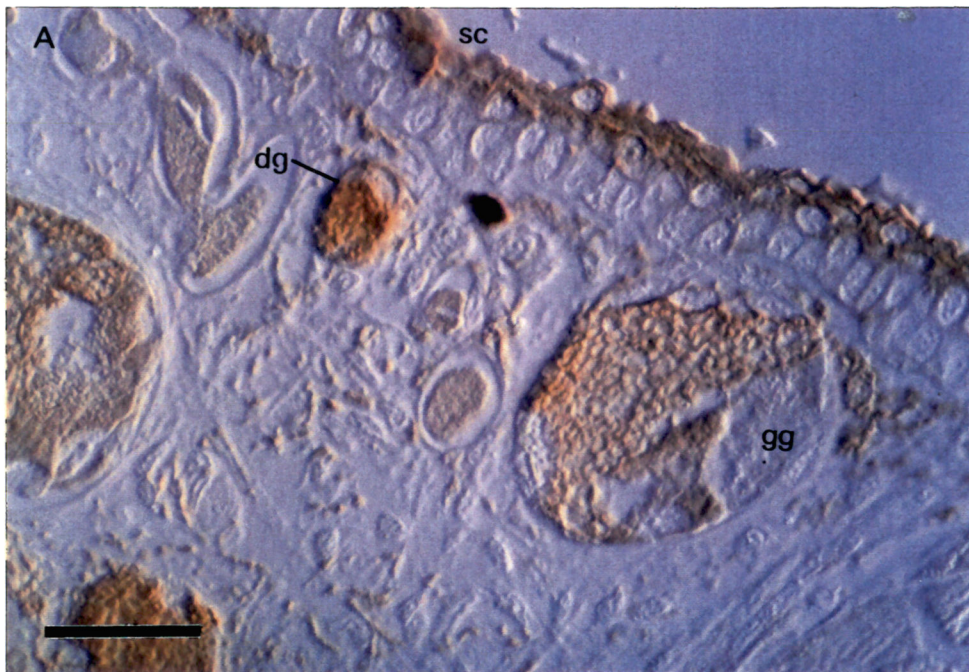


FIGURE 5

A. WGA specific for sialic acid and GluNAc; heavy staining in the s. corneum and on the plasma membrane of other epidermal cells; texture appears rough; domed tubercle (dt).
Original magnification X100. Bar = 51 um.

B. WGA (sialic acid and GluNAc), following prolactin injection the s. corneum (sc) becomes smooth; increase in stain in the corneum; columns of cells stain heavily intercellularly and on the surface (arrow).
Original magnification X100. Bar = 51 um.

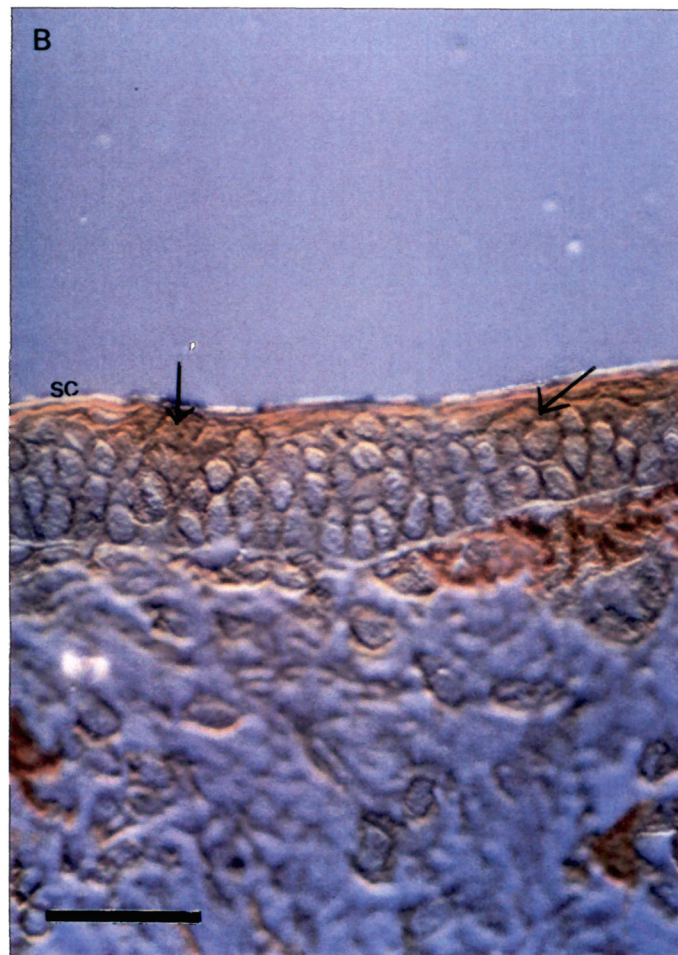
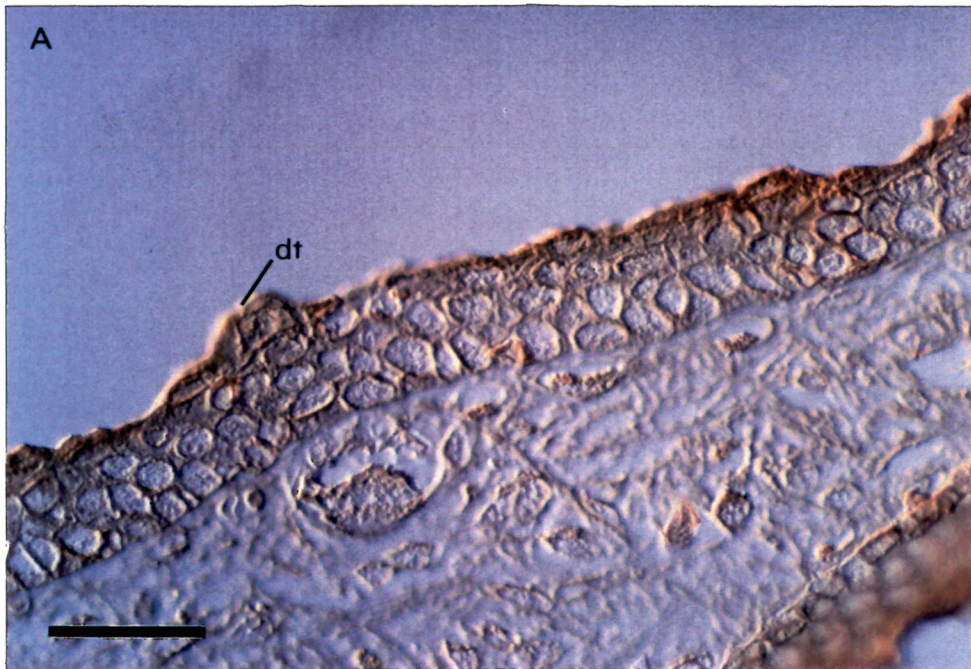


FIGURE 6

A. Heavy stain is evident throughout areas of the epidermis for PNA (Gal-1,3-GalNAc); transitional cells (tc) stain heavily; basal cells (bc) also stain heavily on the surface, intracellularly no stain is evident; portions of granular gland (gg) stain heavily; basement membrane (bm) stains heavily.
Original magnification X100. Bar = 51 um.

B. PNA (Gal-1,3-GalNAc); prolactin injected animals show considerable decrease in stain throughout epidermis and the basement membrane (bm), although portions of granular gland stain heavily, s. corneum (sc).
Original magnification X100. Bar = 51 um.

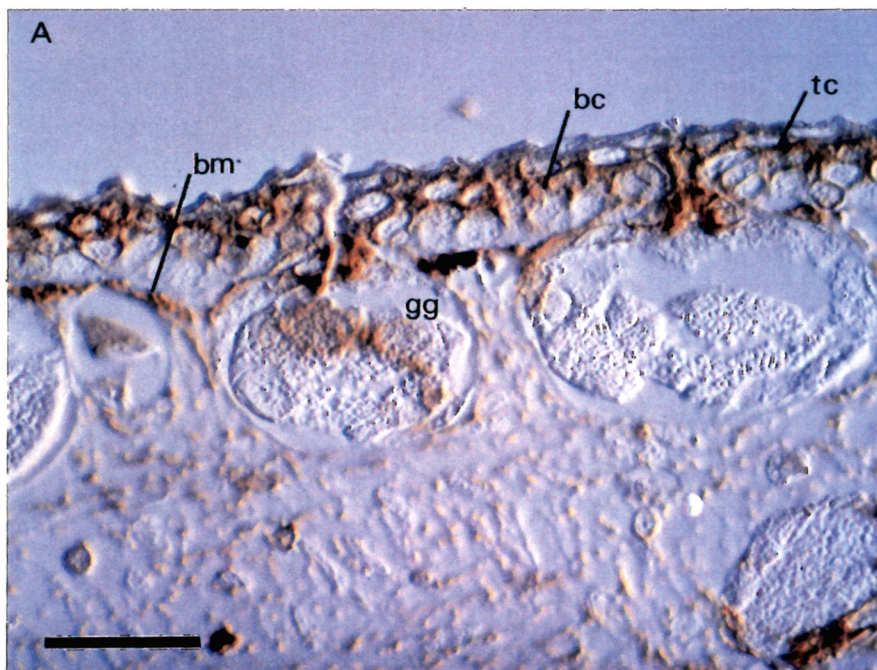
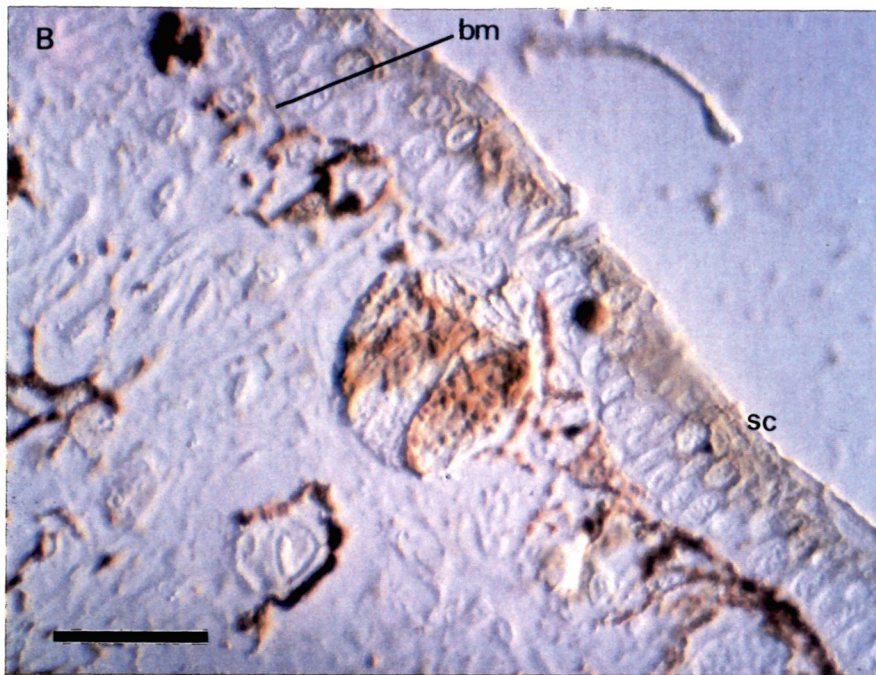


FIGURE 7

A. LTA (fucose) at day 3 of conditioning; s. corneum stains lightly and continues to have rough appearance at day 3; epidermal melanophore (em) present as well as dermal melanophores (dm); no evidence of stain in granular glands (gg).

Original magnification X100. Bar = 51 um.

B. LTA (fucose) at day 6 shows heavy stain in the s. corneum (sc); the surface of some transitional cells stain heavily (arrow).

Original magnification X100. Bar = 51 um.

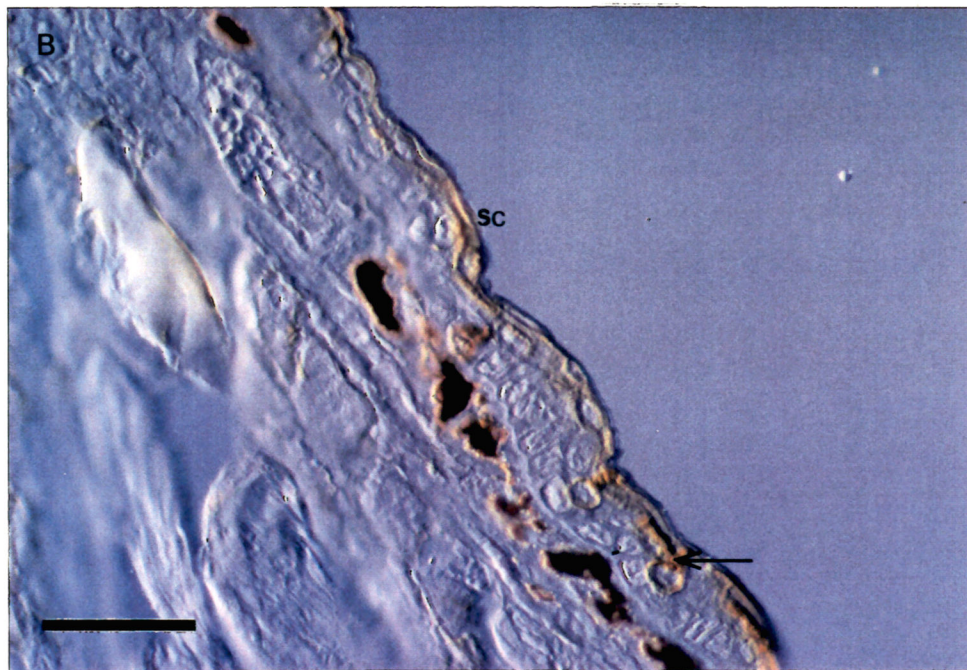
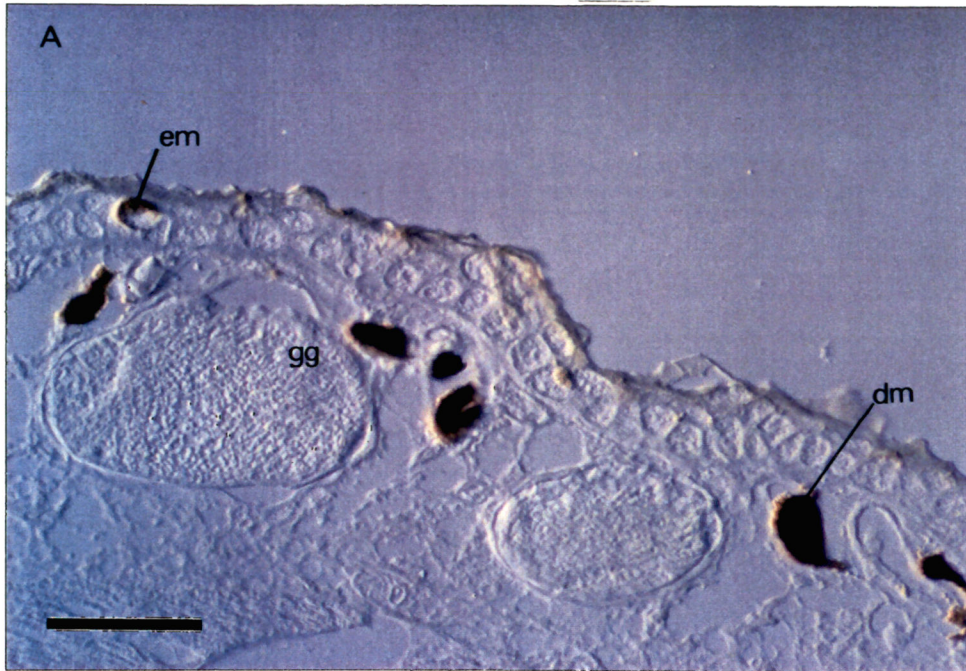


FIGURE 8

A. SBA (GalNAc) at day 3 of conditioning there is moderate stain throughout the epidermis; basal cells (bc) stain lightly; s. corneum (sc).
Original magnification X100. Bar = 51 um.

B. SBA (GalNAc), by day 9 the surface of the epidermis appears rough and the s. corneum (sc) stains heavily; the surface of transitional cells also stain heavily; basal cells (bc) stain moderately on the surface.
Original magnification X100. Bar = 51 um.

C. DBA (GalNAc) very little staining of the epidermis by day 3; upper transitional cells (tc) stain heavily on the surface; portion of granular gland (gg) stained.
Original magnification X100. Bar = 51 um.

D. DBA (GalNAc), day 6 of conditioning; transitional cells (tc) stain heavily on surface; mucous gland (mg) stains heavily; several dermal melanophores present.
Original magnification X100. Bar = 51 um.

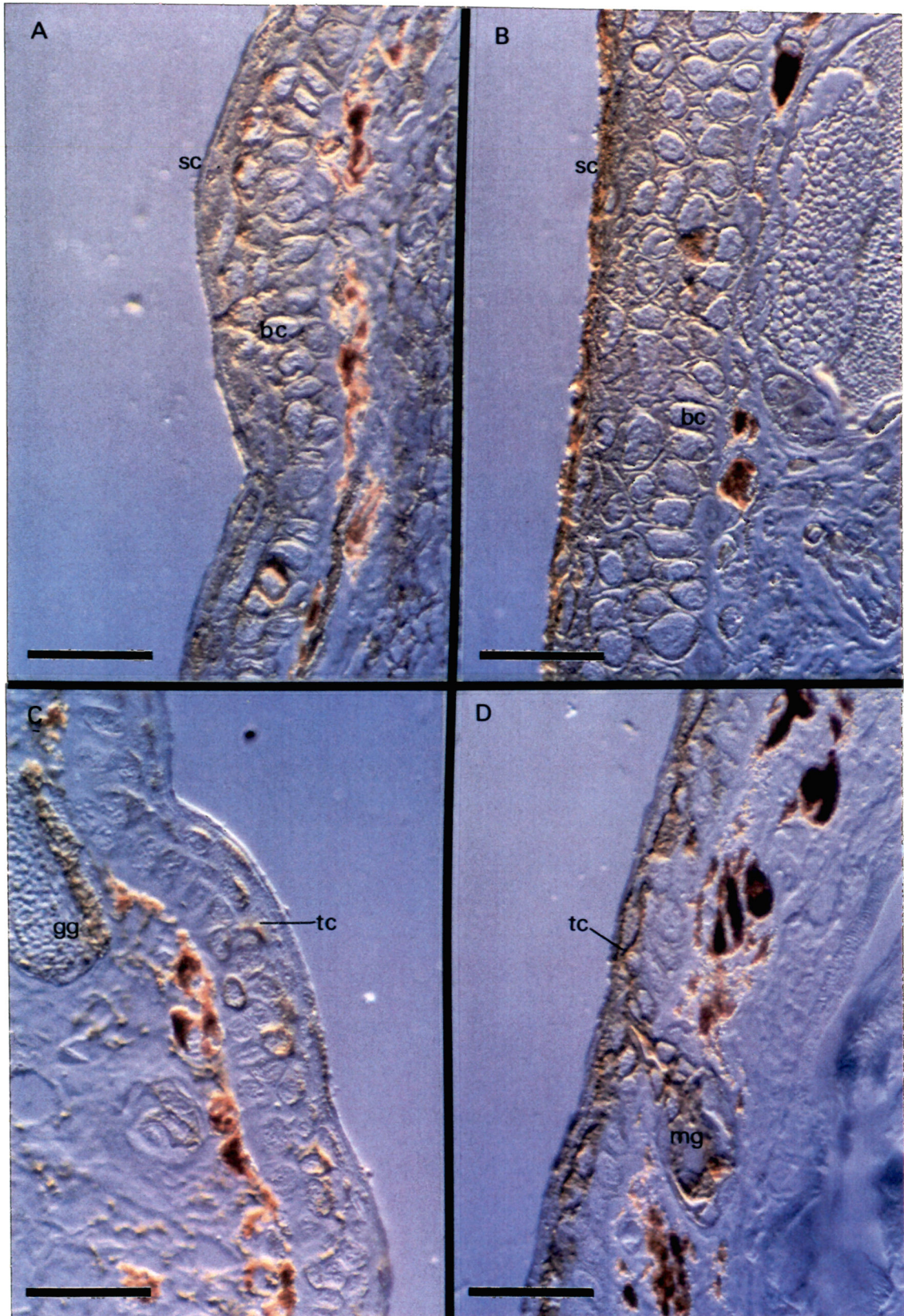


FIGURE 9

A. PNA (Gal-1,3-GalNAc) by day 6, s. corneum (sc) stains moderately; heavy stain is apparent on surface of transitional cells (tc); basal cells show no evidence of stain; dermal melanophores (dm); no stain of basement membrane (bm).

Original magnification X100. Bar = 51 um.

B. Day 12 of PNA (Gal-1,3-GalNAc) stains very heavily in the s. corneum (sc) and the surface of transitional cells; mucous gland (mg) stains lightly granular glands (gg) show no stain; the basement membrane (bm) stains heavily.

Original magnification X100. Bar = 51 um.

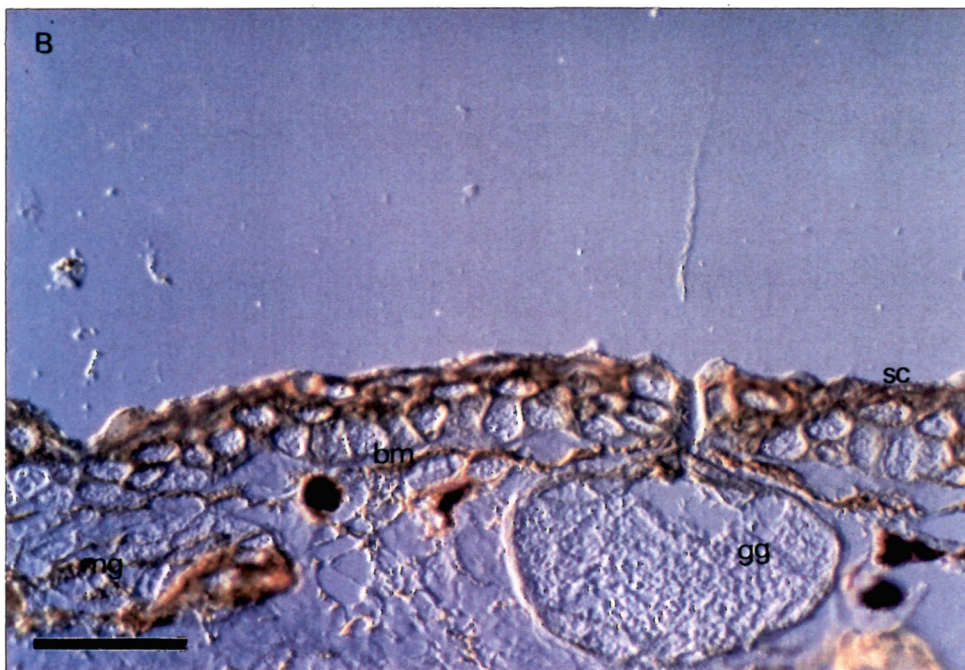
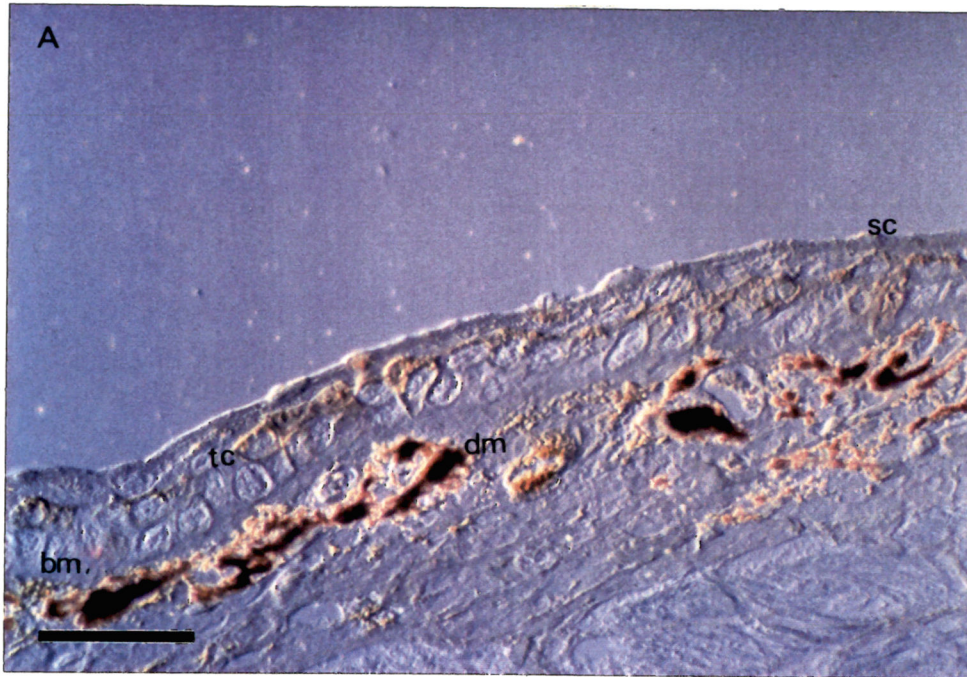


FIGURE 10

A. By day 3 of prolactin injection SBA (GalNAC) demonstrates heavy staining in the corneum (sc) and plasma membranes of some epidermal cells; epidermal melanophore (em) and dermal melanophore (dm); granular glands (gg).

Original magnification X100. Bar = 51 um.

B. SBA (GalNAC); intense staining of the corneum (sc) and heavy staining of other epidermal areas is evident by day 9 of prolactin treatment; melanophores.

Original magnification X100. Bar = 51 um.

C. SBA (GalNAC); diminished stain within the epidermis is seen by day 12 of prolactin injection; portion of a mucous gland (mg) stained heavily; s. corneum (sc).

Original magnification X100. Bar = 51 um.

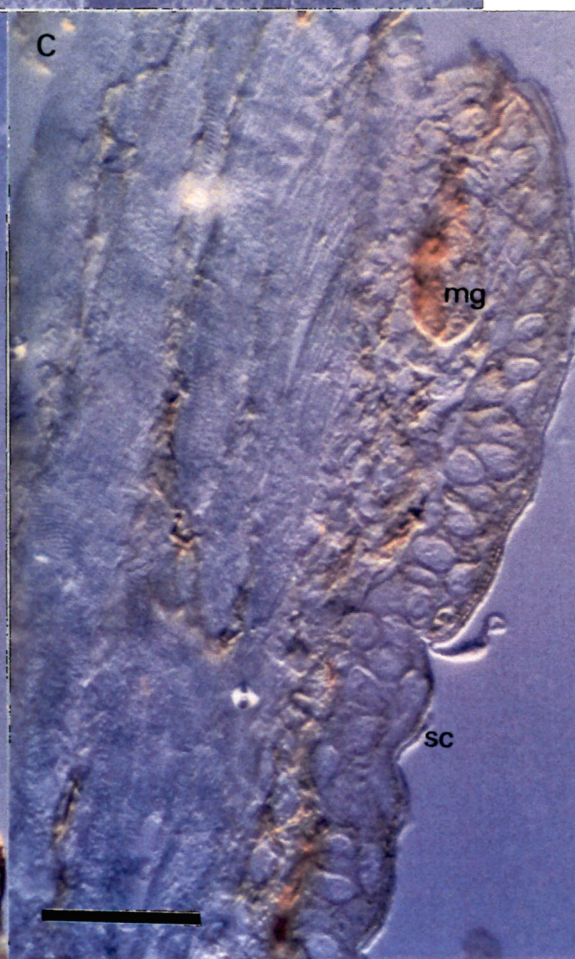
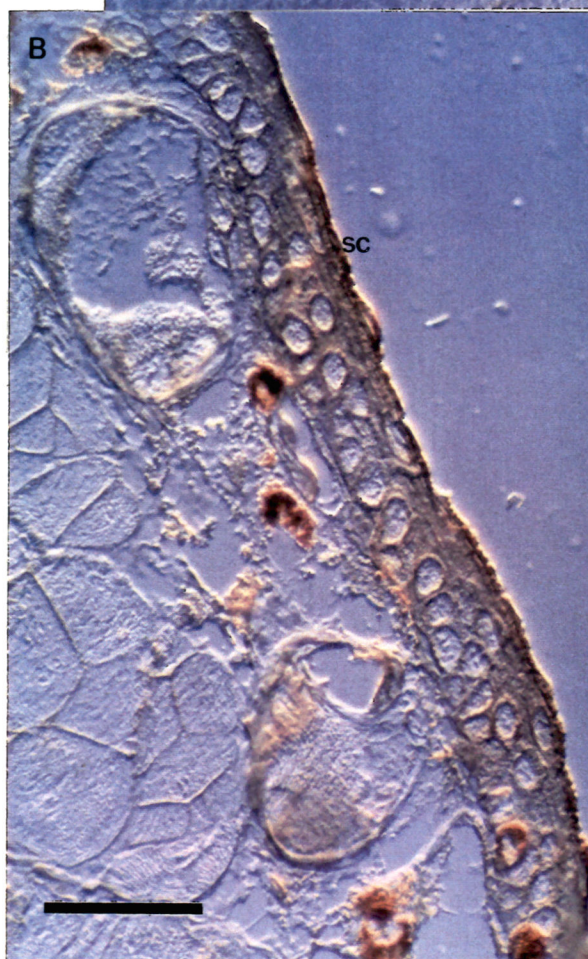
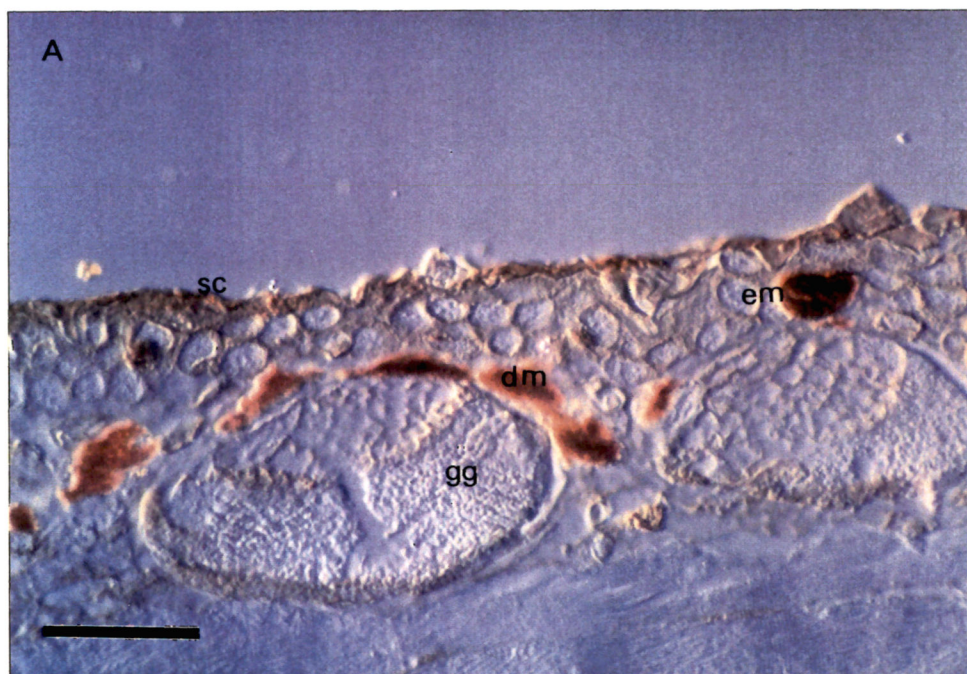


FIGURE 11

A. DBA (GalNAc) demonstrates a decrease in binding in the s. cornuem (sc) by day 9 of prolactin injection; the basal cell show no evidence of lectin binding; several granular glands are located in the dermis (d). Original magnification X100. Bar = 51 um.

B. DBA (GalNAc) by day 12, shows an increase in stain in the upper transitional cells (arrow); basal cells reveal no apparent stain; dermal melanophores (dm). Original magnification X100. Bar = 51 um.

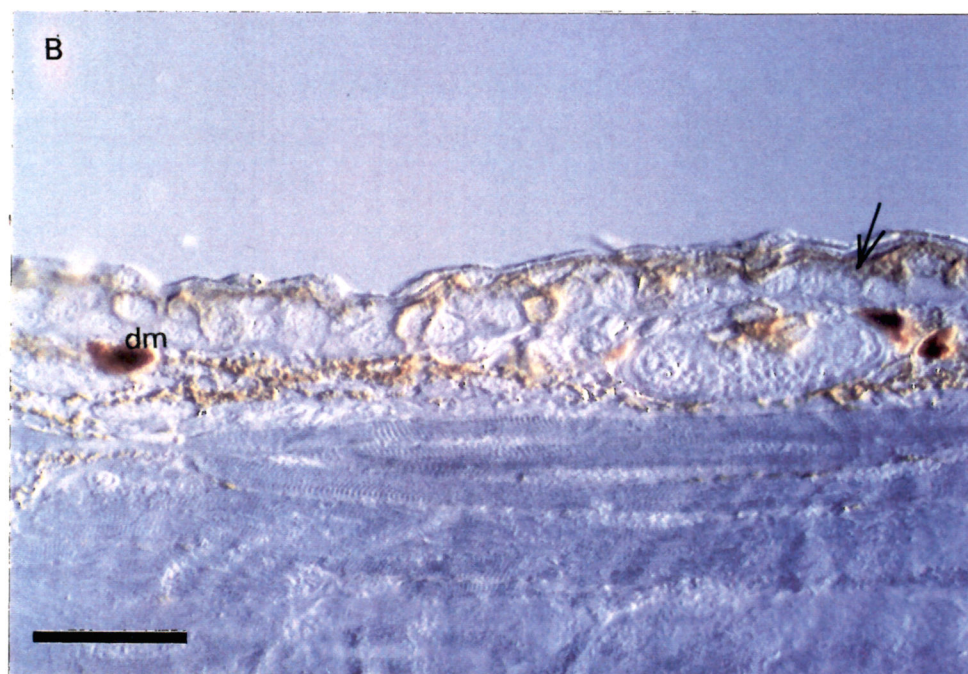
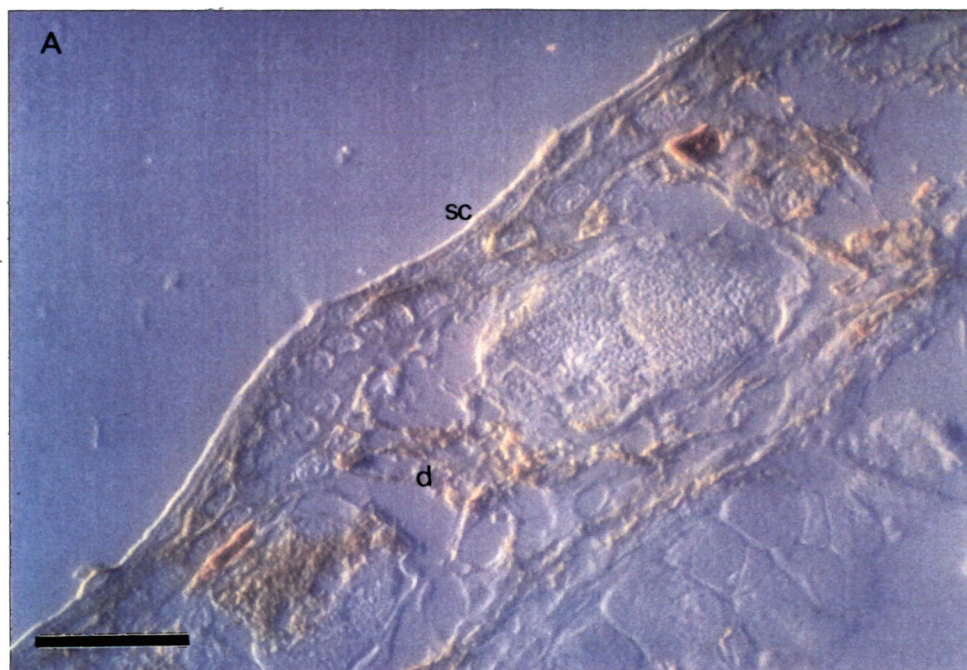


FIGURE 12

A. WGA (sialic acid, GluNAc); moderate stain is seen within the epidermis of prolactin treated newts by day 9; s. corneum (sc); epidermal (em) and dermal (dm) melanophores.

Original magnification X100. Bar = 51 um.

B. WGA (sialic acid, GluNAc); by day 12 of prolactin injection the s. corneum (sc) shows intense stain; portion of granular gland (gg) stains heavily; dermal melanophores (dm).

Original magnification X100. Bar = 51 um.

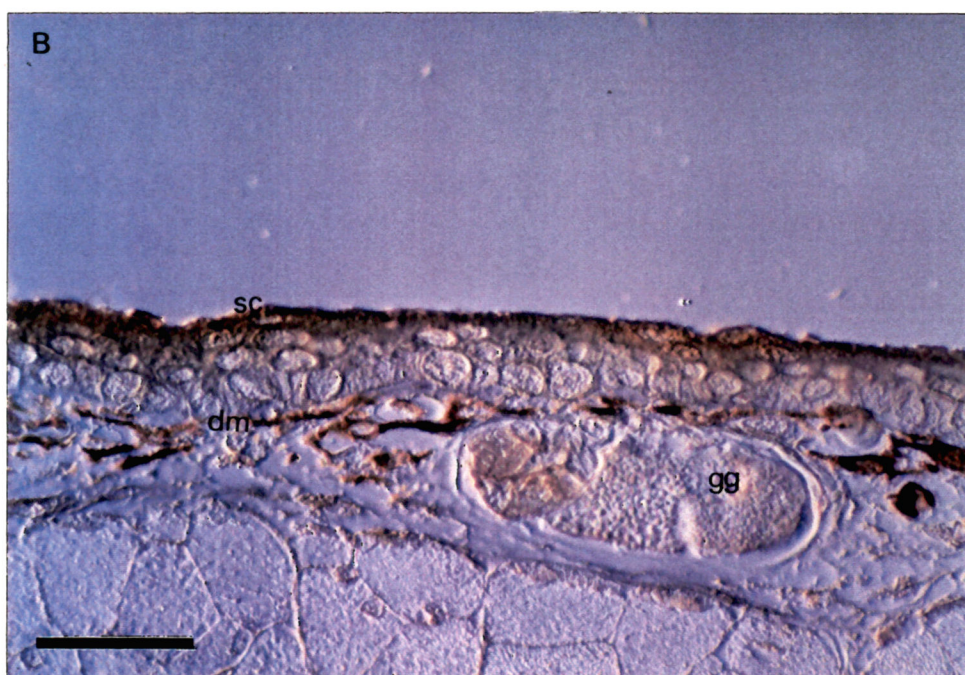
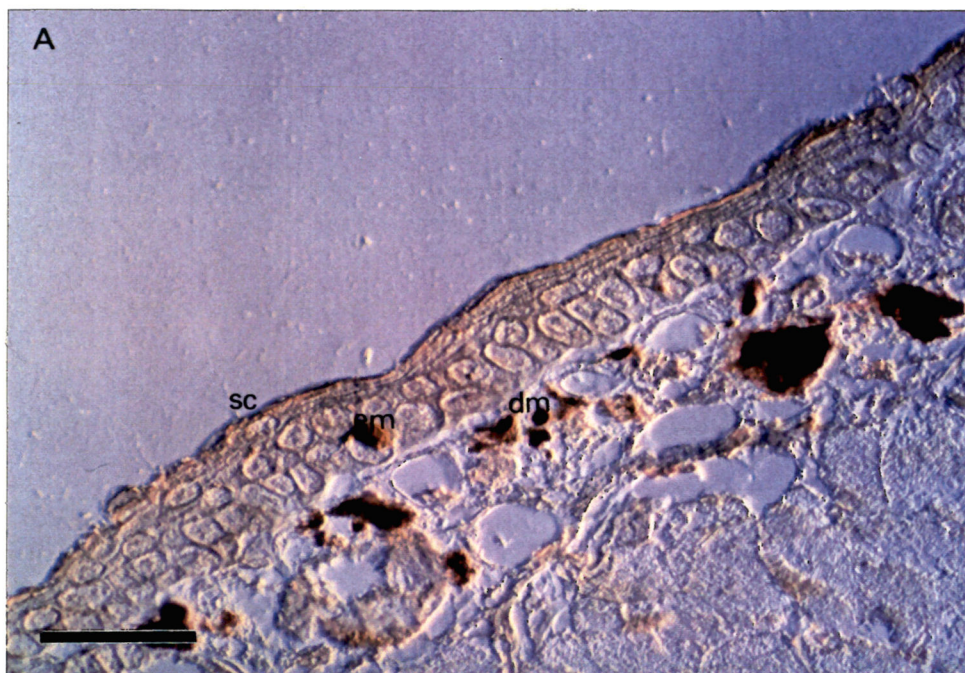


FIGURE 13

A. Prolactin injection by day 3 demonstrates intense stain of the s. corneum (sc) for PNA (Gal-1,3-GalNAc); upper transitional cells (tc) stain heavily; basal cells (bc) stain lightly.

Original magnification X100. Bar = 51 um.

B. By day 12 of prolactin injection the corneum (sc) reveals decrease in stain; basal cells (bc) show no evidence of stain.

Original magnification X100. Bar = 51 um.

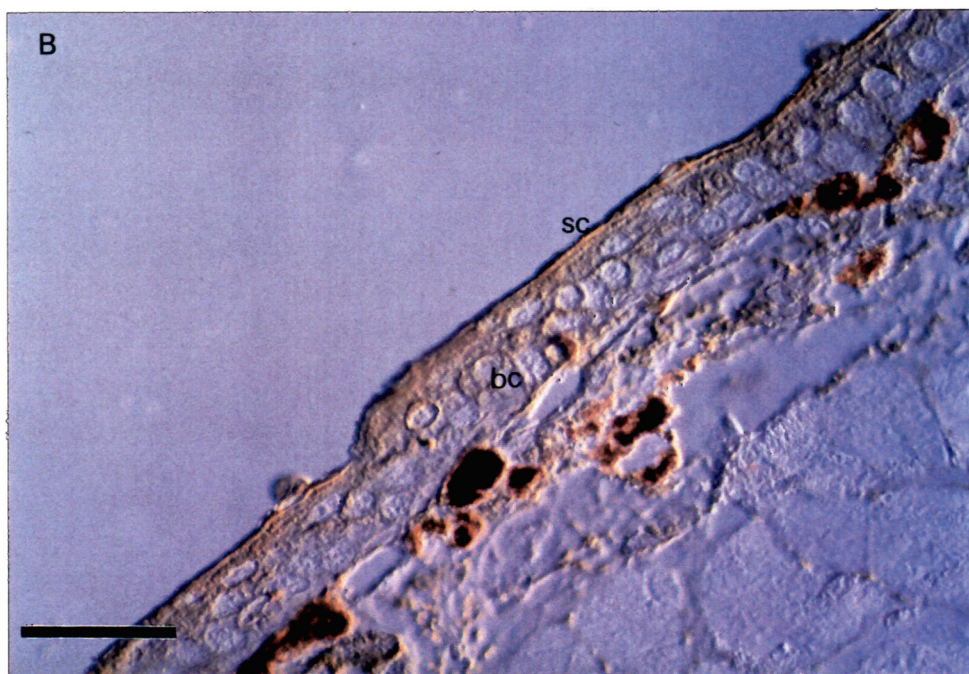
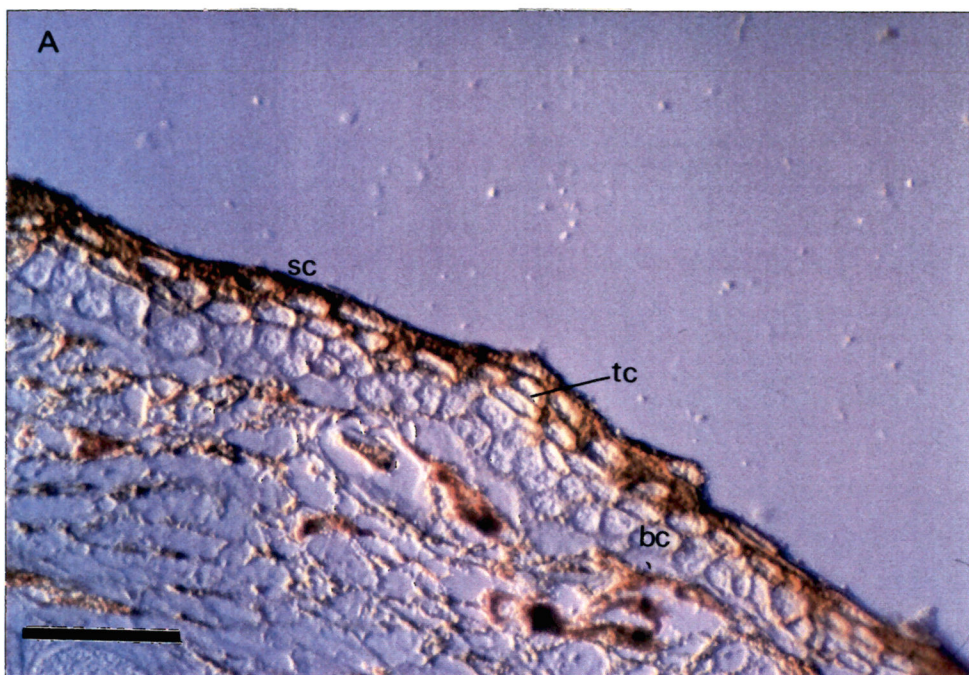


FIGURE 14

A. SBA (GalNAc), epidermis of laboratory conditioned newt; s. corneum (sc) has rough appearance; moderate staining of the surface of transitional cells; granular gland (gg) stains lightly; dermal melanophores (dm) present.

Original magnification X100. Bar = 51 um.

B. SBA (GalNAc), epidermis of winter freshly collected newt; s. corneum (sc) has smooth appearance; moderate stain throughout most of epidermis; dermal melanophores (dm).

Original magnification X100. Bar = 51 um.

C. SBA (GalNAc), epidermis of PRL-treated (X14 days) newt; lightly staining of most of epidermis; transitional cells (tc) stain moderately on the surface; s. corneum (sc)

Original magnification X100. Bar = 51 um.

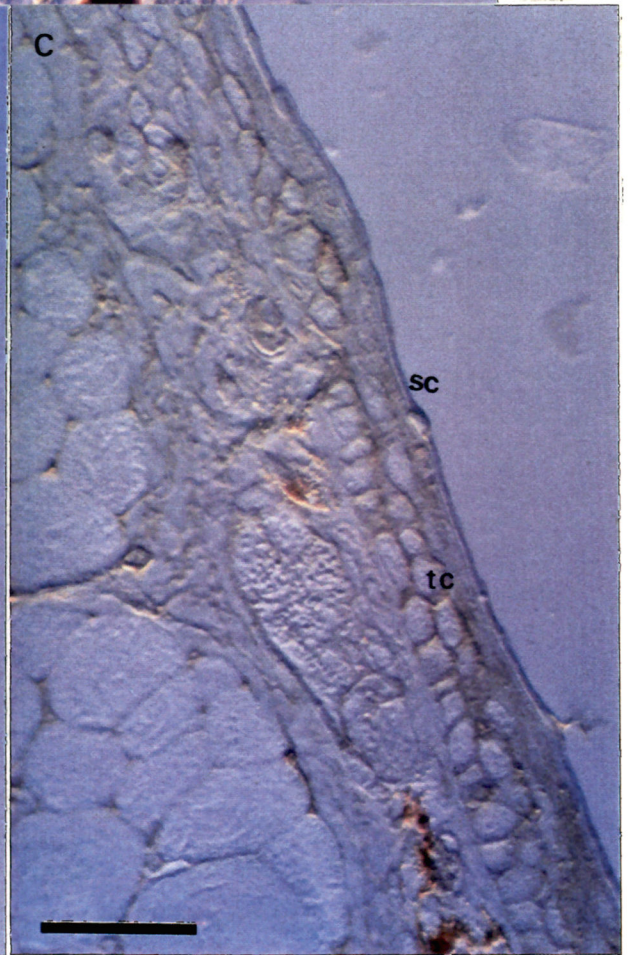
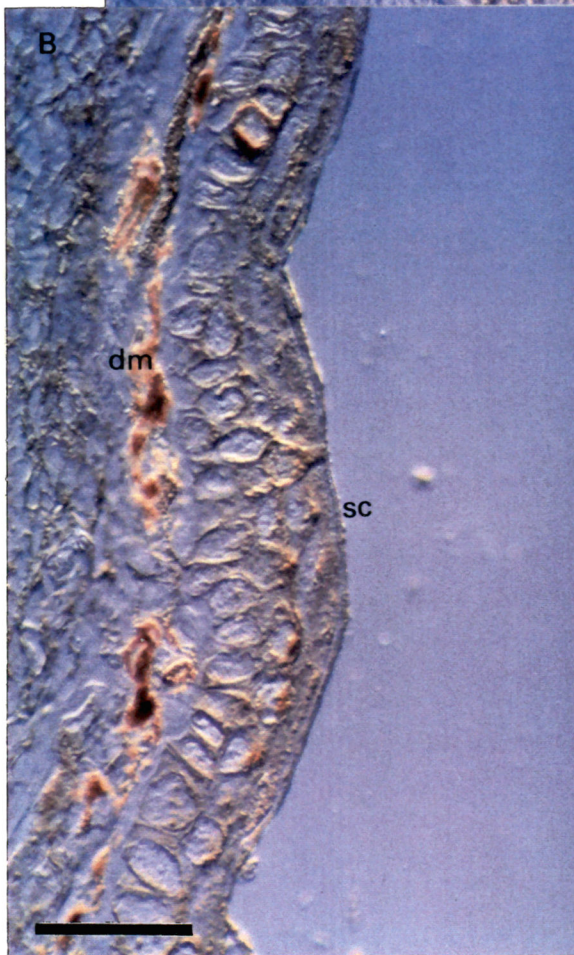
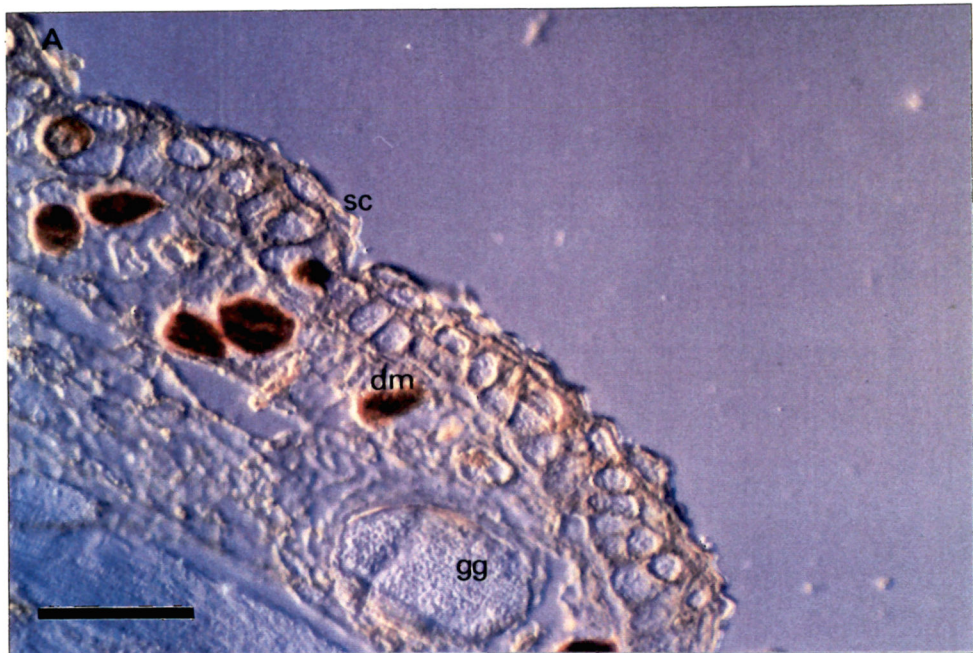


FIGURE 15

A. DBA (GalNAc), epidermis of laboratory conditioned newt; heavy staining of rough-textured s. corneum (sc); upper transitional cells stain heavily at surface; portion of granular gland (gg) stains moderately; dermal melanophores.

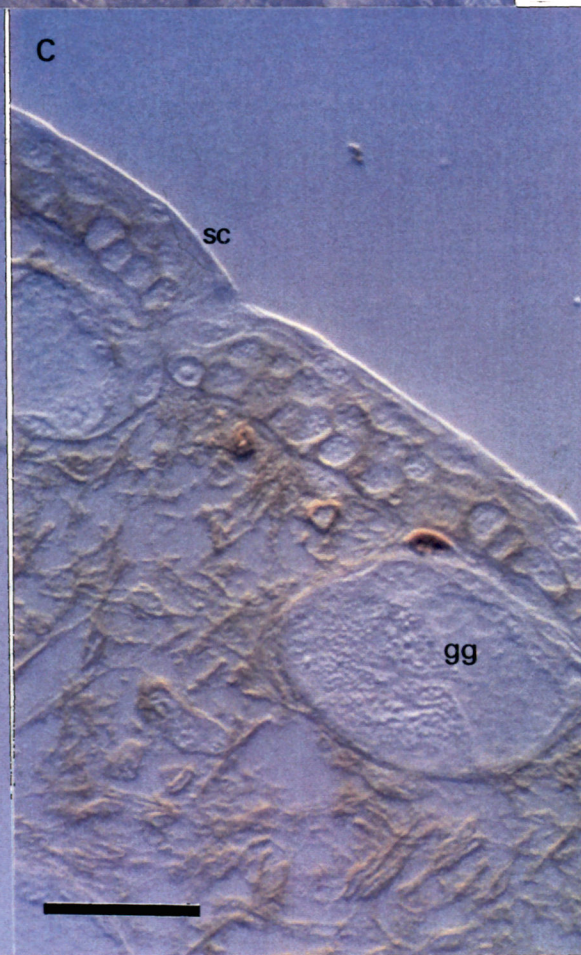
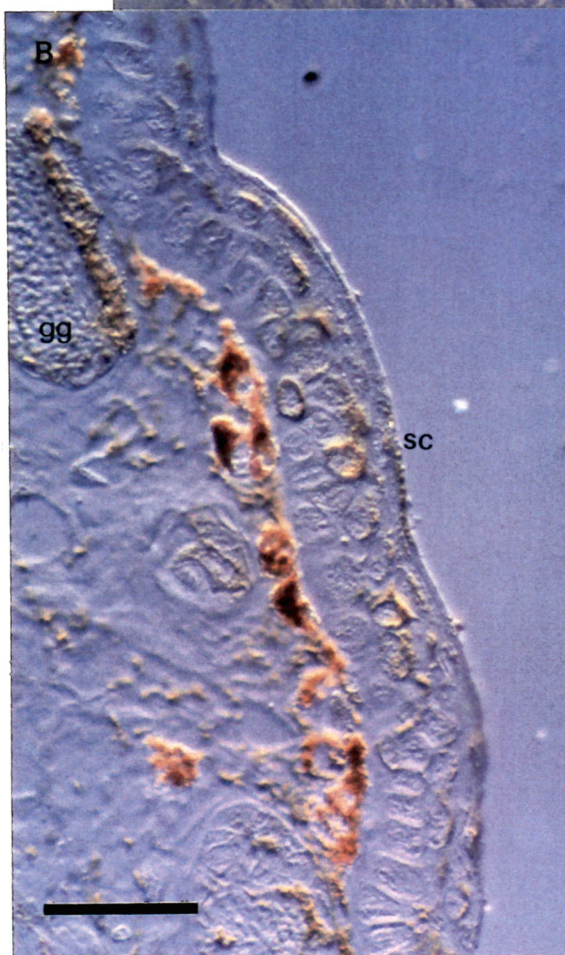
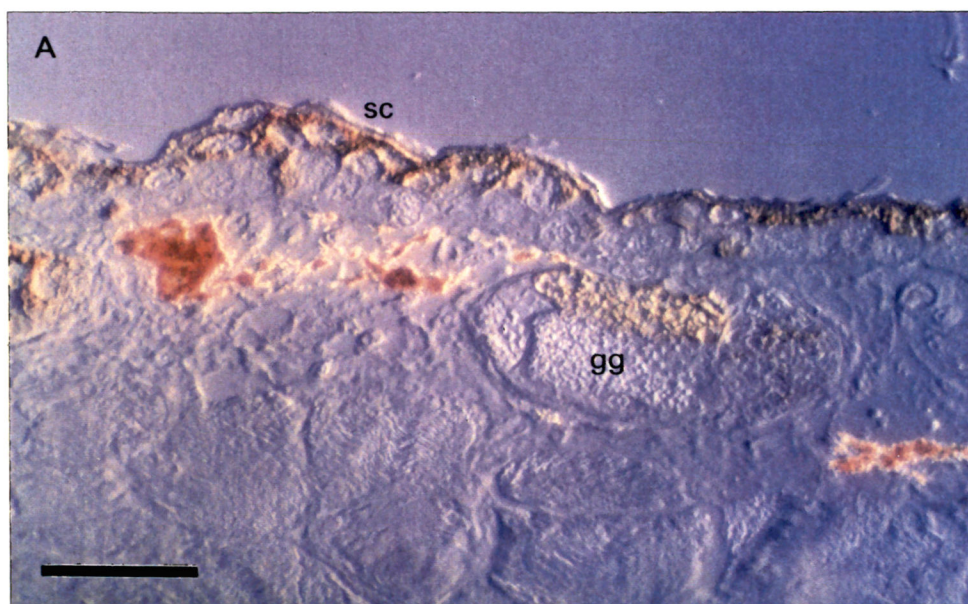
Original magnification X100. Bar = 51 um.

B. DBA (GalNAc), epidermis of winter freshly collected newt; no staining is evident of smooth-textured s. corneum (sc); portion of granular gland stains.

Original magnification X100. Bar = 51 um.

C. DBA (GalNAc) epidermis of PRL-injected (X14 days) newt; s. corneum (sc) appears smooth and with out stain; other epidermal areas show moderate to no stain; granular glands (gg) present.

Original magnification X100. Bar = 51 um.



DISCUSSION

The specificity of lectins for glycoconjugates includes both glycoproteins and glycolipids. However, clearing agents used in processing the tissue resulted in the extraction of glycolipids prior to lectin staining procedures. Hence, lectin binding only demonstrates the presence of structural and secretory glycoproteins.

The ability of prolactin (PRL) to elicit increased cutaneous secretion and smoothness in skin texture in the red-spotted newt and other urodeles has been well established (Adams, 1932; Chadwick, 1941; Grant and Grant, 1958; Dent, 1966; Dent et al., 1973) and is consistent with results obtained in this study, in which ovine PRL administered to conditioned animals for a two week period caused substantial increase in cutaneous secretion and smoothness in skin. In this study Mallory's triple stain reacted in the corneum of rough-skinned, control newts, indicating the presence of basic proteins (Figure 2:1-2). This staining pattern of the corneum may have been due to the presence of keratin since prolactin treated newts developed smooth skin and did not stain similarly.

Smoothness in skin texture may be due to the synergistic action between thyroxine and PRL (Dent et al., 1973; Dent, 1975). Dent (1975) has suggested that prolactin facilitates molting by stimulating cutaneous secretion, and the actual

casting off of the corneum (ecdysis) is controlled by thyroxine. An explanation for the differential staining of Mallory's that is seen between control and prolactin treated animals may be that the administration of endogenous PRL stimulates cutaneous secretion, which induces cornified cells to slough off in minute portions and is not detectable as a molt per se (thyroxine was not administered in this study). Another explanation may be that prolactin inhibits cornification, as prior studies have reported that prolactin depresses keratinization in conditioned crested newts (Vellano et al., 1970).

Enhanced secretory activity resulting in the sliminess of skin emanates from the epidermal glands. Hoffman and Dent (1978) report that mucous glands release their contents when stimulated by PRL. Dent and coworkers (1973) report that during winter months, secretory material resembles the contents of the granular glands. In the present study, glycoconjugates containing GluNAc and sialic acid (WGA) residues showed an increase in reactivity in the corneum following PRL treatment but not the epidermal glands. While these findings indicate that increased cutaneous secretion subsequent to PRL injection may be explained by the release of secretory product stored within epidermal glands on the surface of the corneum, there is no evidence that the activity of the gland itself is affected.

This study has shown that carbohydrate moieties

available for lectin binding which are associated with glycoconjugates in control newt epidermis differ from that of prolactin treated newts. GalNAc (SBA,DBA), Gal-(1,3)-GalNAc (PNA), galactose (GSAI-B4), glucose and mannose CON A, and fucose (LTA) residues showed a decrease in lectin binding in the epidermis of prolactin treated newts as compared to that of control animals.

It has been reported that PRL stimulates mitogenesis in the s. basale (Chadwick and Jackson, 1948). Cells migrate upward as differentiation occurs from the mitotically active basal layer to the transitional layer, and finally the corneum where keratinization is complete. In PRL-injected animals, decreased lectin binding may be the result of differential expression (e.g., composition, distribution) of glycoconjugates that occur during cell differentiation. The cytoplasmic staining was comparatively lighter than surface staining, which may be related to transport of synthesized glycoproteins. Similar observations have been made in canine keratinocytes, where lectin binding patterns were found to be dependent upon the functional state and the degree of differentiation of cells (Suter et al., 1991). Several other studies have demonstrated a relationship between cell differentiation and binding of lectins to glycoconjugates (Brabec, 1980; Zieke and Bernstein, 1982; Navas et al., 1987).

Dent (1966) reported that the effects of pituitary

autografting, and the succeeding release of endogenous PRL in the red-spotted newt, includes increased cutaneous secretion, sliminess and smoothness in skin texture. He concluded that in intact animals, the pituitary hormone PRL is under the influence of PRL inhibiting factor (PIF, dopamine) from the hypothalamus. It is likely that endogenous PRL levels decrease during conditioning, perhaps in response to PIF. In the present study, within a two week period of conditioning, lectins revealed differential binding in the epidermis by day 6. During conditioning, several carbohydrate moieties increase in availability for lectin binding. Gal-(1,3)-GalNAc (PNA), mannose and glucose (CON A), galactose (GSAI-B4), GalNAc (SBA, DBA), fucose (LTA) residues exhibited an increase. Hence it is possible that the gradual increase in staining that was seen with most lectins during conditioning may be associated with a decrease in endogenous levels of PRL, which in turn affects the expression of glycoconjugates. Additionally, a decrease in lectin binding of GluNAc and sialic acid residues supports the likelihood of prolactin's involvement, since in newts treated with exogenous PRL (X 14 days) an increase of WGA binding is evident.

Prolactin-induced changes in the temporal expression of epidermal glycoconjugates first became obvious by days 6 and 9. By day 15, binding of most lectins showed an overall decrease, although WGA (sialic acid, GluNAc) revealed an increase to very heavy binding in the corneum. Again, this

may indicate that the s. basale is mitotically stimulated and as cell differentiation occurs, epidermal glycoconjugates change, as reflected in altered lectin staining patterns.

Winter freshly collected newts demonstrate increased cutaneous secretion, smoothness and sliminess of skin, and in males, increase tailfin height. Prolactin injected newts show comparable characteristics. Conversely, summer newts exhibit decreased cutaneous secretion, roughness of skin and decreased tailfin height. Laboratory conditioning of freshly collected newts results in characteristics similar to that of summer animals. The present study and several others (Chadwick, 1941; Grant and Grant, 1958; Dent, 1966; Singhas and Dent, 1975) have elucidated a cyclic relationship between PRL, possibly other hormones acting synergistically, and the appearance of physical characteristics and behavior in the red-spotted newt. This may explain a decrease in lectin binding seen in winter freshly collected animals as compared to summerlike, laboratory conditioned animals. The decrease suggests that endogenous PRL levels may be higher in winter animals.

This investigation elucidates PRL's ability to alter the expression, and distribution of structural and secretory epidermal glycoconjugates in the red-spotted newt. Additionally, this study establishes that changes in glyconconjugates begin to occur primarily between days 3 and 6 in laboratory conditioning and also in PRL injection

Finally, there is evidence of seasonal variation in the expression of glycoconjugates, which may correlate to endogenous PRL levels.

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Figure 1. Lectin reactivity of LTA and SBA in the epidermis of the red-spotted newt during conditioning.

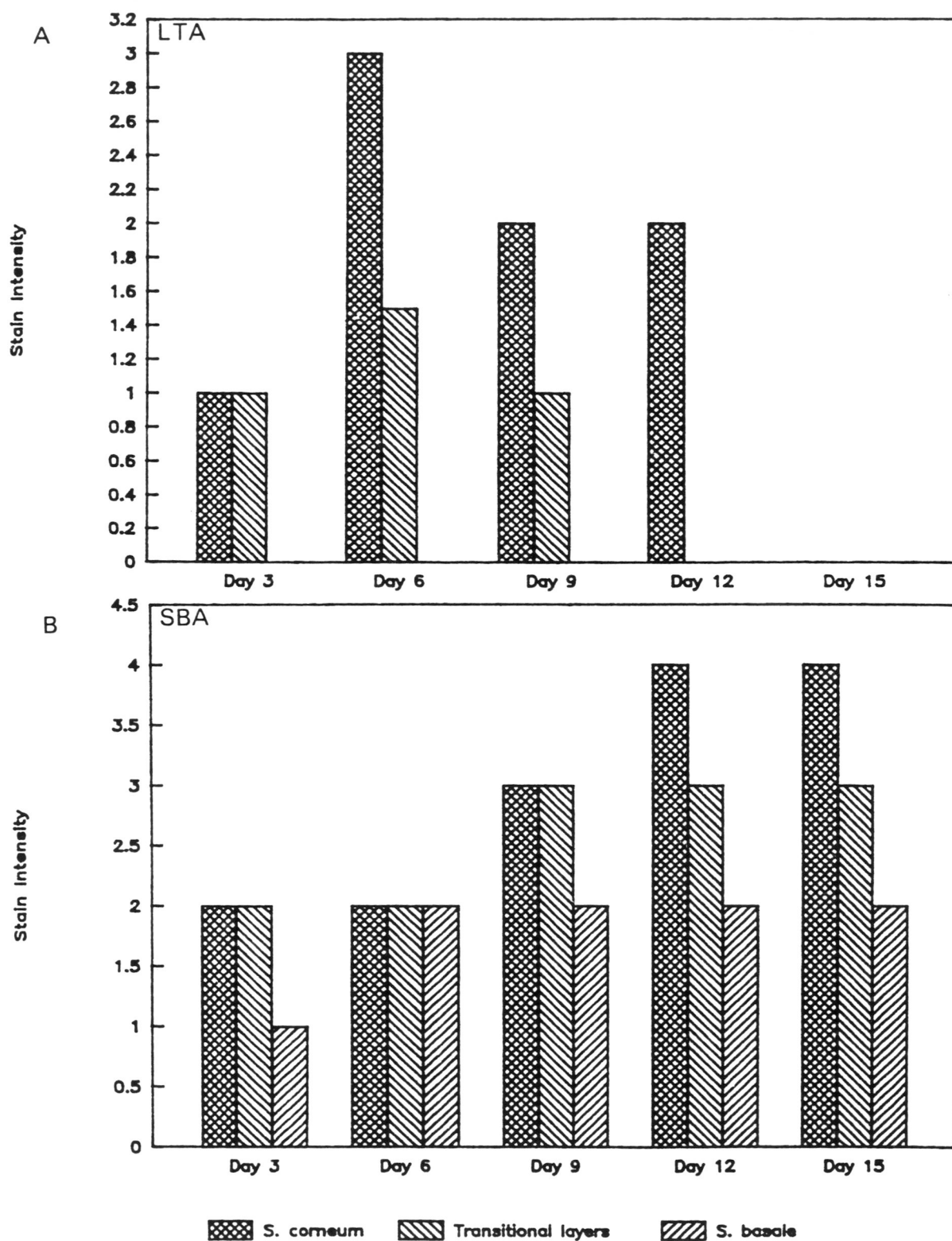


Figure 2. Lectin reactivity of DBA and GSAI-B4 in the epidermis of the red-spotted newt during conditioning.

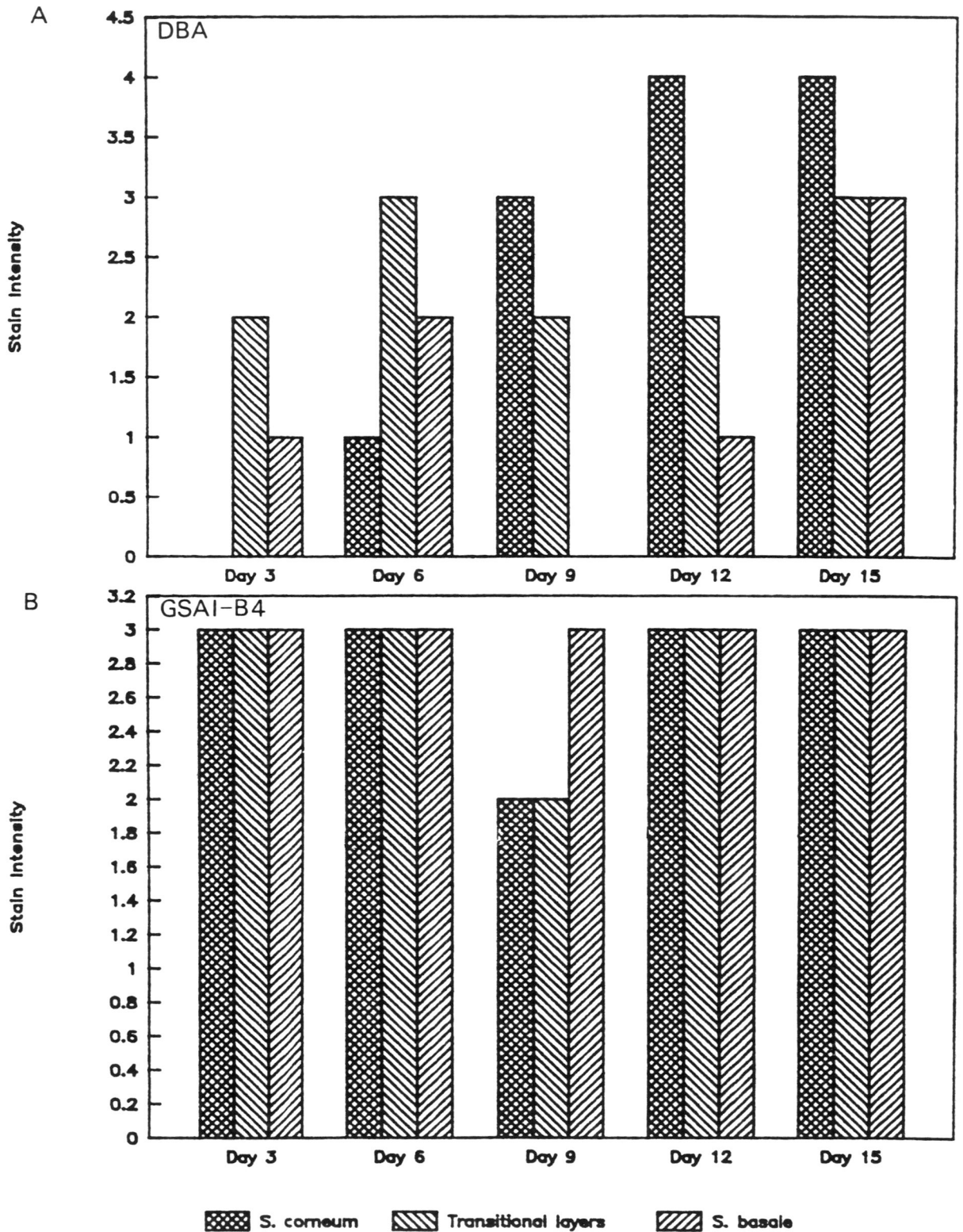


Figure 3. Lectin reactivity of CON A and WGA in the epidermis of the red-spotted newt during conditioning.

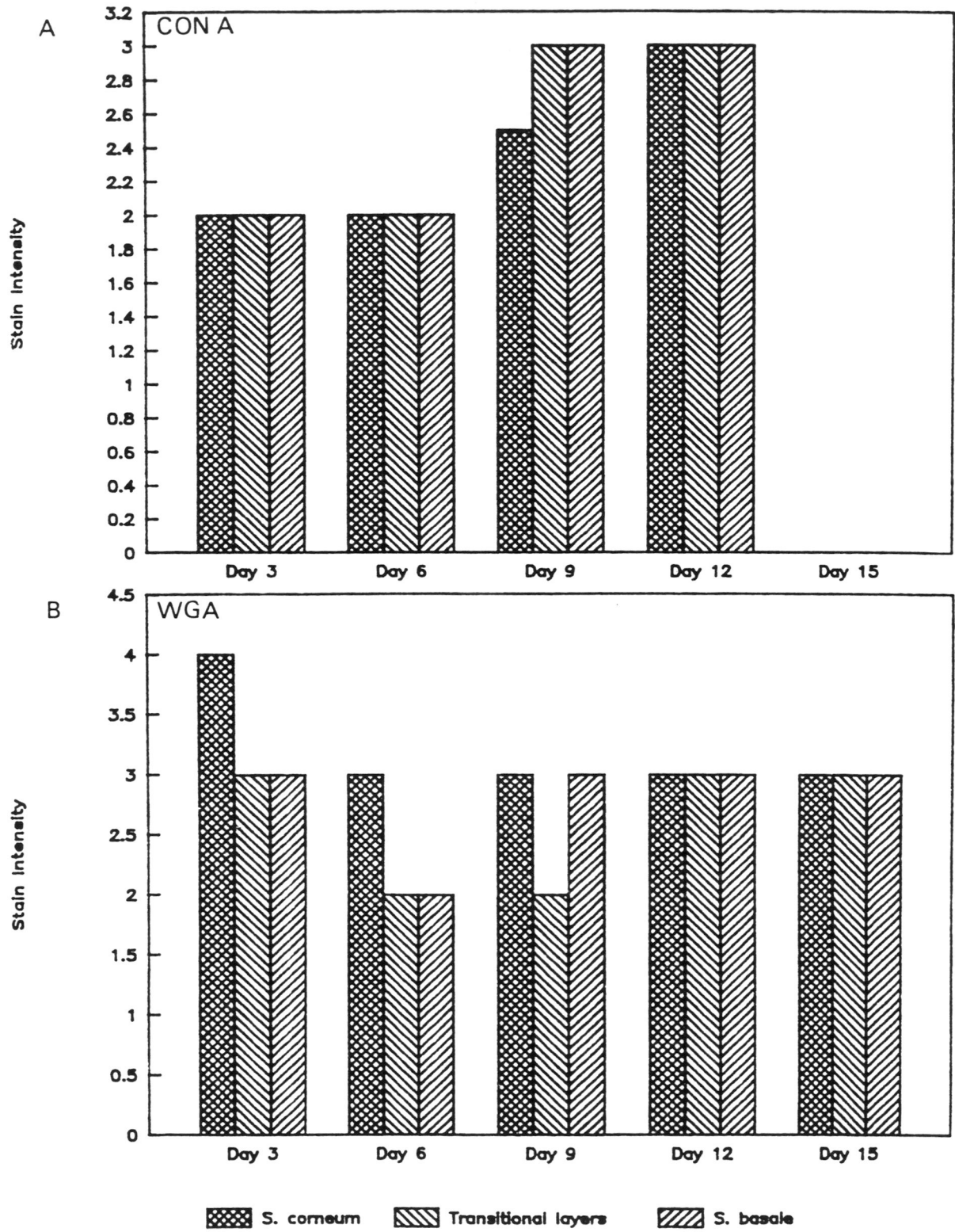


Figure 4. Lectin reactivity of PNA in the epidermis of the red-spotted newt during conditioning.

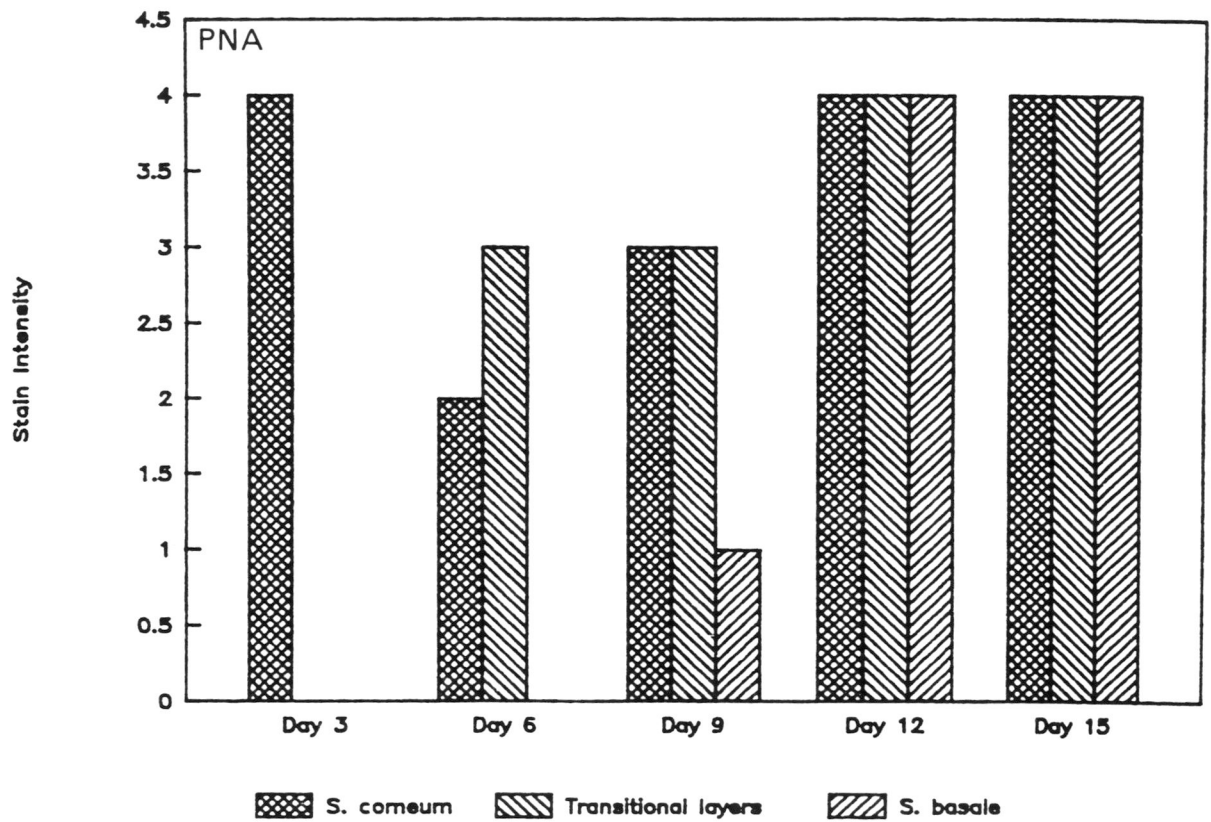


Figure 5. Lectin reactivity of LTA and SBA in the epidermis of the red-spotted newt during prolactin injection (X 14 days).

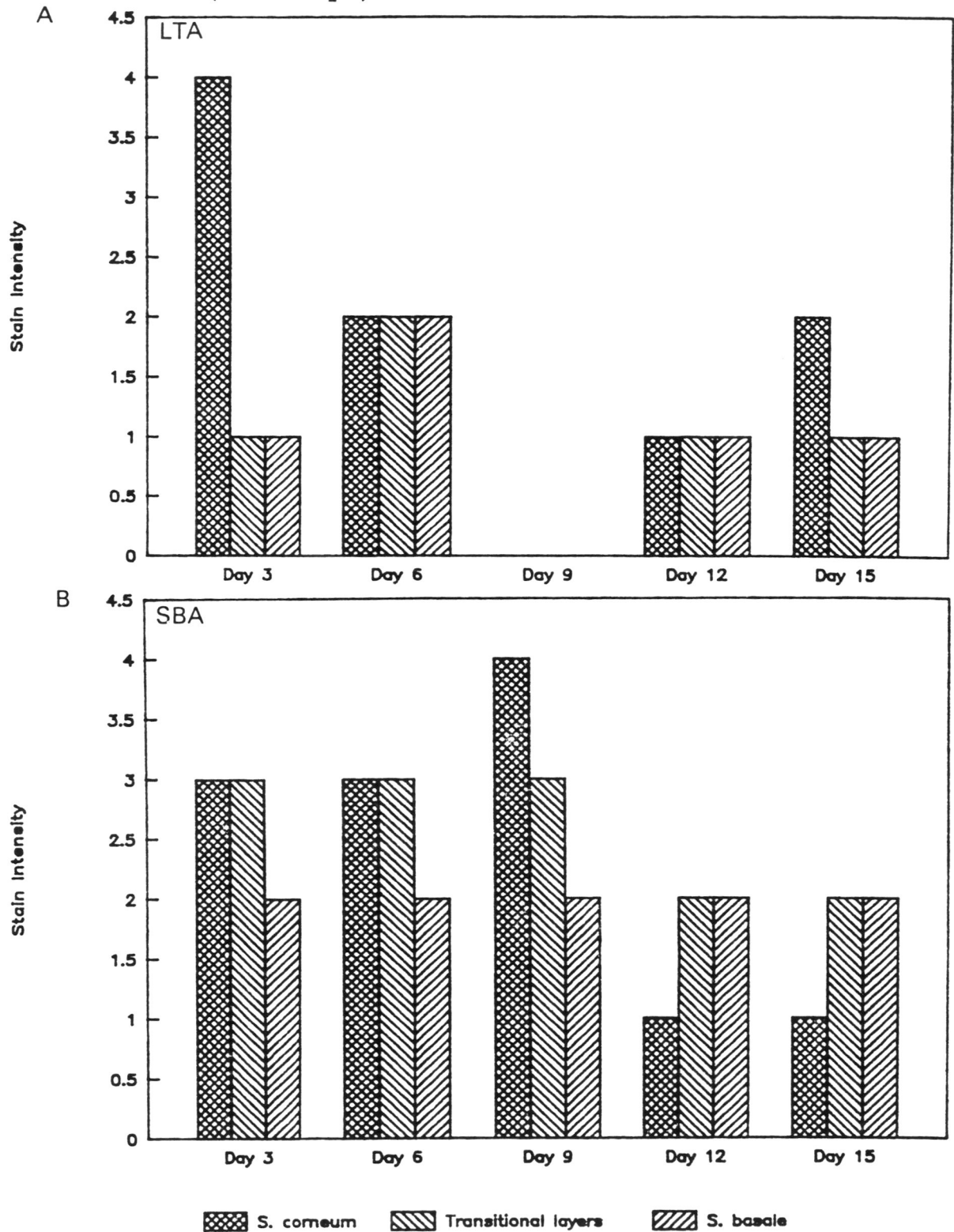


Figure 6. Lectin reactivity of DBA and GSAI-B4 in the epidermis of the red-spotted newt during prolactin injection (X 14 days).

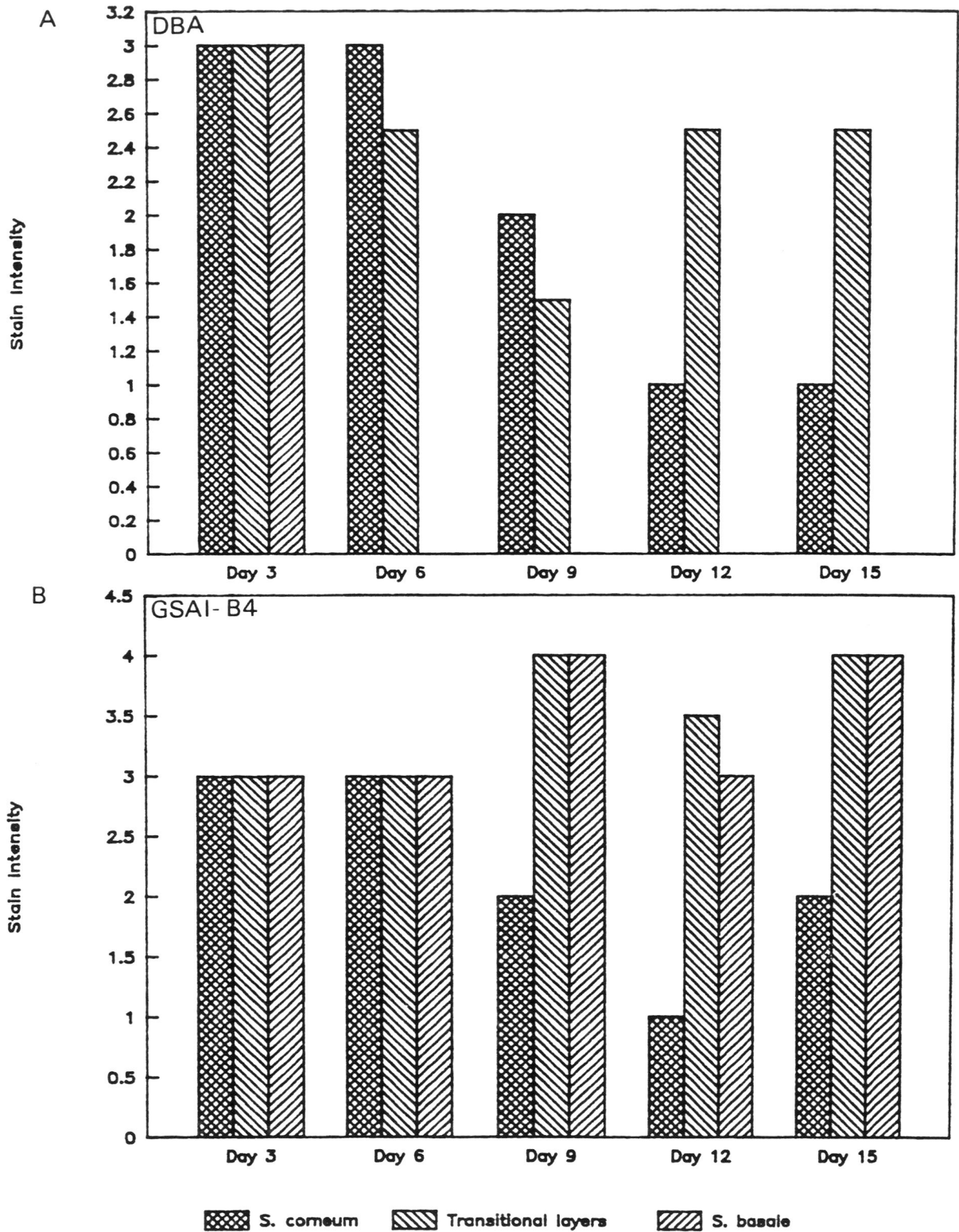


Figure 7. Lectin reactivity of CON A and WGA in the epidermis of the red-spotted newt during prolactin injection (X 14 days).

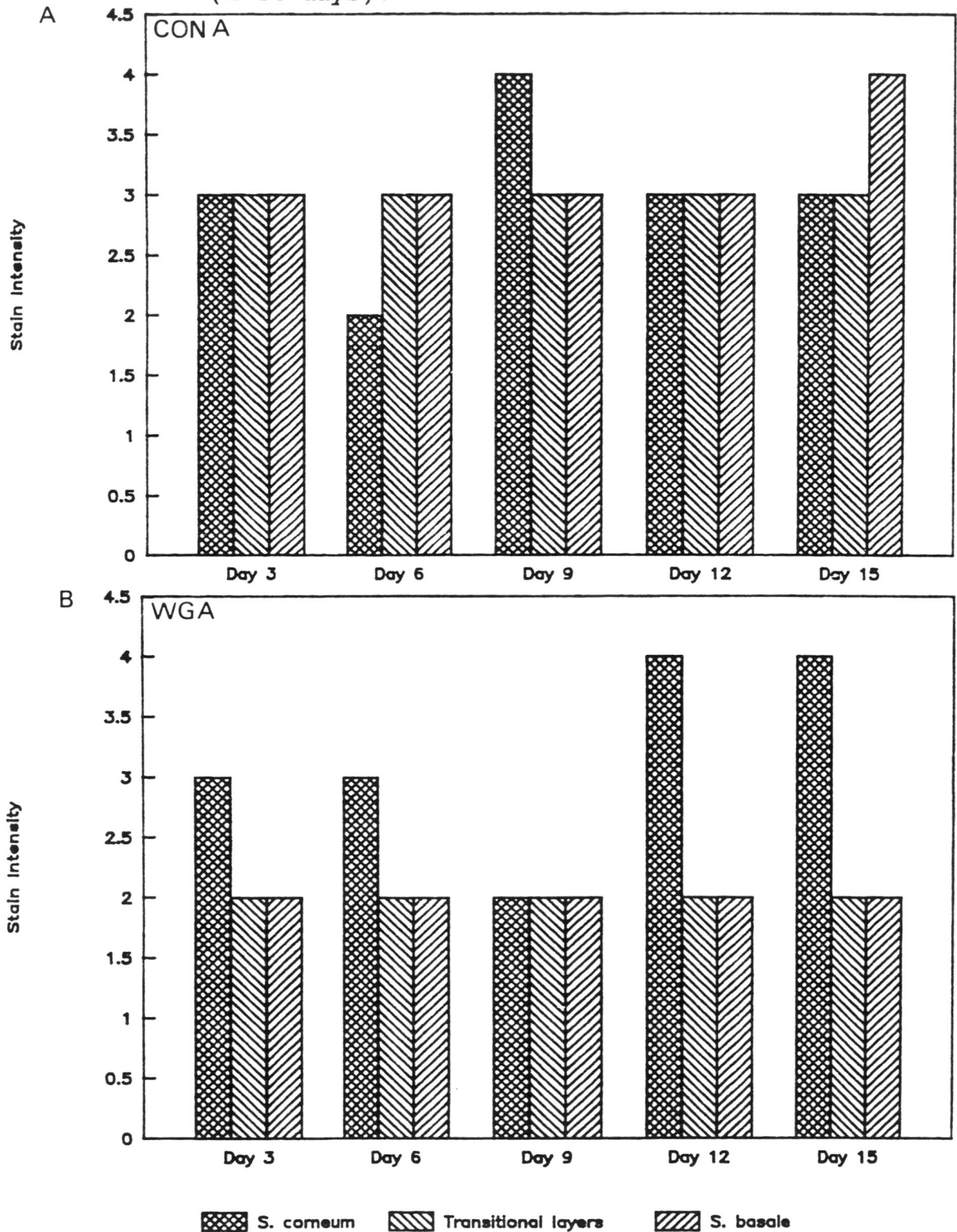


Figure 8. Lectin reactivity of PNA in the epidermis of the red-spotted newt during prolactin injection (X 14 days).

