cell body. He was unable to trace the course of this axon. I have observed short, blunt processes extending from the anterior side of the cell body for very short distances—less than 15 μm. If these extensions synapse with other cells, the LG axons are bipolar.

The branches of both the MG and LG fibers were very similar in different segments. The lengths of branches M1 and M3 varied, but no systematic differences in branching patterns between anterior and posterior regions of the worm occurred. No identified structures of the MG or LG fibers explain the different thresholds in the anterior and posterior regions. Bullock and Horridge (*2*) mention that the cross-over point of the behavioral response (normally segment 40) may change with repeated stimulation, so the polarizing mechanism may be the distribution of sensory input to the giant fibers.

The failure of dye to cross septa except where they had been ruptured by the pressure of injection supports Coggeshall's conclusion (*7*) that the septum is a complete cell boundary in which no pores or gaps occur. This is unlike the segmental synapses of *Procambarus*, which are slightly permeable to Procion Yellow (*8*). Procion Yellow is believed to bind covalently with carbohydrates and proteins (*4*), and dye molecules so bound would diffuse more slowly than would molecules in solution, but even after 12 hours there was no visible fluorescence past earthworm septa that had not ruptured. However, the synapse between the two L1 branches in each segment is very slightly permeable to the dye. I do not think this permeability can be dismissed as an artifact, but rather it indicates that the segmental synapses of earthworms have a structure different from that of the L1-L1 synapse. Otherwise, the LG fibers of earthworms are remarkably similar to the LG fibers of *Procambarus clarkii* (*5*). Both are formed by chains of segmental axons which make electrotonic junctions (*3*, *6*) with the corresponding axons in the adjacent segments. They have one cell body per axon located on the ventrolateral edge of the ganglion contralateral to the axon. In each segment, they both have major branches on the neurite on the side ipsilateral to the axon and make electrotonic connections with the contralateral LG axon.

It would be very interesting to know if the giant axons of earthworms make electrotonic connections with motor neurons similar to those in crayfish, but like many other basic features of the physiology of these everyday animals, the connections between the giant fibers and motor neurons are unknown.

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Harderian Gland: Development and Influence of Early Hormonal Treatment on Porphyrin Content

Abstract. *The porphyrin content of the rat Harderian gland remains low until 12 days of age at which time both porphyrin content and concentration rapidly increase. Intraperitoneal administration of tetraiodothyronine (thyroxine) into newborn animals advances the appearance of porphyrin in the gland. Conversely, a single injection of cortisol acetate into newborns retards the appearance of porphyrin. The time of porphyrin appearance in the gland parallels the time for maturation of the evoked cortical response to visual stimulation in normal and hormone-treated animals.*

The Harderian gland is a large, bilobed, saccular gland located in the orbit of reptiles, birds, and mammals. It is rudimentary or lacking in Anthropoidea (*1*). In some rodents the gland has a characteristic appearance due to its high porphyrin content (*2*). Information on the development of the Harderian gland and on its function is sparse. Müller has studied the histological changes in the gland from birth to 13 weeks (*3*). He reported a rapid, pronounced alteration in cellular morphology and increased secretory activity at about the 14th day of age which he correlated to eye-opening. He also found evidence of porphyrins in the gland at 8 days and the appearance of yellowish-brown pigments at day 9. Boas and Scow (*4*) reported atrophy of the Harderian gland in thyroidectomized or hypophysectomized adult rats, but Figge and Davidheiser (*5*) failed to observe any effect of hypophysectomy on porphyrin synthesis of the gland. Rohonyi and Kelényi (*6*) found the porphyrin content of the Harderian gland of young rats to be lower than that of adult animals. Klüver (*7*) found that a fluorescent spectrum similar to that of porphyrin first appears in the nervous system during a period coincident with vascularization and development of motor activity and suggested a possible relation between porphyrins and myelinization.

We have suggested that the Harderian gland may act as a secondary phototransducer in blinded suckling rats, because removal of the gland affects the light-dependent variation of serotonin in the pineal gland (*8*). The normal maturation of evoked cerebral cortical potentials in response to sensory stimuli in the rat can be altered by early hormonal treatment (*9*). Such treatment particularly affected the following response in the visual cortex. This study was undertaken to determine the normal development of porphyrin in the rat Harderian gland, the influence of early hormonal treatment on such development, and the relation of the development of the Harderian gland to the maturation of the visual cortex.

Harderian gland weight, porphyrin content, and porphyrin concentration were determined in 153 male and female Sprague-Dawley rats bred in our laboratory and ranging in age from 4 to 26 days. The infant rats were divided

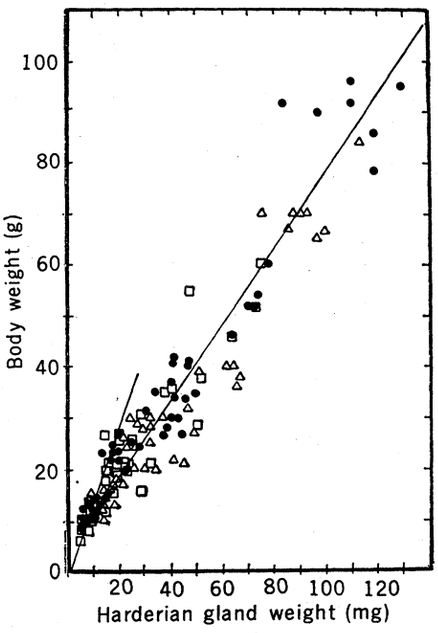

Fig. 1. Relationship between Harderian gland weight and body weight. Circles, controls; triangles, animals treated with thyroxine; squares, animals treated with cortisol. The lines are based upon least-squares fit of the control values between 4 to 10 days of age and 14 to 26 days of age.

into three groups according to neonatal treatment.

To one group ($N = 51$) thyroxine (1 μg/g body weight) was administered intraperitoneally daily for 3 consecutive days beginning at birth. A second group ($N = 44$) received a single subcutaneous injection of 500 μg of cortisol acetate at birth. The control group ($N = 58$) received saline intraperitoneally at birth. The rats were decapitated at the appropriate times, the brains were removed, and the heads were frozen at -76°C until assayed. The heads were then thawed and the Harderian glands were removed (*8*), weighed, and homogenized in 10 ml of a mixture of ethyl acetate: glacial acetic acid (4 : 1). A portion of the homogenate was diluted 1 : 100 with 1.5*N* hydrochloric acid and centrifuged for 5 minutes. Porphyrin content was determined by fluorescence at 602 nm upon activation at 405 nm. Tetramethylcoproporphyrin (Harleco) stock standard (0.5 μg/ml) was used as reference substance, since it was commercially available. The total porphyrin content of the gland was calculated as coproporphyrin equivalents even though the major porphyrin component of the Harderian gland is protoporphyrin (*10*).

In normal animals the weight of the Harderian gland increases more slowly than does body weight for the first 10 days of life (Fig. 1). This is followed by a spurt between days 12 to 26 during which the Harderian gland increases in weight faster than does the rest of the body. A similar time course was found in animals treated with thyroxine. However, the growth spurt of the Harderian gland of the animals treated with cortisol was delayed until day 15.

Both porphyrin content (μg/gland) and porphyrin concentration (μg/100 mg of gland) of the Harderian gland are low during the first 10 days after birth, and both rise rapidly between 12 and 21 days of age (Fig. 2). After this period porphyrin content reaches a plateau while porphyrin concentration declines. Thyroxine treatment results in a precocious appearance of porphyrin in the gland and a slow continuous rise in both content and concentration. Thus, animals treated with thyroxine at day 10 have a porphyrin content in the Harderian gland equal to that of 13-day-old controls, whereas at day 15 porphyrin content is the same for both groups. Cortisone treatment retards the appearance of porphyrin in the gland, and the porphyrin concentration in these animals does not equal that in 16-day-old controls until 26 days of age.

It may also be seen from Fig. 2 that thyroxine treatment, although initiating porphyrin development in the Harderian gland at an earlier age, also retards the subsequent attainment of control amounts. Cortisone treatment, on the other hand, while retarding the initial appearance of porphyrins, results in a later increase in porphyrin content of the gland. Similar patterns of acceleration and retardation by these hormones have been previously observed on behavioral parameters such as the development of the startle reflex (*11*) and swimming ability (*12*), on anatomical parameters such as development of dendritic spines (*11*), on learning ability in a light-dark discrimination task and a Lashley-3-maze (*11*), and on maturation of the electroencephalogram (*11*). The relative significance of this apparent reversal of the first action of these hormones during later development is not yet known.

These changes are temporally coincident with the pattern of development of the visual system (*9*). Treatment of neonates with either thyroxine or cortisone leads to premature eye opening, but, whereas treatment with thyroxine results in the precocious appearance of cortical evoked potentials in response to light, cortisol treatment delays this neurophysiological response (*9*, *11*). Thus, evoked potentials first appear in the normal animal at about day 12 or 13, in animals treated with thyroxine they appear at about day 9 or 10, and in animals treated with cortisol they appear at about day 15 or 16. It is of interest that the porphyrin content in the Harderian glands of 10-day-old animals treated with thyroxine, 13-day-old controls, and 16-day-old animals treated with cortisol is identical.

It appears then that the development of porphyrins in the Harderian gland coincides with the maturation of evoked cortical responses to visual stimuli, but not with the time of eye opening. Whether this signifies a functional relation between maturation of the visual system and porphyrin content of the Harderian gland, or simply coincidental development, is not known. In addition, these hormonal effects on por-

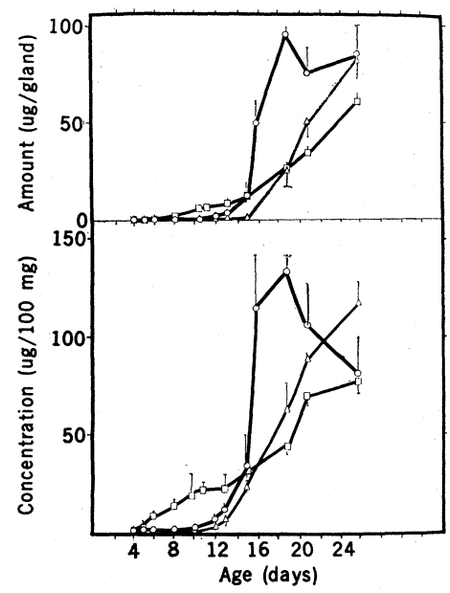

Fig. 2. Total porphyrin content (top) and concentration (bottom) in the Harderian gland of the neonatal rat. Circles, normal animals injected with saline; squares, injected with thyroxine (1 μg/g) 1, 2, and 3 days after birth; triangles, injected with 500 μg corticol acetate at birth. Each point represents the mean value and the bars show the standard error of the mean. The porphyrin content (μg/gland) in the animals treated with thyroxine differs significantly ($P < 0.05$) from controls on days 5, 6, 7, 8, 10, 12, 13, 19, 21, and 26 and the concentration (μg/100 mg) in these animals differs significantly ($P < 0.05$) on days 6, 7, 8, 10, 12, 19, and 21. In the animals treated with cortisol the porphyrin content differs significantly ($P < 0.05$) on days 12, 13, 19, and 21 and the porphyrin concentration differs significantly ($P < 0.05$) on days 19 and 26.

phyrin biosynthesis may be of value both in preparing models for the study of human porphyrias (*13*) and for investigating the mechanisms for regulation of porphyrin biosynthesis.

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Colicin-Tolerant Mutants of Escherichia coli: Resistance of Membranes to Colicin E1

Abstract. *Colicin E1 blocks proline accumulation by membrane vesicles prepared from wild-type sensitive* Escherichia coli. *Two classes of mutant cells are unaffected by colicin. Vesicles from colicin-resistant strains are sensitive to colicin E1, whereas vesicles from colicin-tolerant strains are unaffected by colicin E1. These results suggest that the colicin E1 receptor is on the cell membrane and that colicin-tolerant strains have altered membranes while colicin-resistant strains have altered cell walls.*

Although all colicins appear to be small macromolecules (predominantly proteins) which are adsorbed to and remain on the surface of the cell which is being affected, the mode of action of colicins differs. Some affect DNA metabolism, others ribosomal function, and still others oxidative phosphorylation (*2*, *1*). Colicin E1, with which our studies are concerned, appears to affect oxidative metabolism and active transport in *Escherichia coli* (*3*).

Mutants of *Escherichia coli* which are no longer sensitive to colicin E1 fall into two classes: (i) colicin-resistant mutants which have lost the ability to adsorb the colicin onto the cell surface and are therefore not killed by the action of colicin, and (ii) colicin-tolerant mutants which, although they retain the adsorption receptors for colicin, are not killed by colicin (*1*, *4*). The distinction between these two classes of mutants on the basis of adsorption of colicin (along with additional properties of colicin-tolerant mutants, such as increased sensitivity to lipid-active detergents) has led to the hypothesis that colicin-resistant mutants have altered cell walls such that the cell-wall binding sites will no longer fix the colicin, and that colicin-tolerant mutants have altered cell membranes through which the colicin cannot transmit its lethal action to the intracellular target (*1*, *4*). The experiments reported here with isolated subcellular membrane vesicles (*5*) provide direct support for the distinction between resistant (wall) and tolerant (membrane) mutants, and also show that the cellular binding site for colicin E1 is on the cell membrane and not the cell wall. The closed membrane vesicles lack most of the cell wall material, DNA, RNA, and the soluble enzymes of the original cell, but they are still capable of several membrane-associated functions, such as active

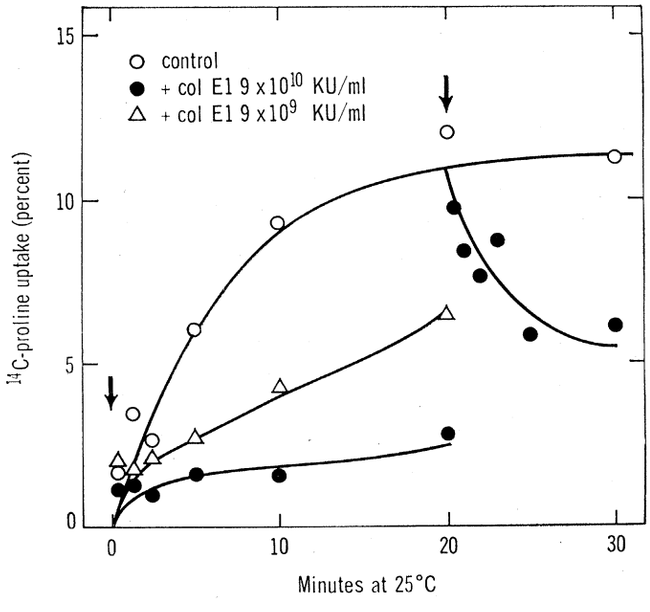

Fig. 1. Effect of colicin E1 on [^{14}C]proline accumulation by isolated membrane vesicles from the colicin-sensitive strain of *E. coli*. Colicin E1 at either 9×10^9 or 9×10^{10} killing units (KU) per milliliter was added to membrane vesicles (2 mg/ml, dry weight) from *E. coli* strain K-12 either 1 minute before or 20 minutes after the addition of [^{14}C]proline. The fraction of [^{14}C]-proline in the vesicles (on the filters) was determined by counting direct samples of the reaction mixture. Colicin titers in "killing units per milliliter" were determined by standard methods (*6*).