posure to the 16:8 regimen. However, early exposure to the 12:12 photocycle apparently was able to produce a certain degree of inhibition of the endocrine centers; otherwise, the intercalation of the 16:8 photoperiod would have produced sufficient activation of these centers to allow all the larvae to proceed to the pupal stage without diapause.

Larvae produced under treatments 1 and 4 with both the 14:10 and 16:8 photoperiods were observed until they had either pupated or reached 4 months of age. Most of the nondiapausing larvae had pupated by the time they were 20 days old. As shown in Fig. 2, the various photoperiodic regimes apparently had no effect on the time required for these individuals to achieve the pupal stage.

The rate of pupation of the diapausing larvae is shown by records made between the 40th and 120th days. These observations provided a measure of the differences in the intensity of diapause produced by the various photoperiodic exposures. The few diapausing larvae produced in treatments having 14:10 photoperiods proved to have a less intense diapause than those produced under regimes having 16:8 cycles. The treatments which produced the greatest percentages of diapausing larvae also caused the most intense state of diapause. Therefore, it appears that the intensity of diapause is dependent, at least partially, on the quantity and quality of the inductive stimuli previously experienced by the insect. However, because within each treatment group, there was a considerable difference in the rate of pupation of the various individuals, the genetic factors taking part in the diapause phenomena should not be ignored.

The manifestations of larval diapause—cessation of growth, atrophy of the gonads, increase of fat, and lowered respiration—are indicative of an endocrine deficiency (*1*, *3*). Research by Williams (*4*) on pupal diapause has demonstrated that the primary cause of these happenings is a failure of the brain to supply the hormone required to activate the endocrine organs and, more particularly, the prothoracic glands. Also, it has been found in certain species that the endocrine processes are under environmental control (*3*, *4*). Undoubtedly, the diapause of the pink bollworm is provoked when the photoperiod regimen inhibits these endocrine mechanisms.

The full release or activation of the forces controlling growth and development of the pink bollworm apparently is dependent mainly on a night of critical length (*2*). Under this viewpoint, we now see that nights of different length may have different action. This allows one to make certain deductions concerning the dynamics of the system. Although photoperiods having nights 8 or 10 hours long, when given in continuous sequence, appear to be equally effective in preventing diapause, an 8-hour night does not have the force of a 10-hour night in activating previously inhibited endocrine processes. This leaves the impression that the forces causing continued development without diapause gain in strength with an increase in night length from 8 to 10 hours. We also know, however, that as the night increases from 10 to 12 hours, the system undergoes a complete reversal from one that prevents diapause to one that causes it. Thus, it seems, with regard to the length of night, that 8 hours is just barely sufficient for development to continue without diapause, 10 is near optimum and 12 hours is too long.

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Rectal Glands of Marine and Fresh-Water Sharks: Comparative Histology

Abstract. *The rectal glands of elasmobranchs perform the function of salt-excreting organs. These glands are smaller and show regressive changes in specimens of the bull shark,* Carcharhinus leucas *found in fresh-water environment, compared with specimens of this and other species from a marine habitat.*

The physiological significance of the rectal gland was obscure for a long time, though some morphological investigations were made (*1*). Recently the excretion of sodium chloride was reported as the main function of the gland (*2*). Elasmobranchs are marine fish, but a few adapt to the fresh-water environment (*3*). The bull shark, *Carcharhinus leucas* (Müller and Henle), is common in the Gulf of Mexico and Atlantic Ocean, but it also lives in Lake Nicaragua (*4*) and some fresh-water situations in the United States (*5*). This report deals with morphological differences in the rectal glands of marine and fresh-water *C. leucas*.

The rectal glands investigated were from six marine and ten fresh-water specimens of *C. leucas*. Also, for reference, the rectal glands were investigated in the following additional species of marine sharks: blacktip shark, *C. limbatus* (two specimens), tiger shark,

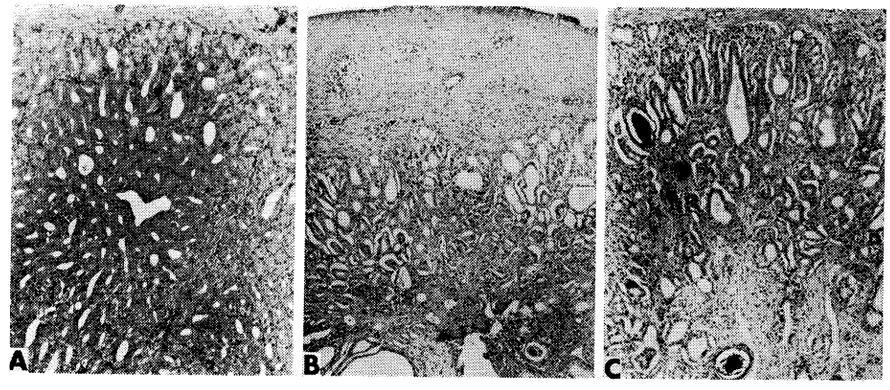

Fig. 1. Rectal glands of bull sharks, *Carcharhinus leucas* (hematoxylin and eosin; × 114). *A*, Marine shark, female (No. 11 in Table 1); *B*, fresh-water shark, male (No. 2 in Table 1); *C*, fresh-water shark, female (No. 3 in Table 1).

Table 1. Rectal glands of fresh-water and marine sharks. The fresh-water species were collected at Lake Nicaragua and Rio San Juan in June and July 1963, the marine species at Cape Haze, July to November 1963.

Animal No.	Sex	Body length (cm)	Rectal gland	
			Length (cm)	Weight (g)
Fresh water: C. leucas				
1	F*	170	4.5	1.1
2	M*	158.4	6.5	0.6
3	F†	188.2	6.5	1.5
4	M†	174	6.7	0.6
5	F‡	155	6.0	0.8
6	M‡	147.5	6.4	0.7
7	F§	(127.2) ‖	6.0	0.35
8	F§	135	3.8	0.35
9	F§	157	4.0	0.4
10	F§	(165.6)	4.2	0.45
Marine: C. leucas				
11	F	215.5		
12	F	231.0		
13	F	246.0	8.2	6.5
14	F	261.2	9.8	15.9
15	M	185.9	7.4	3.8
16	F	200.7	9.1	7.6
Marine: C. limbatus				
17	M	142.5	3.2	1.5
18	F	161.3	5.4	3.0
Marine: Galeocerdo cuvieri				
19	F	196.4	8.5	6.1
20	M	212.7	10.8	8.2
Marine: Negaprion brevirostris				
21	M	265	7.3	12.0

* Collected at El Castillo. † Collected at San Carlos. ‡ Collected at San Juan del Norte. § Collected at Los Cocos near Granada. ‖ Not identified individually.

Galeocerdo cuvieri (two specimens), and lemon shark, *Negaprion brevirostris* (one specimen). Four rectal glands of marine sharks (Nos. 11, 12, 14, and 17 in Table 1) were fixed in Zenker-formol for histological examination. The rectal glands of fresh-water sharks were originally preserved in 70 percent ethyl alcohol, but they were fixed with Zenker-formol for routine histological procedures (*6*, *7*).

Table 1 shows the measurements and other data on the rectal glands of bull sharks used in this study. For reference the data concerning three other species of marine sharks were included. A remarkable difference was observed in the size of rectal glands from marine bull sharks compared to those from Lake Nicaragua and Rio San Juan, even if the smaller size of the fresh-water sharks is taken into consideration. The weight of the rectal gland was greatly reduced, especially in four females from Los Cocos near Granada. In the histological preparations differences were also detected. The rectal glands of marine sharks are compound tubular glands, as shown in Fig. 1*A* which is a section from a female bull shark (No. 11 in Table 1). The cytoplasm of the excretory cells is granular, and it stained in the form of basal filament with eosin or phloxine. Cytological, histochemical, and electron microscope observations were made on the rectal glands of marine elasmobranchs by Bernard and Hartmann (*8*) and Doyle (*9*), and high activity in tubular cells was demonstrated morphologically.

The rectal glands from fresh-water sharks showed regressive changes. Figure 1 (*B* and *C*) shows the rectal glands of male and female sharks caught at El Castillo and San Carlos, respectively (Nos. 2 and 3 in Table 1). The glandular tubules are decreased in number, and the interstitial tissue between these tubules is increased in proportion. Some tubules are shrunken, and others are swollen. Laminated bodies resembling the casts and corpora amylacea found in renal tubules and prostatic ducts of man (*10*) are observed often within the lumina of these tubules (Fig. 1*C*). These bodies stain with PAS, iron-hematoxylin, phloxine, or chrome-hematoxylin, and are associated with tubular dilation and epithelial compression.

Thus, it seems that the rectal glands of Lake Nicaragua sharks become hypofunctional, or quiescent, and finally regressive changes associated with living in a fresh-water environment occur in their structure. These observations support the theory of Burger and Hess that the main function of the rectal gland is to excrete sodium chloride. It is not definitely known whether Lake Nicaragua sharks remain in the lake throughout life or whether they migrate back and forth between fresh water and the Caribbean Sea. The regressive changes were detected also in the rectal glands of sharks caught at the San Juan del Norte (Greytown), at the mouth of the Rio San Juan (Nos. 5 and 6 in Table 1). This suggests that they migrate (up and down) between Lake Nicaragua and the sea.

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Mitotically Synchronized Mammalian Cells: A Simple Method for Obtaining Large Populations

Abstract. *Eliminating most of the ionic calcium from growth medium does not affect cell growth, but it allows simple and preferential detachment of HeLa cells in mitosis from nearly confluent monolayers.*

Biochemical studies of the mammalian cell in mitosis have been severely restricted by the lack of a simple system for achieving large populations in mitotic synchrony in spite of the critical role that biochemistry must play in eludicating the basic mechanisms of mitosis. Through environmental variations in temperature, or use of fluorodeoxyuridine (FUDR), some investigators have obtained what was termed parasynchronous division in mammalian cell cultures (*1*). In these systems a large proportion of the cells doubled their number within a short time interval relative to the total generation time. These techniques, however, are of little use for extensive investigation of the intracellular biochemical profile during the mitotic cycle because the actual increase in the number of mitotic figures present at any one time is usually less than 10 percent of the total population. Terasima and Tolmach (*2*) recently described the first significant improvement in the technique for obtaining mitotically synchronous mammalian cells. They have taken advantage of the relatively tenuous attachment of these