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Introduction

The rectal gland of elasmobranchs secretes large quantities of electrolytes and is of primary importance in the osmoregulatory mechanisms of these animals (Burger and Hess, 1960; Burger, 1962). Analogously, various organs of other vertebrates are also known to be involved in salt secretion (Schmidt-Nielsen, 1965; Bonting, 1970; Bentley, 1971). In general, active ion transport is the basis of this phenomenon; in any case, it requires the presence of structural devices, morphologically and chemically well defined, and of metabolic capabilities which, taken singly, are not specific, but collectively are consistent with active ion transport and salt secretion.

Morphological, biochemical and physiological data on this subject are quite abundant in the literature. The most significant evidence is summarized as follows: (1) Cytochemical evidence includes intense protein and phospholipid staining, the presence of acidic mucopolysaccharides at the cell boundaries, a high level of oxidoreductases and the presence of magnesium ATPase in its sodium activated fraction; (2) ultrastructural evidence includes the infolding of the lateral surfaces of the secreting cells, and abundance of mitochondria and the presence of smooth-surfaced vesicles; (3) biochemical evidence includes the presence of membrane-bound sulfolipids in a rather high concentration, in addition to the activity of the sodium activated ATPase previously mentioned; (4) direct evidence of a substantial presence and transport of electrolytes has also been obtained through the determination of the freezing-point depression of salt gland tissue, the cytochemical demonstration of sodium and chloride ions, especially at the ultrastructural level, and the measurement of exchange of radioactive sodium between the internal milieu and the external environment after suitable stimulation.

Concerning the rectal gland of elasmobranchs, the morphological, biochemical, physiological, ultrastructural and cytochemical data so far obtained by several workers agree with the preceding formulation (Doyle, 1962; Bulger, 1963, 1965; Fänge and Fugelli, 1963; Bonting, 1966; Oguri, 1964; Komnick and Wohlfarth-Bottermann, 1966; Chan and Phillips, 1967; Chan *et al.*, 1967; Gilbert *et al.*, 1967; Stocken *et al.*, 1968; Karlsson *et al.*, 1968; Hansson *et al.*, 1973).

In particular, cytochemical research has been carried out in our laboratory, comparing several species, in order to clarify the problem of the structural basis of salt secretion and osmotic adaptation (Gerzeli, 1967; De Piceis Polver and Bernocchi, 1970). Preliminary reports have also concerned species living in fresh water (Gerzeli and De Stefano, 1968; Gerzeli *et al.*, 1969). In *Carcharhinus leucas* and *Pristis perotteti* from Lake Nicaragua, the authors have demonstrated the morphological atrophy of the gland. However, this atrophy is accompanied by fairly complete preservation of the histochemical features typical of marine species. On the contrary, the gland of another freshwater species (*Potamotrygon brachiurus* from Río Uruguay) appears very peculiar, showing secretory activity histologically, but lacking any cytochemical evidence related to salt secretion, as summarized above. This contrast may be only apparent, in light of the claimed absence of the rectal gland by Goldstein and Forster (1971). It must be noted that this stingray contains only traces of urea in the body fluids (Thorson *et al.*, 1967) and presumably has different osmoregulatory mechanisms (Thorson, 1970). Furthermore, in *Myliobatis aquila*, a related marine species examined by De Piceis Polver and Bernocchi (1970), the histochemical data point to the absence of the structural and metabolic features consistent with electrolyte transport.

Consequently, it appears important to examine the euryhaline and freshwater species of elasmobranchs more closely. In this paper some new cytochemical and biochemical data are presented on the sawfish, *Pristis perotteti*, from Lake Nicaragua, on the bull shark, *Carcharhinus leucas*, from both lake Nicaragua and the Caribbean Sea and on the small blacktip shark, *Carcharhinus limbatus*, from the Pacific Ocean.

Materials and Methods

Several specimens of three species of elasmobranchs were captured in different environments (fresh, brackish and marine water) and used for cytochemical and biochemical analysis; samples of water were also collected and tested for salinity.

Collecting of samples

Animals. Freshwater specimens (10 *Carcharhinus leucas*; 5 *Pristis perotteti*) were captured near San Carlos at the southeast end of Lake Nicaragua. Fresh- to brackish-water specimens (7 *Carcharhinus leucas*) were captured near Barra del Colorado (Costa Rica), where one of the three branches of the Rio San Juan flows into the Caribbean Sea. Of the latter, three individuals were captured in a freshwater lagoon and the other four in the open sea (about 1.5 km from the coast, at the boundary between the river water and the sea). The sea water specimen (one *Carcharhinus limbatus*) was captured in the Pacific Ocean, near Corinto (Nicaragua) about 2 km from the coast.

The captured animals were killed immediately by bleeding, following cutting of the caudal fin. Samples of blood, rectal gland and kidney were taken from all the individuals; samples of somatic (epaxial) muscle were also collected from some specimens of *Carcharhinus leucas*.

The rectal gland was usually divided into four portions, respectively treated as follows: 1) fixed in Bouin's fluid and preserved in 70% ethanol (for histochemical analysis); 2) frozen with a jet of CO_2 and preserved on dry ice (for enzyme histochemistry); 3) placed and preserved in a methanol-chloroform mixture, 1/1 by volume (for lipid analysis); 4) weighed, washed in saline, placed and preserved in 5 ml of 10% trichloroacetic acid (TCA) at 4°C (for chemical analysis).

The samples of other organs were all placed in TCA.

The blood samples, drawn from the caudal vein, were preserved in plastic tubes at 4°C, with and without heparin (5 I.U./ml), for preparation of plasma and serum respectively, for chemical analysis.

Samples of water. Water samples were taken at all of the above-mentioned fishing areas. The water from Corinto and from San Carlos was drawn at the surface; that from Barra del Colorado was drawn both at the surface and at 4 m depth. The samples (1 liter each) were collected in plastic bottles, sealed immediately and preserved at 2°C.

Analytical procedures

Water salinity. The Cl^- analysis was carried out on the water samples according to Smith (1955); the water salinity was subsequently calculated from Cl^- values (by employing conversion tables).

Histochemistry of the rectal gland. The histochemical analysis (for reference see mainly Pearse, 1968–1972) was carried out on fixed and fresh-frozen samples[1].

Paraffin sections of fixed material were tested for:

polysaccharides: PAS reaction alternatively preceded by α-amylase digestion, Alcian blue reaction at increasing $MgCl_2$ concentrations or at different pH;

proteins: Bromophenol blue stain;

polar lipids: acid hematein method, also after methanol-chloroform treatment.

Cryostatic sections of fresh-frozen material were examined for the following enzymatic activities:

oxidoreductases: lactate, isocitrate, glucose-6-phosphate and succinate dehydrogenases (1×10^{-2}M substrate, 1×10^{-3} M coenzyme, when required, 0.25‰ Nitro BT); NADH and NADPH tetrazolium reductases (1×10^{-2} M substrate, 0.25‰ Nitro BT); *hydrolases*: Mg^{++} dependent ATPase, at neutral pH (3×10^{-3} M substrate, 11×10^{-3} M Mg^{++}, 3×10^{-3} M Pb^{++} as trapping agent), with activation control by Na^+ and K^+ and inhibition control by ouabain (3×10^{-5}M) or by Ca^{++} (11×10^{-3}M).

Lipids in the rectal gland. The portion of rectal gland reserved for lipid analysis was homogenized in methanol. Twice the amount of chloroform in volume was added (chloroform/methanol ratio 2/1) and the mixture was then homogenized again and filtered through filter paper; the filter was also washed with a chloroform-methanol mixture; finally the filtrates were combined with the fluid previously used to preserve the samples, until their arrival in the laboratory.

These mixtures (total lipid extracts) were tested for lipid-bound sulfate (by a colorimetric procedure after Kean, 1968), phospholipidic phosphate (by Bartlett, 1959, modification of the Fiske and Subbarow procedure) and cholesterol (by colorimetric procedure after Parekh and Jung, 1970). The urea concentration was assayed by the acetylmonoxime procedure after Natelson (quoted by Varley, 1967).

Chromatographic separation of the polar lipids was also carried out. Precoated 0.25 mm thin layer chromatographic plates (Merck Silica Gel D.C. Fertig Platten ohne Fluoreszenz Indicator) were used.

Samples of lipid extracts were spotted as strips on the plates in comparison with a sulfolipid standard. The solvent system used as developer was chloroform/methanol/water (70/30/5 by volume). The plates were exposed to iodine vapors and then sprayed with anisaldehyde reagent to detect the spots (Bolognani, 1970).

Chemical analysis of blood and tissues. The analysis of blood was carried out directly on plasma or serum previously prepared by centrifugation (whenever possible a few hours after the animals were captured). The analyses of the different organs were carried out on TCA extracts. For this purpose, the samples, preserved in TCA at 4°C, were removed and brought to constant weight at 80°C in an oven. The samples were then homogenized at 0°C in 10% TCA (1 ml TCA/ mg dry tissue). After centrifugation at 2500 rpm for 15 min at 0°C, the supernatant was collected. The sediment was brought to constant weight at 80°C and extracted once more with TCA. After a further centrifugation, the supernatant was combined with the former and with the TCA used to preserve the samples.

Plasma or serum and TCA extracts were tested for the following (for reference see Varley, 1967, and Hawk, Oser and Summerson, 1954): Cl^- (by iodometric method), Na^+ (by volumetric method of Weinbach), K^+ (by volumetric method of Jacobs and Hofmann), Ca^{++} (by volumetric method of Kramer and Tisdall), Mg^{++} (by titan yellow method after Neil and Neely), phosphate (by Fiske and Subbarow method), urea (by diacetylmonoxime colorimetric method after Natelson). Blood serum was also tested for protein concentration by the biuret method after Reinhold.

Results

The environmental conditions of the collecting sites are summarized in Table 1. It should be pointed out that the samples of surface water, collected in the open sea near Barra del Colorado, are strongly affected by the river flow.

The results of the histological and histochemical analyses of the rectal glands are illustrated in Figures 1 through 10.

The rectal gland of specimens of *Carcharhinus limbatus* taken from full strength sea water and of *Carcharhinus leucas* taken in brackish water provide histological and histochemical evidence corresponding to the well-known pattern of other marine species (Gerzeli, 1967; De Piceis Polver and Bernocchi, 1970).

Table 1. Analysis of water

		Cl^- (ppm)	Solutes (ppt)
San Carlos (Lake Nicaragua)		16.5	0.033
Barra del Colorado	(surface water)	50.6	0.09
(Caribbean Sea)	(4 m depth)	1.15×10^4	20.8
Corinto (Pacific Ocean)		1.94×10^4	35.0

[1]The fresh-frozen samples, preserved on dry ice, arrived in Italy by air and were examined within eight days of their collection.

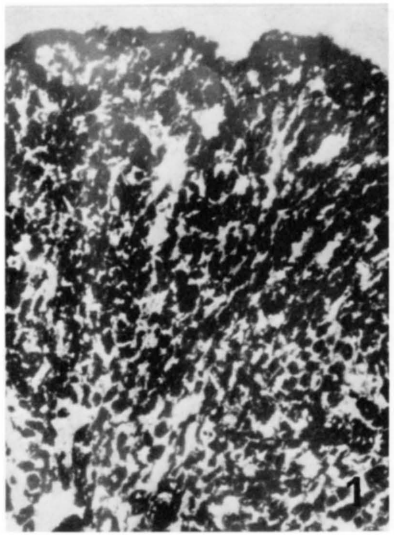

Fig. 1 — *Carcharhinus leucas* (Barra del Colorado – Caribbean Sea); acid hematein method for polar lipids; X 40. The gland is fully differentiated; the reaction of the secretory tubules is highly positive.

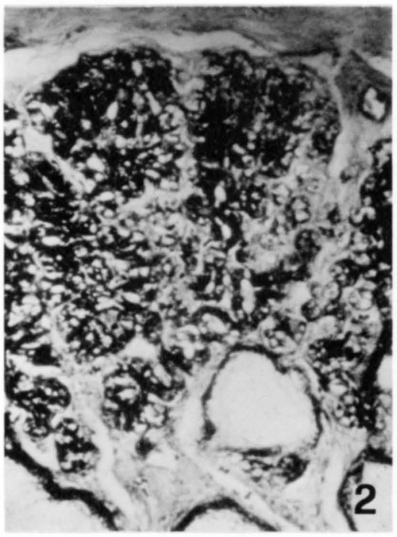

Fig. 2 — *Carcharhinus leucas* (San Carlos – Lake Nicaragua); acid hematein method for polar lipids; X 40. The gland is definitely hypotrophic; nevertheless, the secretory tubules retain the positive reaction.

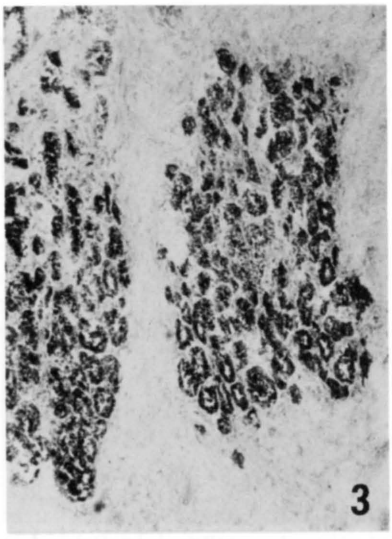

Fig. 3 — *Carcharhinus leucas* (Barra del Colorado – Caribbean Sea); NADH-tetrazolium reductase; X100. The secretory tubules are highly positive; the central excretory duct exhibits a lower degree of positivity.

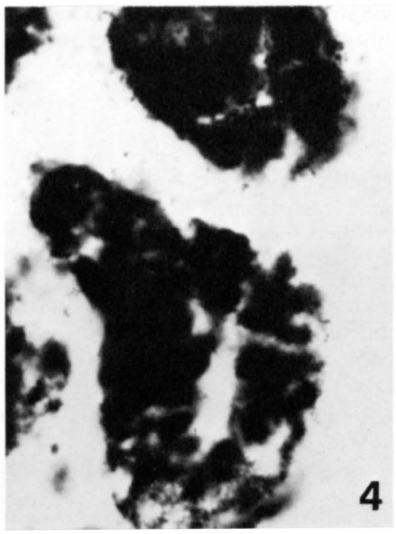

Fig. 4 — *Carcharhinus leucas* (Barra del Colorado – Caribbean Sea); NADH-tetrazolium reductase; X1000. The positive reaction is demonstrated in better detail than in Fig. 3.

The gland is composed of secretory tubules which are normally arranged around the central excretory duct and surrounded by a dense capillary network.

The cells of the secretory tubules are highly positive to the reactions for phospholipids (acid hematein method) and proteins (bromophenol blue method), with a striated granular pattern along the main axis of the cell. The reactions for oxidoreductases are very intense; among them, NADH and NADPH tetrazolium reductases, lactate, succinate and isocitrate dehydrogenases are overwhelming.

Evidence for a positive ATPase reaction is found evenly distributed in the cytoplasm; this reaction is mostly inhibited by ouabain and Ca^{++}. This test indicates the presence of a Na^{+}, K^{+}–activated enzyme.

Cell boundaries are marked by the presence of an amylase–resistant, PAS positive, weakly alcianophilic material.

The glands of the specimens of *Pristis perotteti* and of *Carcharhinus leucas* captured in Lake Nicaragua are reduced in size and weight in comparison with those taken in or near the sea. The connective tissue component is greater and consequently the capsule is thicker; the central excretory duct is enlarged and the tubles around it appear shortened; the capillary network is rather irregular and less abundant.

The glands of the freshwater specimens also have slightly different cytochemical characteristics. Phospholipids, proteins and oxidoreductase reactions exhibit the same fea-

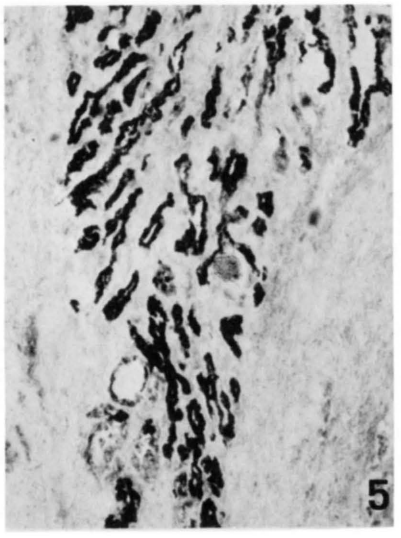

Fig. 5 — *Carcharhinus leucas* (San Carlos – Lake Nicaragua); NADH-tetrazolium reductase; X100. The arrangement of the tubules indicates hypotrophy; nevertheless, single tubules are highly positive.

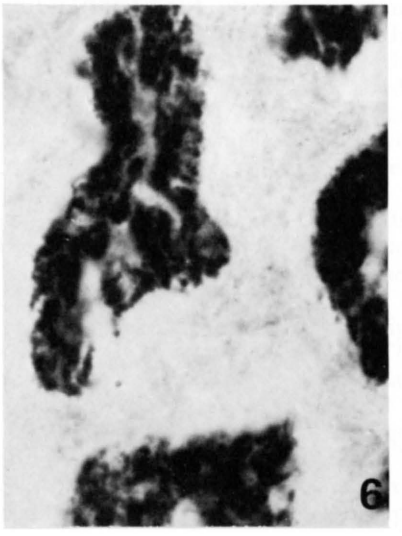

Fig. 6 — *Carcharhinus leucas* (San Carlos – Lake Nicaragua); NADH-tetrazolium reductase; X1000. The positive reaction is demonstrated in better detail.

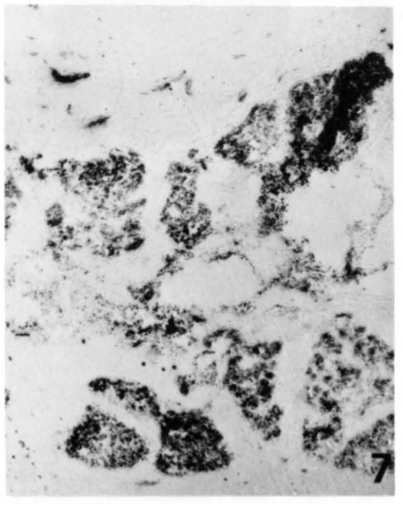

Fig. 7 — *Carcharhinus leucas* (Barra del Colorado — Caribbean Sea); $Na^+-ATPase$; X 40. Different degrees of a positive reaction are demonstrated in the secretory tubules; the blood vessels in the capsule of the gland are highly positive.

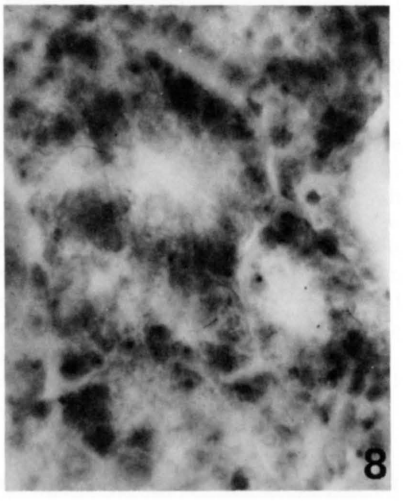

Fig 8 — *Carcharhinus leucas* (Barra del Colorado — Caribbean Sea); $Na^+-ATPase$; X 1000. The distribution of the reaction product is demonstrated at a cytological level.

tures as in the marine or brackishwater specimens. On the contrary, the PAS reaction at the cell boundaries is less appreciable; moreover, the Na^+, K^+-activated ATPase is markedly lowered.

The data concerning the lipid analyses are reported in Table 2. The specimens collected in the lake show lower concentrations of lipids than those captured at the mouth of Río San Juan or in the open sea. Moderate differences were found in phospholipids and cholesterol, whereas very prominent variations were noted in the case of sulfolipids. The quantities vary widely among the specimens captured at the mouth of Río San Juan, suggesting that their recent environmental history was heterogeneous.

Chromatographic separation reveals the lack of substan-

TABLE 2. Lipids in rectal glands of *Pristis perotteti* and *Carcharhinus leucas* (μmoles/gram of dehydrated tissue)

Species	Sample No.	Sulfolipids	Phospholipids	Cholesterol
Pristis perotteti	1	0.39	7.2	2.97
(San Carlos —	4	0.39	9.0	3.60
Lake Nicaragua)				
Carcharhinus leucas	2	0.88	13.6	5.30
(San Carlos —	3	0.85	13.6	5.28
Lake Nicaragua)				
Carcharhinus leucas	5	26.80	23.3	8.60
(Barra del Colorado —	6	6.80	25.8	10.90
Caribbean Sea)	7	12.20	8.6	6.75

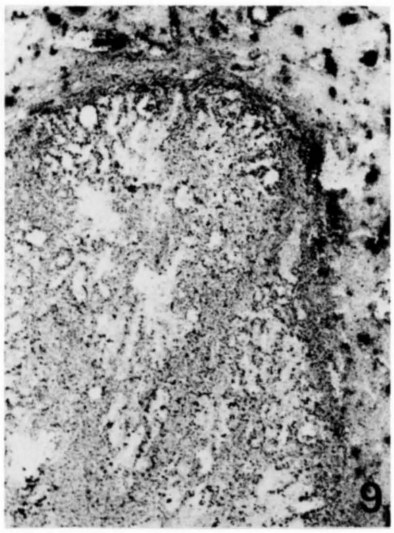

Fig. 9 — *Carcharhinus leucas* (San Carlos — Lake Nicaragua); $Na^+-ATPase$; X 40. No reaction is seen in the secretory tubules; the blood vessels in the capsule are still positive.

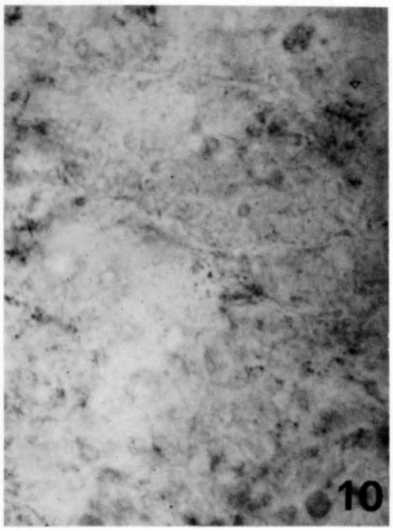

Fig. 10 — *Carcharhinus leucas* (San Carlos — Lake Nicaragua); $Na^+-ATPase$; X 1000. The negative reaction is demonstrated at a cytological level.

tial amounts of sulfolipids (only traces are seen) in the specimens of *Carcharhinus leucas* and *Pristis perotteti* from the lake (Fig. 11). The same figure shows that sulfolipids are evident in the samples of *Carcharhinus leucas* No. 5 and 6 (from Barra del Colorado – Caribbean Sea); these samples, from the same fishing area, have sulfolipids of different polarity (Rf values respectively are 0.45 and 0.49). Among the phospholipids, the following spots are identified: phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine. Other spots belonging to different polar lipids are recognizable, but they are not taken into consideration. Surprisingly, appreciable quantitites of cerebrosides are not indicated in our chromatograms.

Amounts of urea are detectable both in the lipid (chloroform-methanol) extracts and in the total (TCA) extracts. The urea concentrations in serum and in the rectal glands of some specimens are reported in Table 3. The data indicate that the amount of urea that can be extracted by a lipid solvent is relatively constant among the specimens and is lower than that extracted by TCA. On TLC plates it has been observed that urea migrates together with some polar lipids, and it is possible to demonstrate their corresponding spots on the developed chromatographic plates sprayed with anisaldehyde and left to oxidize at room temperature (Fig. 12).

Comprehensive data on the electrolyte, urea and protein pattern of plasma and of certain organs are reported in Tables 4 through 8. By comparing the data from animals living in different environmental media, it is apparent that the most marked changes concern urea; its concentration in the blood of marine *Carcharhinus limbatus* is three times that of freshwater *Pristis perotteti*.

Electrolyte concentrations are also higher in the blood of the marine than the freshwater specimens, but it should be pointed out that the differences are not very great (*Carcharhinus leucas* from the Caribbean Sea and *C. limbatus* from the Pacific Ocean have approximately 15 and 20% more electrolytes respectively than *C. leucas* from Lake Nicaragua). Brackish water specimens are very heterogeneous as indicated by higher standard deviations. On the other hand, attention must be paid to the high electrolyte and total urea concentration in the kidney and rectal gland, compared to the low values observed in the muscle. Furthermore, the electrolyte content of rectal gland progressively increases from freshwater to marine animals, while it remains more nearly constant in the kidney.

Discussion

The glands of specimens taken in the lake show morphological and histological signs of hypotrophy, as noted by Oguri (1964); however, the main histochemical features are preserved in our preparations. In particular, strong activity of several oxidoreductases indicates the continued capability to supply energy for metabolic requirements, even in the

Table 3. Urea in serum and rectal gland of *Pristis perotteti* and *Carcharhinus leucas*

Species	Sample No.	Urea in serum (mM/l)	Urea in rectal glands (mg% dehydrated tissue)		
			TCA extracts (A)	Lipid extracts (B)	B/A %
Pristis perotteti (San Carlos — Lake Nicaragua)	1	108.56	2.06	1.03	50.0
	4	123.21	2.24	1.07	47.7
Carcharhinus leucas (San Carlos — Lake Nicaragua)	2	156.51	2.58	1.07	41.5
	3	131.20	3.04	1.07	35.2
Carcharhinus leucas (Barra del Colorado — Caribbean Sea)	5	185.65	8.07	1.40	17.3
	7	199.80	6.86	1.35	19.7

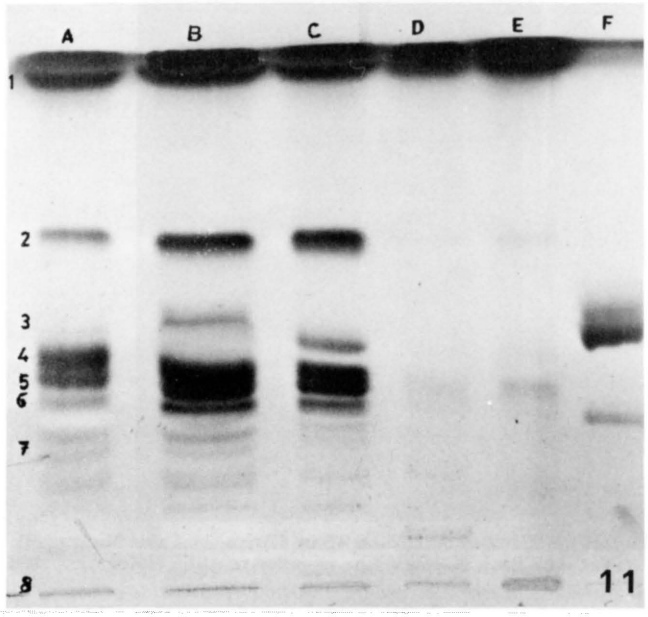

Fig. 11 — T.L.C. separation of lipids in chloroform-methanol extracts of rectal glands (sprayed with anisaldehyde and immediately recorded). A) *Carcharhinus leucas* (San Carlos — Lake Nicaragua), sample 2. Sulfolipids are not detectable; B) *Carcharhinus leucas* (Barra del Colorado — Caribbean Sea), sample 5. Sulfolipids are evident among other polar lipids; C) *Carcharhinus leucas* (Barra del Colorado — Caribbean Sea), sample 6. Sulfolipids with lower Rf are evident; D) Phospholipid standard mixture; E) *Idem*, plus phosphatidylserine and urea; F) Sulfolipid standard mixture.

1) Cholesterol; 2) Phosphatidylethanolamine; 3) Sulfolipids; 4) Phosphatidyl choline; 5) Sphyngomyeline; 6) Phosphatidylinositol; 7) Gangliosides; 8) Phosphatidylserine.

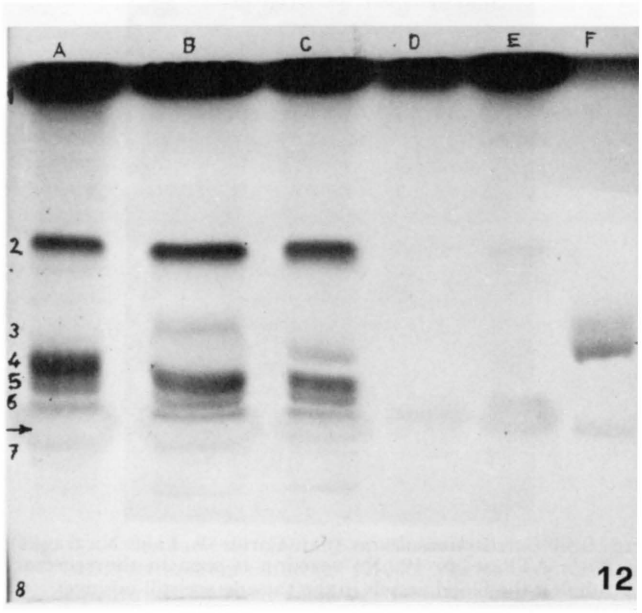

Fig. 12 — T.L.C. separation of lipids in chloroform-methanol extracts of rectal glands (sprayed with anisaldehyde and left to oxidize at room temperature). Letters and numbers as in Fig. 11. Between fractions 6 and 7, an arrow indicates the appearance of a yellow spot attributable to urea.

presence of an anatomical condition which may hinder the diffusion of metabolites, namely, the increased interstitial connective tissue and lower density of the capillary network observed by Oguri (1964), Gerzeli and De Stefano (1968) and Gerzeli *et al.* (1969).

On the other hand, two characteristics are quite different from those of the marine animals: (1) ATPase activity has almost completely disappeared and (2) PAS positive and alcianophilic material (likely due to acidic hexosaminoglycans) at cell boundaries are no longer evident. To sum up, the changes involve cytochemical markers which appear to be directly or circumstantially related to an active ion transport.

It should be mentioned that the specimens from brackish water correspond histochemically to those from the sea, although they appear heterogeneous from various other points of view.

The lipid analysis has been mainly oriented to the evaluation of cholesterol and strongly polar lipids such as phospholipids and sulfolipids. Some differences in cholesterol and phospholipid concentration seem to be related to the environmental situation, but a heterogeneous pattern in the distribution of the data is also evident. Other factors should be considered in this connection, such as species specificity and connective and vascular tissue components in the sample. In any case, cholesterol and phospholipids are not present in considerable amounts.

The sulfolipid content of the rectal gland of marine specimens is very high, as observed by Karlsson *et al.* (1968) in spiny dogfish and by ourselves (unpublished data) in common dogfish. Such a concentration is not usually found except in organs where Na^+, K^+–ATPase activity occurs, related to an active ion transport. This level of sulfolipids is comparable with that of the mammalian kidney (0.1‰), widely studied from the morphological and physiological point of view, as an organ involved in active transport (Mårtensson, 1966). Furthermore, sulfolipids have been reported to be present in other salt secreting glands such as the lachrymal glands of Chelonia (Gerzeli *et al.*, 1971) and the supraorbital glands of birds (Karlsson *et al.*, 1969, 1971; Hansson *et al.*, 1973). These polar lipids should be considered one of the prerequisites needed for osmotic work, together with other histochemical and ultrastructural features. The very low sulfolipid content (up to 50 times less) in the glands of specimens from the lake, where high salt

TABLE 4. Chemical contents of serum, rectal gland and kidney of *Pristis perotteti* taken at San Carlos (Lake Nicaragua)

	Serum (meq/l)	Rectal gland (mg % dehydr. tissue)	Kidney (mg % dehydr. tissue)
Na^+	204.49 ± 14.34	1.51 ± 0.03	1.34 ± 0.02
K^+	3.77 ± 0.43	0.17 ± 0.01	0.23 ± 0.01
Cl^-	235.80 ± 27.69	3.73 ± 0.62	---
Ca^{++}	4.84 ± 0.88	0.10	0.10
Mg^{++}	4.39 ± 0.59	0.11 ± 0.01	0.19 ± 0.03
PO_4	1.01 ± 0.15	0.16 ± 0.01	0.63 ± 0.04
Urea (mM/l)	115.89 ± 7.00	2.15 ± 0.09	1.92 ± 0.11
Total proteins (g% in plasma)	2.06 ± 0.21		

TABLE 8. Chemical contents of serum, rectal gland and kidney of *Carcharhinus limbatus* taken at Corinto (Pacific Ocean)

	Serum (meq/l)	Rectal gland (mg % dehydr. tissue)	Kidney (mg % dehydr. tissue)
Na^+	262.48	4.09	2.93
K^+	4.00	0.89	0.77
Cl^-	246.93	6.84	3.40
Ca^{++}	12.80	0.10	0.10
Mg^{++}	8.71	0.61	0.26
PO_4	5.82	0.14	0.40
Urea (mM/l)	445.39	8.91	4.66
Total proteins (g% in plasma)	3.76		

SUMMARY

Histomorphological, histochemical and biochemical analyses have been carried out on rectal glands of *Pristis perotteti* (Lake Nicaragua), *Carcharhinus limbatus* (Pacific Ocean) and *Carcharhinus leucas* captured in different environments (Lake Nicaragua and Caribbean Sea). The main differences in the freshwater as opposed to marine specimens include: (1) the lower concentration of polar lipids, with very prominent variations in sulfolipids; (2) the strong decrease of ATPase activity demonstrated histochemically.

The electrolyte and urea pattern of blood and of certain organs have also been determined. The urea concentration changes markedly in relation to the environment; the electrolytes are more concentrated in rectal gland and kidney than in muscle.

The biological meaning of the observed changes is discussed. In particular, the maintenance of a very similar electrolyte pattern in the internal milieu under different environmental situations may be related to a very intense ion flux, especially in the rectal gland. In this organ, an increment of sulfolipids from freshwater to marine specimens is related to the enzymatic activities (ATPase, oxidoreductases), since these molecules represent the prerequisites for an active ion transport.

RESUMEN

Se practicaron análisis histomorfológicos, histoquímicos y bioquímicos en la glándula rectal de *Pristis perotteti* (Lago de Nicaragua), *Carcharhinus limbatus* (Océano Pacífico) y *Carcharhinus leucas* capturados en diversos medios (Lago de Nicaragua y el Mar Caribe). La principal diferencia en los ejemplares de agua dulce, comparados con los marinos, incluye: (1) concentraciones menores de lípidos polares, con variaciones prominentes en sulfolípidos, y (2) una fuerte reducción de la actividad de ATPasa, demostrada histoquimicamente.

También se determinó el patrón de electrólitos y urea en la sangre ye en ciertos órganos. La concentración de urea cambia marcadamente en relación con el medio ambiente; los electrólitos están mas concentrados en la glándula rectal y en los riñones que en los músculos.

Se discute el significado biológico de estos cambios. En particular, el mantenimiento de un patrón muy similar de electrólitos en el medio interno, estando en condiciones ambientales diferentes, puede estar relacionado al flujo de iones, muy intenso, especialmente en la glándula rectal. En este órgano, el incremento de sulfolípidos de ejemplares de agua dulce a los marinos, está relacionado a actividades enzimáticas (ATPasa, oxirreductasas), ya que estas moléculas representan prerrequisitos para un transporte activo de iones.

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