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LIPIDS IN THE RATHKE'S GLAND SECRETIONS OF HATCHLING KEMP'S RIDLEY SEA TURTLES (*LEPIDOCHELYS KEMPI*)

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Abstract—1. Lipids in the Rathke's gland secretions of hatchling Kemp's ridley sea turtles (*Lepidochelys kempi*) were examined by thin-layer chromatography and gas chromatography–mass spectrometry.

2. Thin-layer chromatograms indicate bands consistent with sterols, free fatty acids, triglycerides, methyl esters, steryl esters, lysophosphatidylcholine, phosphatidylcholine, and phosphatidylethanolamine.

3. Analyses of secretions by gas chromatography–mass spectrometry indicate cholesterol, cholest-3,5-diene, cholest-4-ene-3-one, undecanal, 2,3-dihydroxypropanal, and C_3–C_{26} free or esterified fatty acids.

4. Bacteria in the Rathke's gland exudates were isolated and identified; these may act upon the holocrine secretory products of these glands.

INTRODUCTION

Rathke's glands are paired exocrine organs embedded in the ventrolateral aspect of the trunk of most aquatic turtles (Waagen, 1972). Fluids are discharged from these glands through duct openings in the axillary, inframarginal, and/or inguinal regions, typically, when turtles are disturbed. It is generally hypothesized that these exudates repel predators (e.g. Ehrenfeld and Ehrenfeld, 1973).

The components of Rathke's gland secretions have been characterized in several species. The loggerhead (*Caretta caretta*) and the Kemp's ridley (*Lepidochelys kempi*) sea turtles secrete high concentrations of macromolecules, including, in each, a 55,000–56,000 mol. wt glycoprotein containing glucosamine (Radhakrishna *et al.*, 1989). The glycoproteins of these two turtles possess a similar amino acid composition and an identical sequence for at least the first 15 amino-terminal residues.

Lipids in the Rathke's gland secretions have been identified by gas chromatography–mass spectrometry (GC–MS) in the North American musk turtle (*Sternotherus odoratus*) (Eisner *et al.*, 1977), the Australian snake-necked turtle (*Chelodina longicollis*) (Eisner *et al.*, 1978), and the loggerhead sea turtle (*Caretta caretta*) (Weldon and Tanner, 1990). Fatty acids are indicated in all species' secretions. An analysis by thin-layer chromatography (TLC) of *C. caretta* secretions indicated the presence of a number of other compound classes (Weldon and Tanner, 1990).

We report here on TLC and GC–MS analyses of lipids in the Rathke's gland secretions of hatchling Kemp's ridley sea turtles (*Lepidochelys kempi*). In addition, we provide information on bacteria present in the Rathke's glands of this species. This is the first indication of microorganisms in these chelonian glands.

MATERIALS AND METHODS

Turtles hatched during July from eggs deposited in Rancho Nuevo, Mexico. They were maintained individually in perforated plastic buckets. The buckets were suspended 14–16 cm deep in 6.1 × 1.8 × 0.6 m raceways containing filtered sea-water from the Gulf of Mexico. Water in the raceways was replaced three times a week. Turtles were fed daily 13–15 g of commercially available pellets (Ralston Purina, Richland, IN, USA).

Hatchling *L. kempi* possess five Rathke's gland orifices along the inframarginal scutes, and one in the inguinal region. The shell of the turtles was wiped with a paper towel to remove algae before secretions were collected. Secretion discharge was elicited by wiping the plastral ridge with a paper towel (Fig. 1) or by applying a probe carrying a mild electric current to the axillary and inguinal areas (Radhakrishna *et al.*, 1989).

Secretions for TLC were collected during May by applying a probe to 15 individuals and drawing exudates through the spout of a tube by suction. The secretions were transferred to a vial, which was transported on ice and stored at −90°C. An aliquot of 3.9 g of secretions was extracted with 16 ml of chloroform:methanol (2:1, v/v) within several hours of collection. The solvent was removed under N_2. The residue was dried *in vacuo*. A total of 6.0 mg of material (1.5% of the total secretion) was recovered.

The residue was redissolved in chloroform:methanol for TLC. Aliquots containing 300 μg of residue were applied to 1.5 cm lanes on a 0.25 mm layer of silica gel 60 G on glass plates. Mixed lipid standards (Nu-Chek-Prep, Elysian, MN, USA for non-polar lipids; Supelco, Bellefonte, PA, USA for polar standards) were developed on adjacent lanes of each plate.

Non-polar lipids were resolved by successive development of plates in hexane, then in toluene, and finally in hexane:diethyl ether:acetic acid (80:20:2, v/v/v). Polar lipids were resolved in chloroform:methanol:water (65:35:5, v/v/v). The TLC bands were visualized by spraying plates with 50% H_2SO_4 and charring the lipids on a hot plate.

Secretions for GC–MS were collected from *L. kempi* during September and January. Fluids collected during

Fig. 1. Rathke's gland secretions (indicated by arrows) discharged onto shell of inverted hatchling *L. kempi* after wiping with paper towel.

September were obtained from 202 individuals by brushing a paper towel against the plastral ridge and drawing the exuded fluids into glass capillary tubes. The tubes were broken off into vials containing dichloromethane. The vials were stored at −70°C and shipped on dry ice.

A 1 μl aliquot of the dichloromethane Rathke's gland extract was treated with distilled ethereal diazomethane and injected on a 30 m 5% phenylsiloxane SE-54 polymer-coated capillary column. The column was connected to an LKB-2091 GC–MS operated at 70 EV, 20 μ amp ionizing current. The column was programmed at 10°C/min and the spectrometer scanned continuously.

Secretions were obtained during January by applying an electrified probe to 22 individuals. Secretions were pooled in a glass vial without solvent. The vial was placed on dry ice and stored at −70°C before analysis.

A 0.5 ml aliquot of Rathke's gland secretion was added to 0.5 ml of deionized H_2O and brought to pH 1. NaCl was added to saturation. The sample was extracted once with 5 ml of chloroform:methanol (2:1, v/v), then twice with 2 ml of chloroform. The combined extracts were dried under a stream of N_2.

A 1 ml aliquot of methanolic HCl was added to the dry sample and heated at 100°C for 1 hr. After cooling, 0.5 ml of deionized H_2O was added and the solution was extracted twice with 1 ml of hexane. The organic layer was dried under N_2 and dissolved in 100 μl of hexane prior to analysis.

Three drops of 6N NaOH, then 0.5 ml of ethoxyamine (0.1 g/ml in pyridine), were added to another 0.5 ml sample of Rathke's gland secretion for quantitative analysis. The sample was heated at 60°C for 30 min. After cooling, 30 drops of HCl were added and the solution was saturated with NaCl. Five milliliters of chlorobenzoic acid (1 mg/ml) were added to provide an internal standard. The solution was then extracted with 3 ml of ethyl acetate. The organic phase was dried under N_2 and derivatized with 200 ml BSTFA:pyridine (3:1, v/v) for 30 min at 60°C to generate trimethylsilyl derivatives.

The hexane extract was analyzed on a 15 m SE-54 fused silica capillary column programmed at 100–285°C (8°C/min). The silylated materials for quantitative analysis were analyzed on a 60 m DB-1 fused silica capillary column. Both columns were interfaced with a Finnigan MAT INCOS 50 mass spectrometer. All compounds were characterized by comparison of their mass spectra with the literature database.

RESULTS AND DISCUSSION

Thin-layer chromatograms of the Rathke's gland secretions of *L. kempi* display bands consistent with sterols, free fatty acids, triglycerides, methyl esters, steryl esters, lysophosphatidylcholine, phosphatidylcholine, and phosphatidylethanolamine (Fig. 2). A band (R = 0.13) in the non-polar chromatogram corresponds to a component less polar than cholesterol. These chromatograms are similar to those obtained with extracts of the secretions from hatchling loggerhead turtles (*C. caretta*) (Weldon and Tanner, 1990).

Mass spectral analysis of esterified, hexane-soluble components of *L. kempi* secretions indicates cholesterol, cholesta-3,5-diene, and the following fatty acids: 14:0, 15:0, 16:0, 16:1, 17:0, 18:0, 18:1, 18:2, 19:0, 20:0, 20:1, 20:2, 20:3, 20:4, 22:0, 22:1, 22:4, 22:5, 23:0, 24:0, 24:1, 24:2, 26:4 (Fig. 3). A similar analysis of *C. caretta* secretions indicated cholesterol, cholest-3,5-diene, and a comparable array of fatty acids, including a 22:3 compound not observed in *L. kempi* (Weldon and Tanner, 1990). The C_{17} acid present in trace amounts in *L. kempi* was not detected in *C. caretta*.

Cholesterol, cholest-4-ene-3-one, undecanal, and phenylacetic, tetradecanoic, hexadecanoic, and octadecanoic acids were indicated by GC–MS in the diazomethane-treated extract of *L. kempi* secretions.

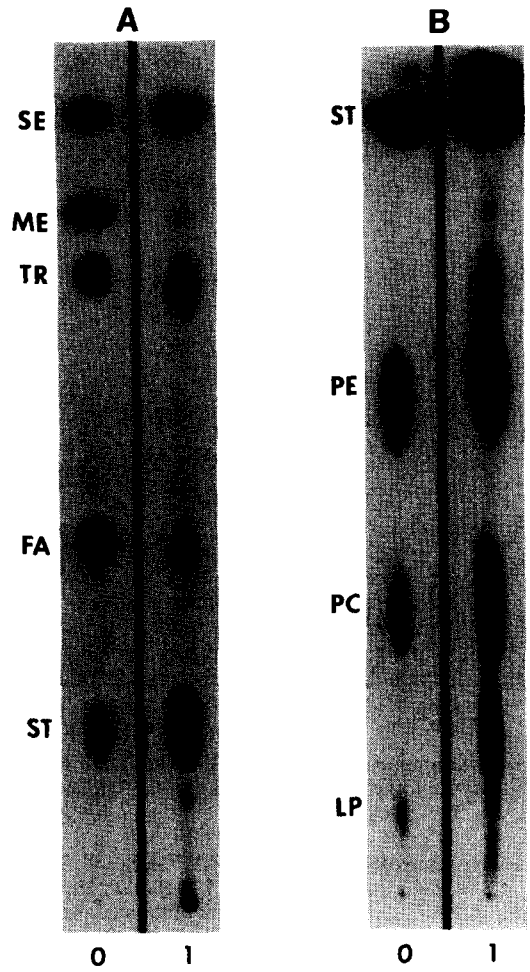

Fig. 2. (A) Thin-layer chromatogram of non-polar Rathke's gland lipids from *L. kempi*. Lane 0 contains lipid standards: ST (sterol; cholesterol), FA (fatty acid; oleic acid), TR (triglyceride; triolein), ME (methyl ester; methyl oleate), and SE (steryl ester; cholesteryl oleate). Lane 1 contains Rathke's gland secretion extract. (B) Thin-layer chromatogram of polar Rathke's gland lipids from *L. kempi*. Lane 0 contains lipid standards: LP (lysophosphatidylcholine), PC (phosphatidylcholine), PE (phosphatidylethanolamine), and ST sterol; cholesterol). Lane 1 contains Rathke's gland secretion extract.

Quantitative GC–MS analysis of the silylated and *o*-ethyloxine derivatized components indicated a large quantity of 2-hydroxypropanoic (lactic) acid (2437 μg/ml) in the concentrate. Other compounds in the extract were 2-oxo-4-methylpentanoic (325 μg/ml), 2-oxo-butanoic (184 μg/ml), 2-hydroxybutanoic (128 μg/ml), 2-oxo-propanoic (43 μg/ml), hydroxyacetic (41 μg/ml), methyl succinate (35 μg/ml), 2-oxo-3-methylbutanoic (20 μg/ml), 2,3-dihydroxypropanoic (15 μg/ml), butanedioic (15 μg/ml), 2-oxo, 3-methylpentanoic (11 μg/ml), 3-hydroxybutanoic (5 μg/ml), 2-oxo, 3-methylpentanoic (5 μg/ml), and 2-methylpropanedioic acids (4 μg/ml). 2,3-Dihydroxypropanal, hexadecanoic, and butenedioic acids were detected, but their concentrations in the extract were not determined.

Histological studies of the Rathke's glands of a variety of turtles indicate that they exhibit holocrine secretion (Ehrenfeld and Ehrenfeld, 1973; Solomon, 1984). Weldon and Tanner (1990) suggested that the fluids produced by these organs may be subjected to microbial action—a common process in the genesis of veretebrate exocrine skin products (Albone, 1984)—but microorganisms have not previously been demonstrated in these glands.

We isolated several bacteria from the Rathke's gland secretions and from the plastron of eight 6-month-old *L. kempi*, obtained and maintained as described for the secretion donors. Isolates from the plastron were obtained by streaking the shell surface with a swab immediately after the turtles were removed from the water; those in the secretions were obtained after wiping the shell surface with a towel treated with hydrogen peroxide, rinsing it with sterilized water, and stimulating turtles with an electrified probe to elicit secretion discharge. The following species were indicated in the exudates and (*) on the plastron: *Aeromonas salmonicida*, *Bacillus cereus*,* *Micrococcus luteus*, *Salmonella enteritidis*,* *Staphylococcus aureus*,* *S. epidermidis*,* *Streptococcus* sp.,* and *Vibrio fluvialis*.* Several bacteria from the plastron, *Aeromonas punctata caviae*, *Pseudomonas aeruginosa*, *P. putrifaciens*, and *Vibrio alginolyticus*, were not indicated in the glandular exudates. Whether the bacteria found in the secretions normally reside in the glands or are transient organisms

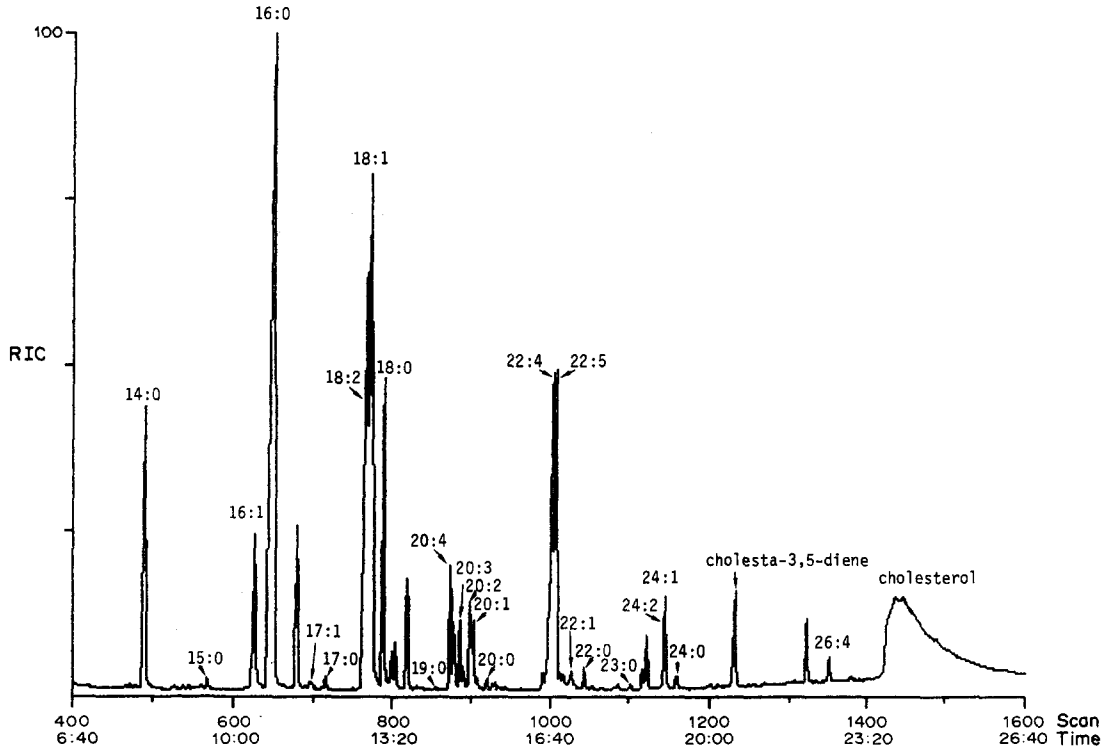

Fig. 3. Reconstructed ion chromatogram of *L. kempi* Rathke's gland secretion extract, indicating cholesterol, cholesta-3,5-diene, and C_{14}–C_{26} fatty acids. Retention time in min.

needs to be determined, as does their contribution to Rathke's gland products.

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