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12. We have found that a higher alkyl analog of uridine, 5-hexyluridine, undergoes a similar reaction to yield uridine, on irradiation at 254 nm, in a yield of 50 to 60 percent (E. Sztumpf-Kulikowska and D. Shugar, in preparation). Furthermore, one can obtain the 1 : 1 photoadducts of thymidine with ethylene and propylene. These exhibit spectral and photochemical properties similar to those of the 1 : 1 adduct of uridine with propylene.

13. We thank Dr. B. Fiszer for the samples of 5-propyl- and 5-isopropyluracil used in the preparation of the corresponding nucleosides; Dr. S. Braunstein of the National Research Council of Canada, Ottawa, for the NMR spectra; and the World Health Organization, The Wellcome Trust, and the Agricultural Research Service, U.S. Department of Agriculture, for partial support of this and related investigations.

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Sea Snakes: An Unusual Salt Gland under the Tongue

Abstract. *The posterior sublingual gland of sea snakes is a salt gland. It secretes a fluid surpassing seawater in sodium chloride concentration. The gland lies on the ventrolateral surfaces of the tongue sheath and empties through multiple ducts into the sheath. Fluid is expelled from the sheath when the tongue is extended. For freshly captured* Pelamis, *the plasma concentrations of sodium, chloride, and potassium were 210, 167, and 8 millimoles per liter, respectively. Injections of sodium chloride led to a rise in its concentration in the plasma and to an increase in the rate and concentration of fluid secreted by the sublingual gland. The ultrastructure of this gland is similar to that of other reptilian salt glands. However, the gland is not homologous with any other salt gland. The sublingual gland in* Pelamis *is larger than that in* Laticauda, *and the rate of electrolyte excretion from the larger gland is greater.*

Of all reptiles, only certain poisonous sea snakes have completely broken their ties with the land. Snakes such as *Pelamis*, the pelagic yellow-bellied sea snake, feed at the sea surface and bear their young at sea. These snakes are highly specialized for a marine existence, but little is known about their physiological adaptations to marine conditions.

The sea is a highly concentrated salt solution (about 470 m*M* Na, 548 m*M* Cl) with an osmotic pressure of about 1 osmolal. Most marine vertebrates, including the reptiles, maintain a body fluid concentration about one-third this amount, and they encounter problems in conserving water and excreting excess salts. The reptilian kidney, unlike kidneys of mammals and birds, cannot produce urine more concentrated than the plasma, so accessory organs known as salt glands have developed in marine forms to handle excretion of excess salts. In sea turtles and in the estuarine diamondback terrapin, the salt gland is located behind the eye and secretes "tears" into the orbit (*1*). In the marine iguana the salt gland is nasal and the secretions are sneezed out the nares (*1*). No one had previously found a salt gland in sea snakes, but Dunson had collected fluid more concentrated than seawater from the mouths of *Pelamis* and *Laticauda* (*2*).

At first the sea snake salt gland was thought to be the newly discovered *Laticauda* "natrial" gland (*3*), but we now know this is not the case because *Pelamis* does not have a natrial gland (*4*). Furthermore, the sea snake salt gland is sublingual, not nasal or orbital. Its ultrastructure generally conforms to that of other tissues specialized for electrolyte transport. The gland was cannulated and uncontaminated fluid was collected for analysis. Secretion is probably controlled by receptors for plasma salt concentration or osmotic pressure. Under natural conditions *Pelamis* maintains higher plasma salt concentrations than terrestrial reptiles do. During periods of dehydration, considerably higher plasma salt concentrations can be tolerated. Differences between sea snakes in the size, ultrastructure, and capacity for salt excretion of the salt gland seem to be related to the degree of adaptation for marine life.

Gross dissections of the lower jaw of *Pelamis* revealed an enlarged posterior sublingual gland similar in relative weight to that of known salt glands. In seven *Pelamis* averaging 63 g in body weight, the sublingual gland was 0.043 percent of the body weight compared with the heart which was 0.130 percent of the body weight. Salt glands in sea turtles, the diamondback terrapin, and the marine iguana are about 0.05 to 0.06 percent of the body weight (*5*).

The head of a small *Pelamis* was serially sectioned for examination of the anatomy of the sublingual gland, and particularly of the location of the ducts draining the gland. The head was fixed in formalin, partially skinned, and decalcified in sodium citrate (20 percent) and formic acid (2 percent). It was then dehydrated, embedded, and sectioned at 7 μm. The sections were stained with Masson's trichrome stain. The single large posterior sublingual gland covered parts of the tongue sheath almost completely. The smaller paired anterior sublingual glands lay immediately anterior to the opening of the tongue sheath. Sublabial or infralabial glands were present as were supralabial glands. A large gland, probably the Harderian, lay medial to the eye. The large venom gland was prominent posterior to the orbit (Fig. 1A). Except at the most posterior end, ducts left the posterior sublingual gland at all levels and coursed to the tongue sheath. A typical section near the middle of the gland shows three ducts joining the sheath (Fig. 1B). The sheath and the lower portion of the ducts were lined with keratinized stratified squamous epithelium. The posterior sublingual gland consisted of tubules arranged generally in a radial fashion around the central tongue sheath. At the extreme posterior end of the gland where there were no ducts, the tubules turned and ran longitudinally along the sheath. There was no indication that any other gland emptied into the tongue sheath. The anterior sublinguals emptied into the mouth by two ducts just anterior to the tongue sheath opening. Taub (*6*) reported that a rattlesnake had paired anterior sublingual glands and a single posterior sublingual gland, but in some snakes the glands were not found. In an amphibious sea snake examined in this study (*Laticauda*), the posterior sublingual gland was present, but much smaller than the gland in the pelagic *Pelamis*.

Table 1. Plasma concentrations of sea snakes (*Pelamis*) from two localities in Costa Rica. Values given are means with the ranges in parentheses. The last line gives pooled data.

Locality	Number	Concentration (mmole/liter)			Hematocrit (%)
		Na	Cl	K	
Gulf of Papagayo	27	210.5 (200–230)	171.1 (150–205)	8.1 (7–12)	39.4 (26–52)
Gulf of Dulce	24	209.8 (200–220)	161.9 (147–175)	8.2 (7–10)	35.8 (15–68)
	51	210.2	166.8	8.1	37.5

Since salt glands have a characteristic ultrastructure, we examined sections of posterior sublingual glands from *Pelamis* and *Laticauda* with the electron microscope. The tissues were fixed in glutaraldehyde, treated with osmium tetroxide, dehydrated, and embedded in Spurr resin (*7*). Thin sections stained with lead citrate were examined with a Hitachi HU 11-C-1 microscope. Although our findings are preliminary, it is clear that the posterior sublingual gland bears a striking resemblance to other reptilian salt glands (*1*). The gland consists of a single cell type which contains numerous mitochondria and little rough endoplasmic reticulum (Fig. 2). Adjacent apical cell surfaces seem to be joined by zonulae adhaerens, not zonulae occludens. This is similar to the elasmobranch rectal gland (*8*), but contrasts with the lizard nasal gland in which zonulae occludens are present (*9*). The apparent absence of tight junctions implies a continuity between the interstitial space and the lumen. Numerous villous extensions of the cell surface interdigitate with those of other cells, and there are extensive intercellular spaces. The *Laticauda* gland contains more rough endoplasmic reticulum and a few secretory granules, indications that it is not as highly specialized for salt secretion as the *Pelamis* gland. In contrast, cells of the retroocular (Harderian) gland of *Laticauda* had few mitochondria, there was extensive rough endoplasmic reticulum, and electron-opaque secretory granules were abundant. Thus the ultrastructural evidence supports the other anatomical data indicating that the posterior sublingual gland is a salt gland.

Cannulation of the ducts of the posterior sublingual gland was the only way to conclusively demonstrate that the gland was involved in salt secretion. Since only the many small ducts of the posterior sublingual gland empty into the much larger tongue sheath, the secretion could be collected with minimum contamination directly from the sheath. A technique of cannulation was devised to minimize damage to the gland and its associated neural and circulatory network. A Silastic cannula was inserted into the tongue sheath through the opening in the mouth. The cannula was held in place by sutures through the oral tissue and a ridge of Silastic cement surrounding the cannula tube. The tongue was left intact although its movement was restricted by the cannula. The end of the cannula in the tongue sheath was fenestrated to minimize physical blockage of the ducts as they entered the sheath. This operation was successfully performed on two *L. semifasciata* and two *Pelamis*. Fluid collected from the cannula at about 25°C was analyzed for Na and K by flame photometry (Coleman model 21) and for Cl with an Aminco Cl titrator. In one *Laticauda* (380 g) the NaCl concentration of the cannula fluid rose from about 250 to about 540 mmole/liter 2 hours after subcutaneous injection of 2 mmole of NaCl per 100 g. The K concentration rose from 4 to 6.5 mmole/liter to 8 to 10 mmole/liter. Over the next 37 hours Na, Cl, and K concentrations gradually decreased to the values present before injection of the salt load. In the second *Laticauda* (281 g) the Na concentration of the cannula fluid averaged 330 mmole/liter (range 297 to 393 mmole/liter) over a 10-hour period after injection of 2 mmole of NaCl per 100 g. In both *Pelamis* (114 and 128 g), injection of NaCl led to secretion of a highly concentrated fluid from the cannula (Fig. 3A). Injection of NaCl at 0.5 hour was followed about 1.5 hours later by a sudden increase (300 mmole/liter) in the NaCl concentration of the cannula fluid. The flow rate also went up at this time. Although continuous measurements are not available, flow rates as high as 200 μl/hour were measured at this time, but this rate usually declined within a few hours. The high NaCl concentration was maintained for about 20 hours; then it began to decline to the original value near 300 mmole/liter. The K concentration generally followed the relative changes in NaCl concentration at a much lower level. In the second *Pelamis* two NaCl loads were injected (1.0 and 0.5 mmole/100 g). Injection of the first load led to secretion of 500 m*M* Na. After the second injection the concentration of the cannula fluid rose from about 500 m*M* Na to about 625 m*M* Na. A similar effect on the K concentration was noted, although the maxi-

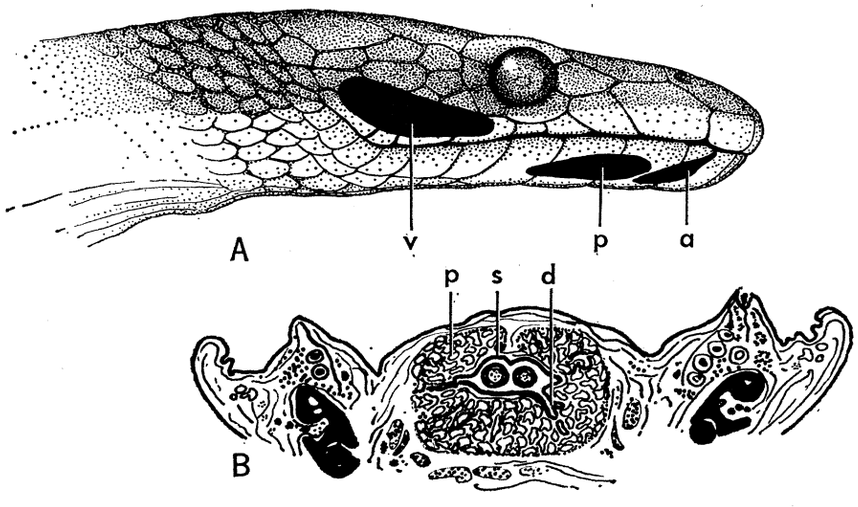

Fig. 1. (A) Side view of the head of the yellow-bellied sea snake *Pelamis platurus* showing the relative positions of the venom (*v*), posterior sublingual (*p*), and anterior sublingual (*a*) glands. The Harderian gland is medial to the eye. (B) A cross section through the lower jaw of *Pelamis* showing the large posterior sublingual gland (*p*) which almost completely surrounds the tongue sheath (*s*). Portions of three ducts (*d*) are shown entering the sheath.

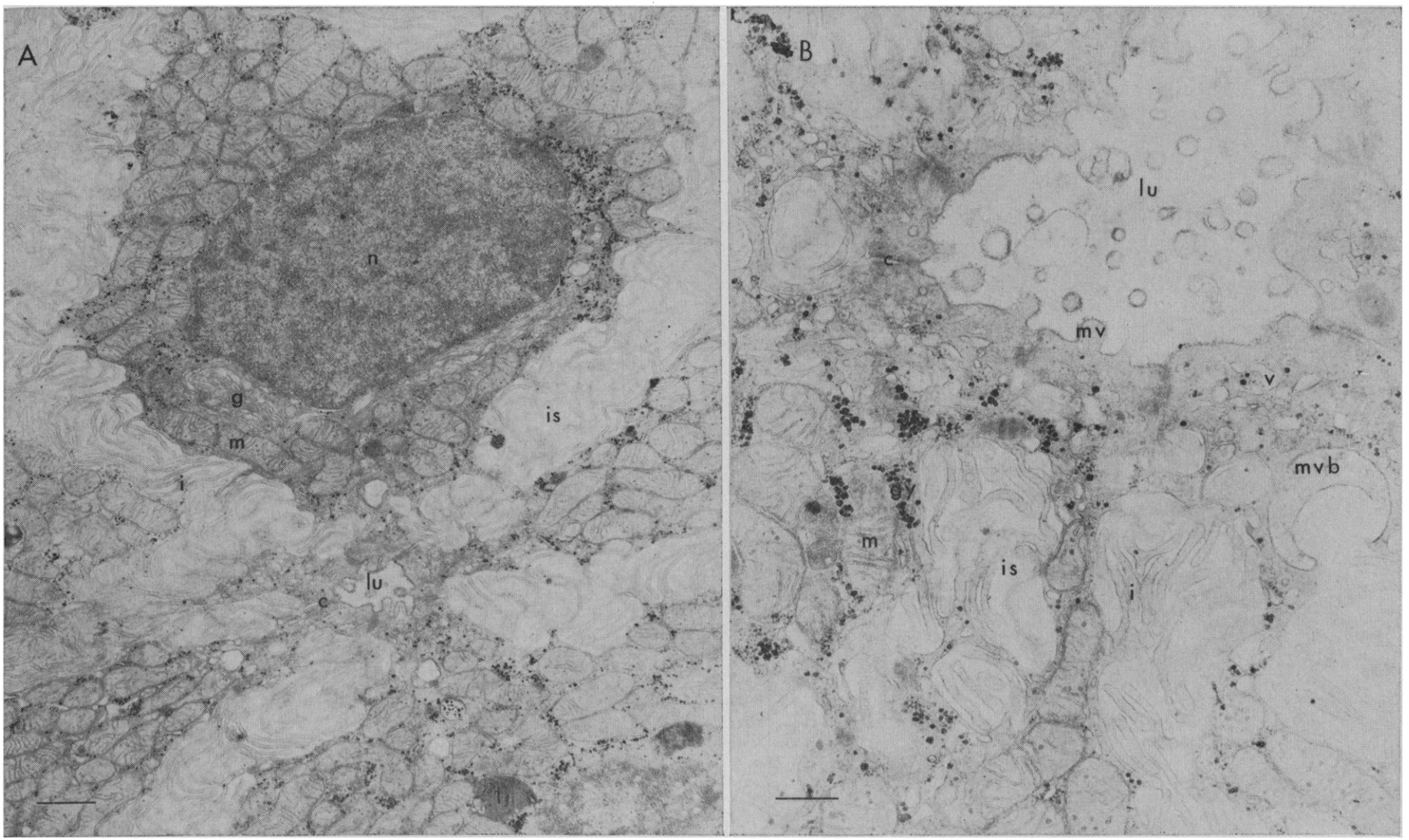

Fig. 2. Ultrastructure of the posterior sublingual salt gland of *Pelamis*. (A) A cross section of a tubule showing five cells around a central lumen. Scale, 1 μm. (B) The apical surface of the secretory cells and the adjacent lumen. Scale, 0.5 μm. Abbreviations: *n*, nucleus; *m*, mito chondrion; *g*, golgi; *c*, junctional; complexes; *gy*, glycogen; *mv*, microvillus; *mvb*, multivesicular body; *v*, vesicle; *is*, interstitial space; *i*, intercellular interdigitations; *l*, lysosome; and *lu*, lumen.

mum value after the second injection was only 20 mmole/liter.

At a flow rate of sublingual fluid of 200 μl/hour and a concentration of 650 m*M* NaCl, *Pelamis* would excrete only about 115 μmole of NaCl per 100 g per hour. A maximum rate of excretion of 218 μmole of Na per 100 g per hour was measured by sampling bath water in which a snake with a catheterized cloaca was placed (*1, 2*). The cannulation procedure apparently decreases the normal flow rate by partially blocking the entrance of the gland ducts into the tongue sheath and by preventing normal extrusion of the tongue which may have a "milking" action on fluid flow. The maximum secretory rate measured in *Laticauda* by the bath technique was 73 μmole of Na per 100 g per hour. Of the marine reptiles, only the marine iguana (255 μmole of Na per 100 g per hour) has a higher capacity than *Pelamis* for excretion of electrolytes through the salt gland (*1, 2*). A marine bird such as the cormorant has only a slightly higher secretory rate (383 μmole per 100 g per hour) (*2*). The salt glands of various gulls secrete at rates many times higher (1160 to 1760 μmole per 100 g per hour) (*2*).

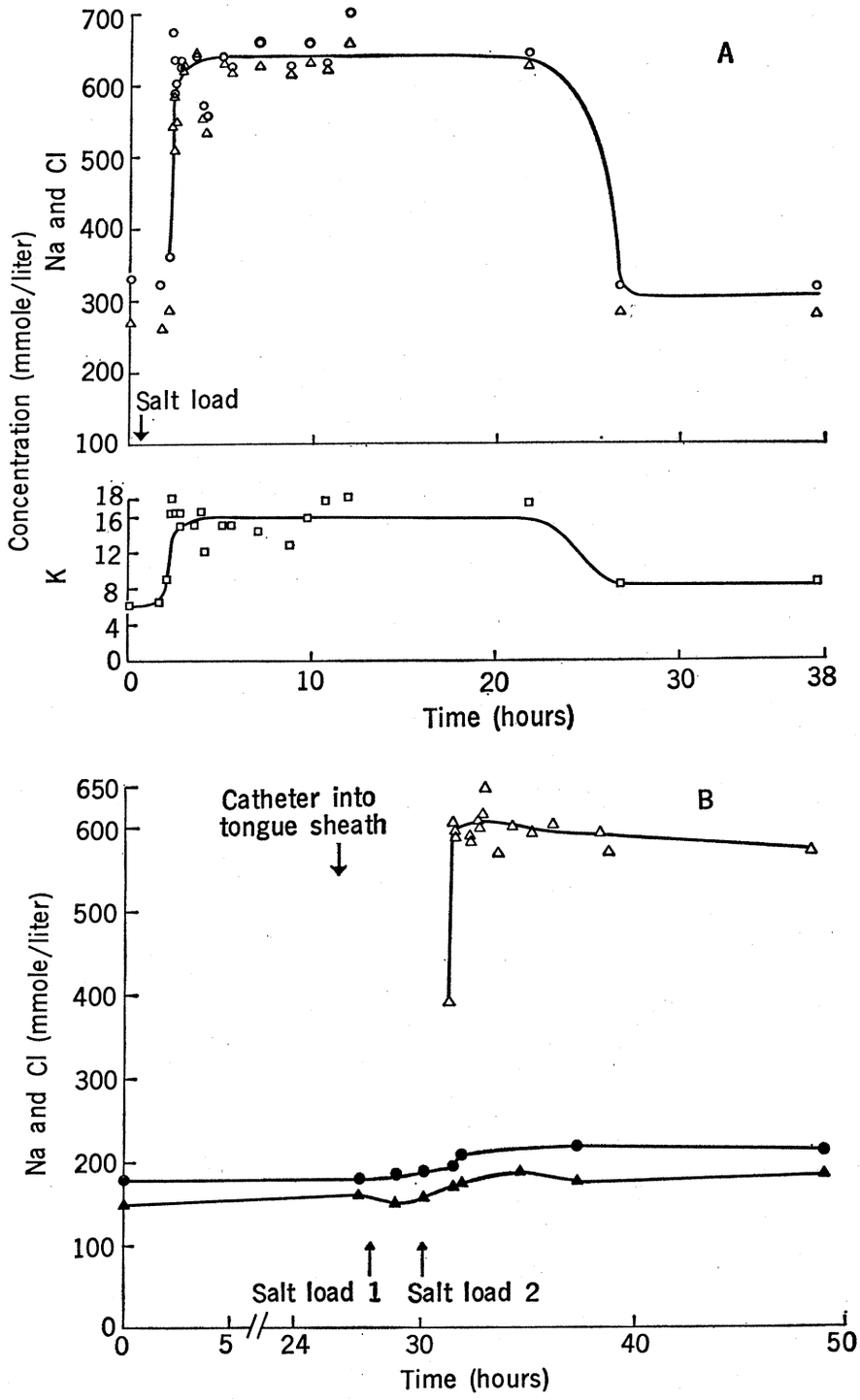

Fig. 3. (A) The effect of subcutaneous injection of a NaCl load (2 mmole/100 g) on the electrolyte concentration of fluid secreted by the posterior sublingual gland of a sea snake (*Pelamis*). Symbols: Na, open circles; Cl, open triangles; K, open squares. (B) The relation between plasma NaCl concentration and the time of initiation of secretion of the posterior sublingual salt gland in *Pelamis*. Salt load 1 was 0.5 mmole of NaCl per 100 g; salt load 2 was 1.5 mmole of NaCl per 100 g. The catheter was inserted into the external jugular vein at approximately time 0. Symbols: tongue sheath fluid Cl concentration, open triangles; plasma Cl concentration, solid triangles; plasma Na concentration, solid circles.

The cannulation experiments showed that the posterior sublingual gland of sea snakes is a salt gland and that injections of NaCl can initiate secretion. It seemed likely that plasma Na concentration would change after salt injections and affect the activity of the salt gland. Plasma concentrations of two groups of freshly captured *Pelamis* were measured (Table 1). Under natural conditions the snakes as a group maintained relatively constant plasma salt concentrations. The plasma Na concentration (210 mmole/liter) remained less than half that of seawater (about 470 mmole/liter). This amount is higher than that found in freshwater and terrestrial vertebrates in which Na concentration is usually less than about 150 mmole/liter (*10*). When the snakes are not fed fish, they lose their major source of water and plasma Na concentrations may increase to 255 or even 307 mmole/liter (*2*). The tolerance of *Pelamis* to dehydration must be an important adaptation for life at sea. The mean hematocrit values of the two groups of *Pelamis* (Table 1) were about the same, but the variability was extreme. We are uncertain what the meaning of this variation is. No hemolysis was observed. Other reptiles tend to have mean hematocrits lower than 35 percent, but there is considerable variation between species and with changing environmental conditions (*10*).

Plasma samples were obtained from two *Pelamis* at the same time that samples of fluid from the posterior sublingual gland were collected from the tongue sheath cannula. The external

jugular vein was catheterized, plasma samples were taken during a control period, and then salt loads were injected subcutaneously. In these two snakes the control plasma Na and Cl concentrations were lower than the mean values reported in Table 1 and the first salt load failed to stimulate salt gland secretion (Fig. 3B). A second salt load stimulated secretion of a highly saline fluid within 1.5 hours. Plasma Na and Cl concentrations increased to values above those in Table 1, and at some critical point near 200 m*M* Na and 170 m*M* Cl salt gland secretion was initiated. Thus an increase in plasma NaCl concentration (and osmotic pressure) leads to increased activity of the salt gland. This response of the gland to differing amounts of salt in the plasma suggests the presence of receptors for salt concentration or osmoreceptors.

There are approximately 50 species of sea snakes divided into two major subfamilies, the Laticaudinae and Hydrophiinae. It will be interesting to compare the structure and physiology of the posterior sublingual glands of species which differ in feeding habits and in the degree of adaptation to the marine habitat. This is especially true of the three species which have secondarily colonized freshwater lakes in the Philippine and Solomon islands and those which feed on fish eggs or invertebrates (prawns and squid) rather than fish.

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Inhibitors of DNA Polymerases of Murine Leukemia Viruses: Activity of Ethidium Bromide

Abstract. *Ethidium bromide, compared on a molar basis, was a more effective inhibitor of the DNA polymerases of the Rauscher and Moloney murine leukemia viruses than either 4-*N*-demethylrifampicin or 4-*N*-benzyldemethylrifampicin. Daunomycin inhibited the polymerases weakly, and chromomycin A_3 inhibited almost not at all. 4-*N*-Benzyldemethylrifampicin was a more active inhibitor than the 4-*N*-demethyl congener.*

The RNA-containing murine and avian oncogenic viruses have both RNA-dependent and DNA-dependent DNA polymerase activities (*1*). The RNA-dependent DNA polymerase activity has now been discovered in visna (*2, 3*) and "foamy" viruses (*3*), and in leukemic cells from patients with acute lymphoblastic leukemia (*4*). Investigation of inhibitors of the nucleic acid–synthesizing enzymes of the RNA-containing tumor viruses may be useful not only in the analysis of the mechanisms of replication of these viruses, but also may provide new drugs for and tests of their efficacy in the treatment of leukemia and other cancers. I now report the effect of several drugs on the DNA polymerase activity of two murine leukemia viruses. The drugs used were 4-*N*-demethylrifampicin (AF/AP; molecular weight, 808.94), 4-*N*-benzyldemethylrifampicin (AF/ABP; molecular weight, 899.07), daunomycin hydrochloride (molecular weight, 560), chromomycin A_3 (molecular weight, 1183; Mann Research), and ethidium bromide (molecular weight, 394; Calbiochem). The rifampicin derivatives are effective inhibitors of the DNA polymerase of the Moloney murine sarcoma virus [MSV(M)] (*5*). Daunomycin, an antibiotic of the anthracycline group, binds to DNA by intercalation (*6*) and is of interest because it is used in the treatment of acute leukemias. Chromomycin A_3 is another antibiotic that binds to DNA but not by intercalation (*6*). The trypanocide ethidium bromide binds to DNA by intercalation, and its effects on the uncoiling of the DNA double helix have been studied (*6*).

The enzyme assay was a modification of that reported by other workers (*1*). The standard enzyme assay consisted of 50 m*M* tris-HCl (*p*H 8.3), 5 m*M* $MgCl_2$, 40 m*M* KCl, 1 m*M* deoxyadenosine triphosphate (dATP), deoxycytidine triphosphate, deoxyguanosine triphosphate (Calbiochem), 20 m*M* dithiothreitol (Sigma), and 2.5 μc [^{3}H]TTP (thymidine triphosphate; New England Nuclear) in 0.1 ml (final volume) of reaction mixture. Portions (containing 20 μg of viral protein) of stock solutions of either the Rauscher [MLV(R)] or Moloney leukemia virus [MLV(M)] (both originally from Electro-Nucleonics Laboratories, and repurified by density gradient centrifugation) were added to the reaction mixture. When included, the nonionic detergent Nonidet P-40 (Shell Chemical) was added to a concentration of 0.01 percent. The mixture was incubated for 120 minutes at 39°C, and the acid-insoluble, alkali stable, macromolecular product was then isolated and counted (*1*). The drugs were added to the final concentrations indicated at the start of incubation. The stock solution of AF/ABP contained 1.25 mg/ml in aqueous ethanol (1 : 1, by volume). The stock solutions of drugs were stored in the dark at −15°C. The enzyme reaction was inhibited either by prior incubation with purified pancreatic ribonuclease or the omission of dATP from the incubation mixture, and the kinetics of [^{3}H]TMP (thymidine monophosphate) incorporation were in agreement with those described (*1*). All experiments were performed in triplicate with the same virus stocks and labeled nucleotide of the same specific activity.

Ethidium bromide was far more effective in inhibiting the DNA polymerase of MLV(R) than either daunomycin or chromomycin A_3 (Fig. 1). Inhibition of the reaction was still complete at a concentration of 20 μg per milliliter of reaction mixture of ethidium bromide (5.1×10^{-8} mole/ml); at 2.50 μg/ml (0.64×10^{-8} mole/ml) 70 percent of the reaction was inhibited. Daunomycin, which, like ethidium bromide, binds to DNA by intercalation, was still more effective than the nonintercalating DNA binder chromomycin A_3. Even at a concentration of 500 μg per milliliter of reaction mixture, chromomycin A_3 showed only 28 percent inhibition of the reaction, while daunomycin inhibited 75 percent of the reaction.

The rifampicin antibiotics inhibit bacterial and mitochondrial RNA polymerases (*7*), inhibit replication of pox and adenoviruses (*8*), block focus formation