a fluid aspirate for protein and by post-perfusion dissection. If the needles were not properly placed or the effluent perfusate was bloody, the experiment was discarded. The plasma osmolality of each fish was measured and an artificial CSF solution (perfusion fluid) of equal osmolality (± 5 mOsm) was prepared. One of the following marker molecules was added to the perfusion fluid: blue dextran (molecular weight = 2×10^6), ^{131}I-serum albumin or ^{3}H-inulin. Perfusion fluid was delivered to the ventricular system by a constant speed infusion pump. The concentration of marker material in the inflow and outflow perfusate was measured by spectrophotometry (blue dextran) or isotope counting. In this experimental situation the rate of CSF production is determined by the amount of dilution of the perfusion fluid which occurs by CSF secretion between the inflow and outflow sites. A standard equation, derived by Heisey *et al.* (Am. J. Physiol. 203: 775-781, 1962), is employed to calculate the production rate.

The CSF formation results (average ± S.D. plus number of animals) obtained with the three markers used are: blue dextran = 1.4 ± 0.7 μl/min., N = 3; ^{131}I-RISA = 1.5 ± 0.7 μl/min., N = 4; ^{3}H-inulin = 2.2 μl/min., N = 1. All three markers combined = 1.6 ± 0.6 μl/min, N = 8.

The rate of CSF secretion reported in this study is approximately one-half that reported by Oppelt *et al.* for the dogfish (Comp. Biochem. Physiol. 12: 171-177, 1964). There are several possible explanations for the discrepancy. In seven out of the eight experiments presented here larger, less diffusible markers were employed, thus smaller but more accurate rates would be expected from these data. Oppelt *et al.* used curare to immobilize their animals whereas pentobarbital was used in the present study. In connection with this reports suggesting that CSF production is partially reduced by anesthetic agents (J. Physiol. 199: 397-425, 1968) have appeared in the last several years. Although not explicitly mentioned by Oppelt *et al.*, the experimental conditions-temperature, perfusate osmolality, total body immersion - probably were different in these investigations. Finally the dogfish used in Oppelt *et al.* study were larger (4-5 kg) than those used in the current work (2-3 kg). At present the relative contribution of each of these factors to the divergence in the two estimates of CSF production is uncertain and a precise evaluation of the rate of CSF secretion of *S. acanthias* cannot be made.

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THE EFFECT OF PURIFIED CHOLERA ENTEROTOXIN AND STAPHYLOCOCCAL ENTEROTOXIN B ON INTESTINAL TRANSPORT IN FLOUNDER

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Recent investigation with an *in vitro* rabbit ileum preparation has indicated that cholera enterotoxin (CT) increases the rate of Cl^- ion secretion from blood to luminal side and that this increase is associated with increases in transmucosal electric potential difference (PD) and short circuit current (Isc) (Field et al. J. Clin. Invest. 51:796-804, 1972). Staphylococcal enterotoxin B (SEB) has been studied in the *in vivo* rat intestine and is thought to stimulate Cl^- transport from serosa (S) to mucosa (M) by nonelectrogenic mechanisms (Sullivan et al. Am. J. Physiol. 220:1793-1797, 1971). The role

that the Cl^- ion plays in the generation of mammalian intestinal PD needs to be elucidated. However Cl^- transport represents a significant component of the electrical activity of the fish intestine (Huang et al. Am. J. Physiol. 220:1734-1738, 1971; J. Pharmacol. Exp. Ther. 180:777-783, 1972). The purpose of this investigation was to study the effect of purified CT and SEB on ion transport and permeability across the flounder intestine and to determine if there is a species difference in response to toxin.

Winter flounder *P. americanus* were kept in a seawater tank for three to seven days before use. Immediately after sacrificing, a section of intestine 3 cm below the stomach and 4 cm above the cloaca were removed, divided into three sections, and each piece mounted in a separate Ussing chamber. The intestinal membrane was bathed with a modified Forster's teleost Ringer's solution containing 5 mM of dextrose and aerated continuously with 95 percent - 5 percent O_2 - CO_2 gas. At steady state conditions the PD across the membrane ranged from 1-4 mV, serosa being negative and the Isc ranged from -20 to -40 μAmp. These control values remained constant for at least three hours. Aliquots of CT and SEB were kept at -70°C and were diluted to the appropriate concentration daily with 0.01 M phosphate buffer (pH 7.4). After a one-hour control period during which steady state conditions were established, either CT or SEB was added to the solution bathing the mucosal side or to both mucosal and serosal bathing solutions.

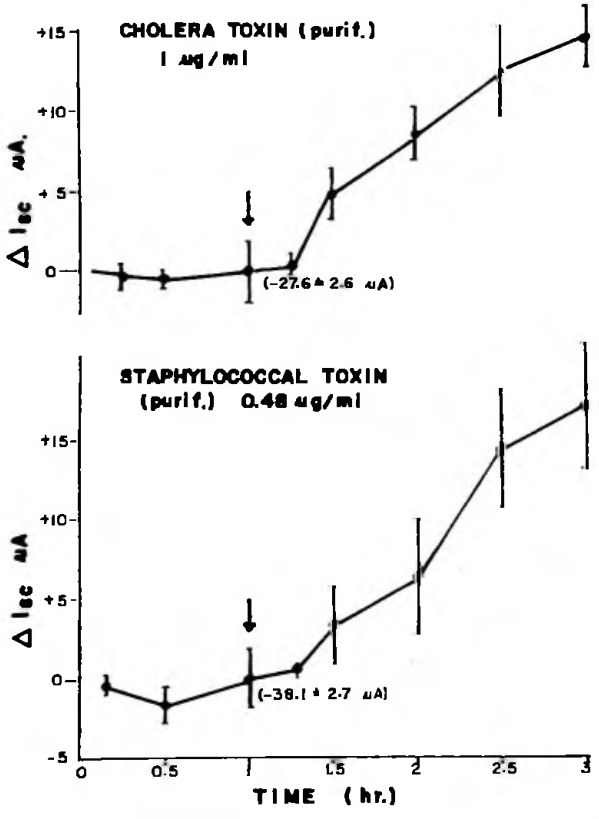

Figure 1. The effect of cholera and staphylococcal enterotoxins on short circuit current applied to the flounder intestine.

As shown in Figure 1 both toxins caused a gradual increase in the Isc as well as an associated parallel change in the PD.

Unidirectional fluxes were determined by the use of ^{22}Na and ^{36}Cl isotopes. Results are summarized in Table 1. CT at a concentration of 1 μg/ml increased the mucosal-to-serosal (J_{ms}) and the serosal-to-mucosal (J_{sm}) flux of Na^+ and Cl^-. The increases for J_{sm} were greater than those for J_{ms} resulting in a decrease in net flux (J_{net}) for both Na^+ and Cl^- which was associated with increases in Isc and PD. SEB at a concentration of 0.48 μg/ml had a similar effect on Na^+ and Cl^- transport but had a greater effect upon J^{Cl}_{net} than that observed with CT.

TABLE 1

The effect of cholera and staphylococcal enterotoxins on ion flux

TOXIN	NO. OF FISH	HOUR AFTER TOXIN	^{22}Na FLUX			^{36}Cl FLUX		
			J_{ms}	J_{sm}	J_{net}	J_{ms}	J_{sm}	J_{net}
			(% of the control)*					
CHOLERA 1 μg/ml								
		C**	(4.4±0.7)	(2.8±0.6)	(1.5±0.4)	(3.8±1.2)	(0.78±0.24)	(3.02±1.1)
both sides	6	1	135	183	46	157	507	67
		2	151	202	50	171	612	57
		C	(4.3±0.7)	(2.7±0.6)	(1.7±0.3)	(4.2±0.8)	(1.2±0.9)	(2.9±0.5)
mucosal	6	1	139	137	120	133	234	91
		2	140	206	39	144	352	59
STAPHYLOCOCCAL 0.48 μg/ml								
		C	(3.8±0.4)	(2.2±0.3)	(1.4±0.3)	(3.7±0.6)	(1.1±0.2)	(2.5±0.5)
both sides	9	1	182	245	109	176	494	35
		2	178	240	106	207	545	60

* The first hour sample before toxin was used as control value = 100
** Control period μEq. cm^{-2} hr^{-1}, mean ± SEM

Preliminary studies were performed using ^3H-water to measure the membrane permeability. In controls, the K_{trans} for ^3H-water was 350±27 x 10^{-7} cm sec^{-1} from mucosal to serosa and 235±20 x 10^{-7} from serosal to mucosa. Addition of CT increased the value by 130 percent and 215 percent respectively. SEB exerted a greater effect on permeability, increasing the K_{trans} to 135 percent and 245 percent respectively.

The data presented here indicate that purified CT and SEB increase unidirectional fluxes of Na^+ and Cl^-, the greater increase being observed in the J_{sm} for each ion, the J^{Cl}_{net} being greater than the J^{Na}_{net}. Both toxins have similar effect on the electrogenic properties of the flounder intestine. The permeability for water is also increased, a finding that is in agreement with mammalian studies

of Love (Gut 10:105-107, 1967) but that disagrees with the work by Lifson et al (Am. J. Physiol. 233:1479-1487, 1972) and Rohde et al. (Gut 13:191-196, 1972). From the limited amount of data presented here one may speculate that the CT has a similar action on flounder intestine to that observed with mammalian intestine and that SEB acts in a similar fashion.

This work was supported by grants from NSF GB-27495 and NIH AM-2217-14. Cholera toxin prepared under contract for the NIAID by R.A. Finklestein, PhD, The University of Texas Southwestern Medical School, Dallas, Texas.

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RENAL UREA AND WATER REABSORPTION IN THE LITTLE SKATE *Raja erinacea*

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In our studies of renal excretion of urea in the dogfish *Squalus acanthias* (Bodil Schmidt-Nielson, et al. Comp. Biochem. Physiol. 42A:13-25, 1972), a constant relationship between urea and sodium reabsorption was found. Thus 1.6μM urea is reabsorbed per μM of Na reabsorbed. The present studies with the little skate *Raja erinacea* were undertaken to determine if this same relationship exists in another species of elasmobranch.

We performed preliminary dissections to determine the position and anatomical arrangement of ureters and urinary vesicle. The anatomy in *R. erinacea* was found to be quite similar to that described by I. Borcea in *R. clavata* (Arch. Zool. Exp. et Générale IV Série, 4:199-484, 1906). In the adult female skate the bilobed urinary vesicle is a diverticulum to the urogenital sinus. It opens through a single canal with a sphincter into the urogenital sinus between the openings of the two oviducts. Four to five ureters from each kidney open into the dorsal side of each of the two lobes of the urinary vesicle. The anatomical arrangement in the male is different but also in the male there are distinct urinary vesicles. We chose to work with the female for the present study.

Twelve adult female skates, *R. erinacea*, were used-body weight 800 - 1000 g. Some of the fish were acclimated to 75 percent seawater for two days prior to the experiment. The skate was anesthetized by superfusion of the gills with a one percent MS 222 solution. A 2 cm incision was made in the skin of the abdomen and a 1 cm incision in the wall of the urogenital sinus. A polyethylene catheter was inserted through the opening into the urinary vesicle and tied in place with a pursestring ligature. The incisions were sutured tightly. Apparently this procedure is not very traumatic to the skate since they could live for several weeks with the catheter in place. The catheter could be irrigated with air through a side tube so that the bladder and catheter could be effectively emptied at the end of each urine collection. ^{14}C labelled polyethylene glycol was used for determination of glomerular filtration rate. The isotope was injected into the caudal vein from eight to 24 hours prior to the start of the experiment. Urine collection periods varied between two and 17 hours. In some experiments trimethylamine oxide (20 ml of 5M TMAO per kg fish) was injected i.v. and in other experiments a similar dose of urea was given. The purpose of these injections was to see if a relative change in the plasma concentration of TMAO or urea would change the ratio of