Table 1. Effect of PBC, PBC aromatic fractions (AR-1, AR-2), dispersant and emulsions on organ weights of Herring Gull chicks

Treatment (n)	Nasal Gland mg/kg	Adrenals mg/kg	Liver g/kg
Control (8)	417 ± 23	155 ± 15	44.6 ± 2.0
1.0 ml oil (7)	654 ± 45	217 ± 7 *	43.8 ± 2.2
1.0 ml equiv. AR-1 (7)	491 ± 34	147 ± 7	46.7 ± 3.1
1.0 ml equiv. AR-2 (6)	741 ± 28 *	267 ± 20 *	60.6 ± 2.3 *
0.1 ml Dispersant (6)	490 ± 34	155 ± 11	41.0 ± 1.1
1.0 ml Emulsion (6)	607 ± 24 *	238 ± 2 *	41.0 ± 2.9

Data expressed as mean + standard error. Birds collected 5 d after dosing. Results of t-test comparisons with controls indicated (* $p<0.01$).

Our preliminary field studies with petrels indicated that chicks (mean initial body weight 33 ± 2 g, 11-12 birds per group) dosed with 0.05 ml of either PBC, Atlantic/Pacific (AP) dispersant or a 10/1 PBC/AP emulsion exhibited no differences in weight gain or other growth parameters from paired controls (3 week growth data, not shown). On a body weight basis, these chicks received oil doses that were roughly equivalent to those causing significant depression of growth in gulls (above). With adult petrels, 0.1 ml doses of either PBC or WSLC (administered during the brood phase of the reproductive cycle) caused significant adrenal and nasal gland hypertrophy (Table 2). Of potentially

Table 2. Effects of ingested crude oil on adult Leach's Storm Petrel organ weights

Treatment (n)	Nasal Gland mg/kg	Adrenals mg/kg	Liver g/kg
Controls (11)	774 ± 22	177 ± 8	25.8 ± 0.6
0.1 ml WSLC (5)	957 ± 77 *	296 ± 40 *	25.7 ± 0.9
0.1 ml PBC (7)	966 ± 29 *	215 ± 6 *	25.4 ± 0.7

Data expressed as mean + SE. Birds collected 14-21 d after dosing. Results of t-test comparisons with controls indicated (* $p < 0.01$).

greater importance, only 53% of the chicks of dosed parents survived, compared with 100% survival for controls. These data suggest that petrel chicks may be considerably less sensitive to ingested oil than gulls or guillemots, and that petrel adults, exposed to oil during the reproductive cycle, are less successful in raising their young. This latter fin in clearly requires further study. Supported by U.S.P.H.S. grant ES 00920.

CHARACTERIZATION OF A STEROID-BINDING PROTEIN IN THE SPINY DOGFISH, SQUALUS ACANTHIAS

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The elasmobranchs are of particular evolutionary importance and great biological interest in their reproductive physiology. Sex steroid hormones have been identified in this group and levels of androgens and estrogens reach con-

centrations which are 50-100 X higher than in mammals (Dodd, Am. Zool. 15:137-171). Identification and characterization of a sex-steroid binding protein in this group is of interest in understanding the evolution of the steroid hormone transport mechanism in the vertebrates.

Blood samples were collected from mature fish and 17-20 cm embryos. Plasma proteins were separated electrophoretically in polyacrylamide gels. Both male and female plasma contained a single protein band which binds to ^{3}H-estradiol and ^{3}H-testosterone but not to ^{3}H-diethylstibestrol or ^{3}H-androstenedione. The molecular weight of this protein was around 150,000 daltons. The sedimentation coefficient as determined on a 5-20% sucrose gradient was 8-9S.

Specific steroid binding was determined by a modified charcoal-adsorption assay. Charcoal stripped plasma (at 1:6 dilution) was incubated with radioactive and unlabeled steroids at 4°C for 30 min. At the end of the incubation, an equal volume of dextrancoated charcoal suspension was added to the samples. Bound and free steroids were separated by centrifugation. Specific binding was calculated by subtracting the radioactivity measured in the presence of 100 fold unlabeled steroid (non-specific binding) from that measured in the absence of cold steroid (total binding). Using this assay it was shown that ^{3}H-estradiol associated with the plasma binder rapidly, apparent equilibrium being reached within 5 min. The dissociation rate was linear and the 1/2 life of ^{3}H-estradiol-steroid-binding protein complex was 100 minutes. The binding affinities and the binding capacities of the plasma for ^{3}H-estradiol, ^{3}H-testosterone and ^{3}H-progesterone were determined.

Table 1. Association constants (Ka) and binding capacity of Squalus plasma SHBP for ^{3}H-estradiol-17β, ^{3}H-testosterone and ^{3}H-progesterone

^{3}H-steroid	Sex	n	Ka(M^{-1})	Binding capacity (M)
Estradiol-17β	F	6	$2.8 \pm 0.23 \times 10^{7}$	$1.8 \pm 0.18 \times 10^{-7}$
	M	4	$2.6 \pm 0.35 \times 10^{7}$	$1.9 \pm 0.20 \times 10^{-7}$
	female embryos	pool	--	1.3×10^{-7}
	male embryos	pool	--	0.8×10^{-7}
Testosterone	F	2	4.0×10^{7}	1.1×10^{-7}
Progesterone	F	2	4.2×10^{7}	1.3×10^{-7}

n denotes the number of different plasma samples from individual animals. Results are expressed as mean $\pm$ S.E.M.s or means only. Embryonic sera from five fish of each sex were pooled together.

The association constants were at the range of $10^{7} M^{-1}$ and affinities for ^{3}H-testosterone and ^{3}H-progesterone were higher than that of ^{3}H-estradiol. In both sexes the capacity expressed as steroid bound per litre plasma (M) for ^{3}H-estradiol was 1.5-2.0 times greater than for ^{3}H-testosterone or ^{3}H-progesterone binding.

The binding specificity of the plasma was evaluated by competitive binding studies (Fig. 1).

Plasma samples were incubated with 10 nM of ^{3}H-estradiol and different concentrations of various unlabeled hormones. Unlabeled testosterone and dihydrotestosterone competed more effectively than unlabeled estradiol, while progesterone competed equally well, but estrone competed less effectively. Corticosterone and estriol were only effective in displacing ^{3}H-estradiol when presented in high concentrations, and diethylstibestrol did not compete at all. The binding capacity of plasma from male and female embryos was similar to that of the adult. However, the binding

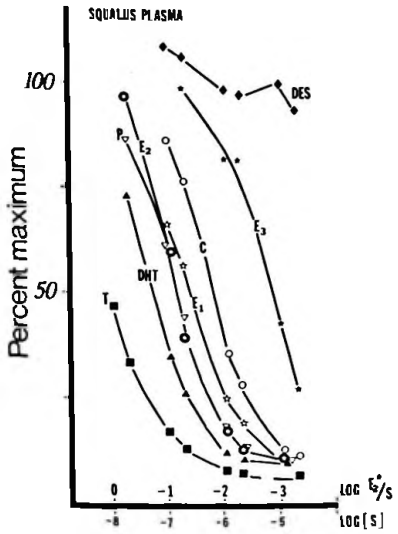

Figure 1. Competition of steroids with ^{3}H-estradiol-17β for binding sites of SHBP from plasma of female dogfish. (T) testosterone, (DHT) dihydrotestosterone-5 , (P) progesterone, (E_2) estradiol-17β, (E_1) estrone, (E_3) estriol, (DES) diethylstilbestrol, [S] = concentration of unlabeled steroids in M. Specific binding of ^{3}H-estradiol in the absence of unlabeled steroid was set at 100%, and the binding of ^{3}H-E_2 in the presence of other unlabeled steroids was expressed as percentage of maximum.

capacity (M) of embryonic plasma for ^{3}H-estradiol was only half that found in mature fish (Table 1), and a sexual difference was apparent; female plasma having a higher binding capacity.

In summary, a high affinity, limited capacity sex-hormone binding protein (SHBP) has been demonstrated in the plasma of mature and embryonic dogfish (Squalus acanthias). This SHBP clearly differs from amphibian and mammalian SHBPs in its steroid specificity as it binds C_{21} steroid (progesterone and corticosterone) as well as C_{18} and C_{19} sex-steroids. The binding affinities for both testosterone and estradiol were ten times less than that of mammalian SHBPs. However, the binding capacities for both steroids were about 100 times higher than those of mammals.

Although the physiological role of high affinity plasma binders for steroid hormones is still speculative, it is likely that they regulate steroid concentration available for receptor binding at the target organs. Supported by NSF grant #PCM 78-08201 to IPC.

INDUCTION OF VITELLOGENIN SYNTHESIS IN THE SPINY DOGFISH, SQUALUS ACANTHIAS

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In non-mammalian vertebrates, estrogens cause hepatic synthesis of a yolk-protein precursor, vitellogenic females. Injection of estrogens into males or non-vitellogenic females readily induces the production of this protein in teleosts, amphibians, reptiles and birds. In the oviparous elasmobranch Scyliorhinus canicula (a continuous breeder), although vitellogenin production continues throughout the year, it is more pronounced from September to February (Craik, Comp. Biochem. Physiol. 60B, 9-18, 1978). We have studied the spiny dogfish, Squalus acanthias, a yolk-sac dependent, aplacental, ovoviviparous species, with a gestation period of 20-22 months (Hisaw and Albert, Biol. Bull. 92, 187-199, 1947). The effect of steroid injections in females at either Stage A (early pregnancy, embryo < 17 mm) of Stage C (late pregnancy; embryos between 17-24 cm) and males were studied as follows:

TABLE 1

	Control (sesame oil)	Estradiol 17β *	Estradiol 17β* + progesterone
Stage A female	2[a]	5	2
Stage B female	2	5	2
Males	2	2	2

*Hormone dosage: estradiol-17β = 3 x 1.0 mg, progesterone = 3 x 2.0 mg. Hormone was administered at days 0, 2, and 4 and plasma samples were taken at day 0, day 3, day 5, day 7, day 14.

[a]denotes the number of animals in the group.