Table 1 presents the species and number of specimens utilized during the period 1979 - 1982. Animals that died following the stress of capture and transport are not included in the figures. The most significant feature of the data is the large number of animals involved. The "Gordon King" operation is now contributing between 1300 and 2000 specimens per season which is as great or in excess of the Squalus collection.

Table 1. Animals collected by Gordon King for use at MDIBL from 1979-82.

Year	Number of collecting trips	Date of first trip	Date of last trip	Skate	Flounder	Sea raven	Longhorn sculpin	Ocean pout	Goosefish	Dogfish	Fish total	Average fish/trip
1979	36	6/10	9/24	437	858	96	170	78	11	4	1654	46
1980	38	6/4	8/26	429	528	160	138	35	5	–	1297	46
1981	44	6/11	10/4	704	947	103	185	12	21	65	2037	46
1982	50	6/3	9/24	654	767	46	311	31	22	20	1851	37

Additional collections over the past four seasons included: wolfish (3), lumpfish (2), hake (2), shorthorn sculpin (7), crabs (14), and squid (15).

A number of other points are apparent from the fish collection records. Foremost, requests for skate, flounder, sea raven, and longhorn sculpin were usually filled within 2 - 3 days of placing the order. In effect, supply kept pace with the demand for these species. The demand for goosefish invariably fell short of the supply. A program dependent upon a large supply of goosefish would require an alternate supply mechanism. This statement also applies to the other species listed in the Footnote to Table 1. Finally, in both 1979 and 1981 there was a period of approximately 10 - 14 days in late July when there were large numbers of fish deaths following transport to the laboratory. The demand for fish and hence holding space was inordinately high during both periods in question.

Although the actual fish collection records are informative in many respects they do not disclose the fine aspects of animal supply. Information on the quality of individual animals with respect to size and condition was sought via a questionnaire during the 1982 season. The consensus of individuals responding was that animal condition was acceptable.

In summary the "Gordon King" operation provides a very high proportion of the specimens utilized at MDIBL and for many species supply is able to meet demand. There appears however to be a need for a mechanism of providing investigators with better quality animals and an increase in holding space. Finally, the collecting committee recommends that because of the current reliance on the "Gordon King" operation a formal and well tested back-up system be established to provide for these species if this service is interrupted.

BILE PIGMENT COMPOSITION AND HEPATIC UDP-GLUCURONYL TRANSFERASE ACTIVITY IN ADULT AND FETAL DOGFISH SHARK, SQUALUS ACANTHIAS

Namita Roy Chowdhury, J. Roy Chowdhury and Irwin M. Arias, Liver Research Center, Albert Einstein College of Medicine, New York, N.Y.

In man and many other mammals, UDP-glucuronyl transferase activity develops in the perinatal period (Lucier, G.W. et al, J. Ster. Biochem., 8:667-673, 1977). Glucuronyl transferase activity towards one group of substrates, including p-nitrophenol (PNP-GT) develops in late fetal life in rats, while the enzyme activity towards several other substrates including bilirubin (B-GT) develops in the neonatal period. In man and Rhesus monkey bilirubin IX_{β} appears in early fetal bile and meconium; bilirubin IX_{α} becomes the almost exclusive pigment in late fetal life (Blumenthal, S.G. et al, Biochem. J. 186:693-700, 1980). Bilirubin IX_{α}-monoglucoside and monoxyloside are the earliest conjugated bile pigments to appear. These are replaced by bilirubin monoglucuronide which is the major bile pigment at birth. Bilirubin diglucuronide becomes the major bile pigment in later life (Fevery et al, J. Clin. Invest. 51:2482-2492, 1972).

Previous studies on several teleost and elasmobranch fish demonstrated that bilirubin glucuronides are the major bile pigments in these fish and UDP-glucuronyl transferase activity towards bilirubin is present in hepatic microsomes of these fish (Roy Chowdhury et al, Comp. Biochem. Physiol., 66B:523-528, 1980). The present study was undertaken to compare the bile pigment pattern and hepatic microsomal UDP-glucuronyl transferase activity in adult and fetal dogfish shark (Squalus acanthias). Fetal and adult dogfish gallbladder bile was collected by needle aspiration from 15 adult dogfish and 100 dogfish fetuses in the second year of gestation. Since bile pigments in the meconium in the distal part of the intestine represent those excreted in early fetal life (Blumenthal et al, Biochem. J. 186:693-700, 1980), the fetal intestines were divided by ligation into proximal and distal halves; the meconium from each half was collected. Bilirubin and its conjugates in the bile samples were separated, identified and quantitated by high performance liquid chromatography (HPLC) using a Waters' μ-Bondapak C-18 column and eluting at 1 ml/min with a concave gradient of methanol (50% to 100%) in sodium acetate, 0.1 M, pH 4.0, containing 5 mM 1-heptanesulfonic acid (Roy Chowdhury, J., et al, Hepatology 1:622, 1981). Bile pigments in meconium were extracted in chloroform/ethanol (1/1) (Blanckaert, N. et al, Biochem. J. 171:203-214, 1978) and analyzed by HPLC after evaporation of organic solvents. Bilirubin monoglucuronide, unconjugated bilirubin, bilirubin diglucuronide and bilirubin monoglucoside were present in all specimens in order of relative abundance, confirming our previous findings (Roy Chowdhury, J., et al., Comp. Biochem. Physiol. 66B:523-528, 1980) (Table 1). There was no significant difference among the proportions of bilirubin monoglucuronide, bilirubin diglucuronide and bilirubin in the adult bile, fetal bile and proximal and distal meconium ($P > 0.2$).

Table I. Bilirubin and its conjugates in bile and meconium

	Total bilirubin (μM)	percent total bilirubin			
		BMGlcAc	UCB	BDGlcAc	BMGlc
Adult bile	300 ± 18	65 ± 6.8	22 ± 2.2	10 ± 2.2	3.5 ± 1.5
Fetal bile	28 ± 3	68 ± 6.6	18 ± 4.0	12 ± 4.1	2.0 ± 0.8
Meconium:					
Proximal	80 ± 11	69 ± 7.2	16 ± 1.5	10 ± 2.8	5.0 ± 1.2
Distal	85 ± 15	60 ± 6.9	18 ± 1.5	9 ± 1.8	13.1 ± 2.9

BMGlcAc = bilirubin monoglucuronide; UCB = unconjugated bilirubin; BDGlcAc = bilirubin diglucuronide; BMGlc = bilirubin monoglucoside. The data represent means of 6 experiments ± S.E.M.

To determine the isomeric composition of the bile pigments, bile and meconium samples were treated with 0.1 M NaOH in the presence of 4 mM sodium ascorbate at 25°C in the dark for 30 min. The pH was adjusted to 2.7 and the unconjugated bilirubin produced by hydrolysis was extracted in chloroform. The extracted pigment comigrated with authentic bilirubin IX_α on thin-layer chromatography (McDonagh and Assisi, FEBS Lett. 18:315, 1971).

Hepatic microsomal B-GT activity was determined as previously reported (Roy Chowdhury, J. et al., Comp. Biochem. Physiol. 66B:523-528, 1980) and PNP-GT was assayed by a radioassay (Tukey, R., et al, Biochem. J. 171: 659-663, 1978). Specific B-GT activity in the fetal and adult liver microsomes were similar ($P > 0.2$). Specific PNP-GT activity was slightly greater in the fetal microsomes, but the difference was not statistically significant ($P > 0.2$) (Table 2).

Table II. UDP-glucuronyl transferase activities in adult and fetal hepatic microsomes

	Bilirubin-UDP-glucuronyl transferase (nmol/mg protein.20 min)	p-nitrophenol-UDP-glucuronyl transferase (nmol/mg protein.15 min)
Adult	2.5 ± 0.8	25.7 ± 2.2
Fetal	2.0 ± 0.4	34.4 ± 3.5

Data represent means of 6 assays ± SEM.

The results indicate that, unlike the findings in the Rhesus monkey and man, isomeric composition of bilirubin in dogfish fetal bile and meconium resemble those in the adult bile. This is consistent with the finding of adult levels of PNP-GT and B-GT activity in the fetal hepatic microsomes.

NEUTRAL NaCl ABSORPTION BY FLOUNDER URINARY BLADDER

John B. Stokes and Ivan B. Lee, Department of Internal Medicine, University of Iowa, Iowa City, Ia.

The urinary bladder of the winter flounder absorbs NaCl by a process independent of electrical activity across the epithelium (Renfro, Am. J. Physiol. 228:52-61, 1975; Renfro, J. Exp. Zool. 199:383-390, 1978). Although in some tissues there is a measurable short circuit current, this current is entirely attributable to K^+ secretion and can be inhibited by mucosal Ba^{++} without affecting NaCl absorption (Dawson and Andrew, Bull. MDIBL 20:89-92, 1980). Among the previously documented characteristics of NaCl absorption are the interdependence of each ion on the other, the similar net absorptive rates for Na and Cl under several conditions, and the dependence on a ouabain-sensitive basolateral membrane Na-K ATPase (Renfro et al., Am. J. Physiol. 231:1735-1743, 1976). The present experiments were designed to further characterize the nature of this absorption process and to examine whether the mucosal-to-cell transport step might be, a) a neutral transport process dependent on mucosal K^+, b) a parallel Na^+-H^+, Cl^--OH^- exchanger, or c) a simple NaCl entry process.