

periment (U/P values below 2), and concomitantly, the clearance ratio increased to values higher than 1.0. The above data may be taken as evidence for a reabsorption of D-galactose from the tubular lumen against a concentration gradient. In two of these experiments, the tissue content of D-galactose was determined at the end of the clearance measurements. In both experiments, the T/plasma ratios were considerably higher than 1.0 (mean value 2.6, 9 analyses). The above data are consistent with the following model for D-galactose transport across the flounder tubular cells: Galactose is first accumulated in the tubular cells by an active transport system localized at the luminal face. Subsequently, the sugar moves in accordance with its concentration gradient from the cells to the plasma by a carrier mediated process.

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1970 #23

PHYLOGENETIC STUDY OF HEPATIC ORGANIC ANION UPTAKE MECHANISMS

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In mammals, organic anions such as bilirubin and various dyes, drugs and metabolites are rapidly and selectively transferred from plasma into the liver. The mechanism of this transfer and its selectivity are unknown. Recently, two organic anion binding proteins, Y and Z, have been isolated from the liver cell cytoplasm of the rat (J. Clin. Invest. 48:2156, 1969). The following characteristics of Y and Z support the theory that they are intracellular acceptors which facilitate hepatic uptake of various organic anions from plasma: (i) Y and Z selectively bind certain organic anions such as bilirubin, sulphobromophthalein (BSP) and indocyanine green (J. Clin. Invest. 48:2156, 1969; J. Clin. Invest. 1970, submitted); (ii) Y, the major organic anion binding protein is present exclusively in the liver (J. Clin. Invest. 48:2156, 1969); (iii) the concentration of Y increases following administration of phenobarbital, DDT and other drugs and concomitantly hepatic anion uptake is increased (J. Clin. Invest., 1970 (submitted); Proc. Nat'l Acad. Sci. 64:168, 1969), and (iv) in the newborn monkey, the concentration of Y and hepatic uptake of BSP, as measured by plasma disappearance rates, are both low and mature simultaneously (The Lancet 2:139, 1969).

A phylogenetic study was performed to investigate further the function of Y and Z. The study included an elasmobranch, the dogfish; several bony fishes; the mudpuppy, a gill-breathing amphibian which spends its entire life confined to water; four species of amphibia which possess functioning lungs and are able to exist for long periods on land; reptiles, birds and several mammals, including man. For each animal, from 2 to 6 mature individuals were studied. R. catesbiana, R. clamitans, and R. heckscherii tadpoles with and without hind limb emergence, as well as adult frogs, were separately studied.

Y and Z were identified and measured in terms of their binding of BSP in vitro (J. Clin. Invest. 48:2156, 1969). The relationship between Y and Z and hepatic organic anion uptake was investigated where possible by determining the half-life for removal of injected BSP from plasma. From 3 to 6 elasmobranchs and teleosts received a single intravenous injection of 10 mg of BSP per 100 g body weight; from 6 to 8 blood specimens were obtained in the subsequent 1 to 60 minutes; BSP content was determined after alkalization and the half-time was derived from a

Table 1

PHYLOGENETIC STUDY OF Y, Z AND MEAN PLASMA HALF-TIMES FOR BSP
(See text for explanation of symbols)

	No. animals studied	Y	Z	No. animals studied	Mean plasma BSP t 1/2 (min)
ELASMOBRANCH					
dogfish (<i>Squalus acanthias</i>)	6	-	-	4	44
TELEOSTS					
mackerel (<i>Scomber scombrus</i>)	6	-	-	4	36
carp (<i>Cyprinus carpio</i>)	3	-	±		NT
eel (<i>Anguilla rostrata</i>)	2	-	±		NT
flounder (<i>Pseudopleuronectes americanus</i>)	2	-	±		NT
AMPHIBIANS					
mudpuppy (<i>Necturus maculosus</i>)	3	-	±		NT
Congo eel (<i>Amphiuma means</i>)	2	+	±		NT
tiger salamander (<i>Ambystoma trigrinum</i>)	2	+	±		NT
toad (<i>Bufo marinus</i>)	3	+	±		NT
frog (<i>Rana clamitans</i> , <i>Rana catesbeiana</i> , <i>Rana heckscherii</i>)					
early tadpole	36	-	±		NT
late tadpole	44	±	+		NT
adult	18	+	+		NT
REPTILES					
turtle (<i>Pseudemys scripta elegans</i>)	2	+	+		NT
alligator (<i>Alligator mississippiensis</i>)	2	+	+		NT
iguana (<i>Iguana iguana</i>)	2	+	+		NT
king snake (<i>Lampropeltis getulus</i>)	2	+	+		NT
BIRDS					
chicken (<i>Gallus domesticus</i>)	2	+	+	2	4.8
seagull (<i>Larus atricilla</i>)	2	+	+		NT
MAMMALS					
mouse (<i>Mus</i> , 9 different strains)	16	+	+	12	2.7
rat (<i>Rattus</i> , 4 different strains)	21	+	+	21	3.8
kangaroo rat (<i>Dipodomys merriami</i>)	2	+	+		NT
rabbit (<i>Oryctolagus cuniculus</i>)	3	+	+	5	8.4
guinea pig (<i>Cobaya cobaya</i>)	10	+	+	3	6.7
goat (<i>Capra</i>)	2	+	+	2	6.4
sheep (<i>Ovis aries</i>)	3	+	+	2	11.1, 5.2
monkey (<i>Macaca mulatta</i>)	10	+	+	4	9.6
man (<i>Homo sapiens</i>)	4	+	+	10	8.9
seal (<i>Phoca vitulina</i>)	2	+	+		NT

NT = not tested.

logarithm plot of the plasma disappearance curve. From two to 10 representatives of each species of bird and mammal studied received a single intravenous injection of 5 mg of BSP per 100 g body weight and the plasma disappearance curve was similarly determined. These studies were not technically possible in amphibia or reptiles.

Bile duct cannulation and, where indicated, cholecystectomy were performed in from 2 to 10 rats, guinea pigs, goat, sheep, chicken, dogfish and mackerel. Twenty minutes after intravenous injection of the same dose of BSP content was determined in bile and liver.

In the rat, BSP was localized to two prominent fraction, Y and Z, which results from a specific Y and a specific Z protein (J. Biol. Chem. 1969 (submitted)). The dogfish pattern exhibited neither a Y nor a Z peak. Dogfish liver supernatant did not inhibit Y or Z dye binding by rat liver supernates. With rat liver, the dye binding method detected Y and Z when 10% as much liver was used as in the studies with fish liver.

Comparative evaluation of Y and Z in all animals studied is presented in Table 1. In Table 1 + denotes a prominent Y or Z peak as illustrated by the elution pattern in the rat; - indicates the absence of a Y or Z peak as illustrated by the dogfish elution pattern, and ± signifies a trace of BSP binding in the Y or Z region but no clearly defined peak. In elasmobranch and teleost fish, as well as the gill-breathing amphibian mudpuppy, Y was not detectable and Z was either not detectable or present in trace amount. In liver from tadpoles of R. catesbiana, R. clamitans, and R. heckscherii prior to hind leg emergence, Y and Z were either not detectable or present in trace amounts. Following hind leg emergence and prior to complete tail resorption, tadpoles of each species had prominent Z and trace or nondetectable Y. Liver from adult frogs of each species had prominent Y and Z peaks. All lung breathing amphibians, reptiles, birds and mammals studied had prominent Y and Z binding proteins.

Values of $t_{1/2}$ for the plasma disappearance of intravenously administered BSP are also shown in Table 1. In mammals and birds, $t_{1/2}$ ranged from 2.7 to 11.1 min. The effects of temperature, blood flow and plasma proteins were not systematically studied.

Twenty minutes after injection of BSP, 68-88% of the injected dose was recovered in the liver and bile from rats, guinea pigs, goat, sheep and chicken, whereas only 2-8% of the administered dose was recovered in liver and bile from dogfish and mackerel.

It has been assumed that fish gills, and/or skin, are permeable to lipid soluble compounds including organic anions, although few data are available on the subject. If this thesis is correct, in the evolutionary transition from aquatic to terrestrial life, certain species replaced gills with lungs and the liver assumed an important role in the metabolism and excretion of various non-polar organic anions such as drugs, dyes and steroids. In the present study, phylogenetic and ontogenetic appearance of Y and Z coincided with the transition from water to land, as well as with development of mechanisms for selective hepatic uptake of BSP. Similar ontogenetic development of Y, the major mammalian organic anion binding protein, has been demonstrated in guinea pigs and monkey (The Lancet 2:139, 1969; N. Eng. J. Med., 1970 (in press)).

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1970 #24

ASPECTS OF NICHE DIVERSIFICATION IN TWO SPECIES OF HERMIT CRABS

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Shell selection by hermit crabs can be considered a specialized case of habitat selection (Reese, E. S., Anim. Behav. 10:374-360). Studies of two sublittoral, sympatric species of hermit crabs (Pagurus pubescens and P. acadianus) were conducted in an attempt to define their respective niches in terms of this major parameter.