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Elimination and Distribution of Different Substituted Phenols by Frog *(Rana temporaria)* and Crayfish *(Astacus leptodactylus)*

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The fate of phenols in fish has received increased attention during the past years. KOBAYASHI & AKITAKE (1975 a,b,c) and KOBAYASHI et al. (1976) investigated the uptake, turnover and excretion of phenol and pentachlorphenol by goldfish. NAGEL & URICH (1980) studied the elimination of different substituted phenols by goldfish.

But there are almost no informations about the disposition of phenols by amphibia and crustacea, except ZABEL (1971) who explored the elimination of phenol by crayfish.

MATERIALS AND METHODS

Labeled compounds: [U-^{14}C]- phenol (368 Ci/mg), [G-^{3}H]- 3,5 diethylphenol (37 mCi/mg), The Radiochemical Centre Amersham; [U-^{14}C]- 4 - aminophenol (125 Ci/mg), Hoechst; [U-^{14}C]- 3 - nitrophenol (100 Ci/mg), Schering.

Determination: Tissue samples were oxidized by TRI-CARB OXIDIZER 306 (Packard Instruments). Radioactivity was measured by liquid scintillation counting.

Application: Frog: Phenols were administered via lymph sac (0,25 mL/5 mg/kg) according to THER (1965). The average body weight of the frogs used was 28 $\pm$ 8 g. Three frogs were placed in a 10 L glass tank containing 1 L water and kept at 20°C.

Crayfish: Phenols were injected into the pericard (0,25 mL/3 mg/kg). The average body weight of crayfish was 53 $\pm$ 13 g. Each crayfish was placed in a 10 L glass tank containing 2 L tap water and kept at 20°C.
With the exception of unsubstituted phenol, the substances were solved in one volume Cremophor EL [Sigma, Germany] and three volumes water. To prevent the oxidation of 4 - aminophenol ascorbic acid was added.

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Routes of excretion: Frog: The concentrations of phenols in urine, gallbladder and gut were measured.
Crayfish: Gills, gut and antennal glands are to be considered as routes of excretion. To determine the relative importance of the different routes the crayfish were fixed. Over the tail a rubber tube filled with water was pulled. A plastic cup with an additional hole was turned up the cephalothorax. The gaps were sealed up in a way that the pores of the antennal glands remained open. The separation of the nonpolar excretory products from the polar was carried out by continuous liquid-liquid extraction (pH 6.5; diethylether).

Pharmacokinetics: A two compartment model was used to describe the levels of the phenols in frog and crayfish (NAGEL & URICH 1980). In this model is k_{10} = rate constant of elimination from compartment I (non polar, presumable unconjugated phenols),
k_{20} = rate constant of elimination from compartment II (polar, presumable conjugated phenols)

RESULTS AND DISCUSSION

Distribution: Table 1 + 2 show the retention of phenols in various tissues of frog and crayfish. The concentrations of phenols gradually decreased in each organ, except the gall bladder of the frog and the contents of the gut. The results with crayfish correspond with the findings of ZABEL (1971).

Kinetic of elimination:

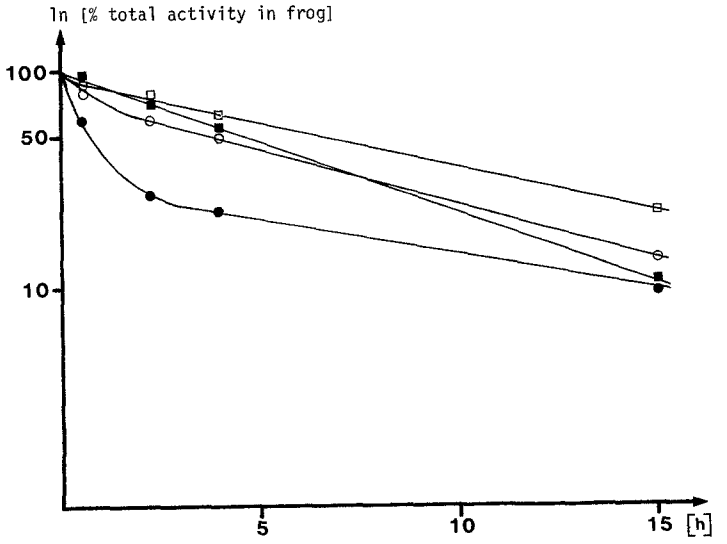

Figure 1. Retention of phenols in frog during culture in water after injection (5 mg/kg)

● = Phenol, ○ = 3-nitrophenol, ■ = 3,5 diethylphenol
□ = 4-aminophenol

TABLE 1

Distribution of phenols [µg/g] in various tissues of frog after injection (5 mg/kg)

	0.5 h				2 h				4 h				15 h			
	Ph	NP	DP	AP	Ph	NP	DP	AP	Ph	NP	DP	AP	Ph	NP	DP	AP
Blood	11.6	8.9	16.5	11.3	5.8	6.4	13.3	10.5	2.1	5.6	15.9	7.1	1.3	0.8	2.7	4.4
Gut	4.9	2.3	7.2	7.1	3.2	3.5	7.2	7.0	1.5	4.0	7.2	8.7	1.2	1.6	7.8	3.5
Brain	3.7	3.9	9.4	5.9	3.0	1.5	3.5	3.5	1.1	1.4	1.9	3.5	0.5	0.1	0.5	2.2
Skin	4.3	16.5	8.8	5.5	3.1	3.9	5.4	4.2	1.4	1.9	3.8	3.8	1.5	0.4	0.9	1.9
Heart	5.2	6.3	9.6	6.7	3.4	3.0	5.9	3.9	1.2	2.4	4.6	3.2	0.8	0.3	1.1	1.5
Liver	6.5	5.5	8.3	12.9	2.7	4.4	8.9	6.7	1.4	3.9	7.5	5.6	0.9	1.2	2.2	2.0
Lung	5.4	7.1	10.7	10.2	2.6	4.2	7.9	5.6	1.7	3.3	5.7	6.0	0.9	0.7	1.9	3.8
Stomach	4.8	2.7	8.3	6.4	2.3	3.3	7.1	4.8	1.3	2.8	5.3	4.3	0.5	0.4	1.0	1.5
Spleen	8.0	2.4	9.7	6.9	4.7	4.6	10.3	5.2	1.5	3.4	7.2	4.9	1.1	0.4	2.1	2.5
Kidney	8.9	16.0	17.9	10.7	12.4	15.1	13.6	6.3	7.8	18.4	9.2	5.8	2.4	8.1	2.9	3.1
Pancreas	6.2	4.3	10.5	7.2	5.0	4.9	10.4	4.5	1.5	2.5	7.5	4.5	1.1	0.7	1.7	2.0
Remainder	2.9	4.2	4.6	4.0	1.8	2.9	4.3	3.5	1.1	2.0	3.1	3.6	0.6	0.3	0.8	1.1
Gall bladder	6.1	4.8	14.3	15.5	2.4	11.9	113	29.4	7.3	73.8	287	69.8	10.0	60.4	126	54.0
Contents of gut	1.8	8.3	3.8	3.6	3.4	0.9	8.8	9.7	1.4	12.7	7.4	20.9	7.7	10.4	84.3	30.0

TABLE 2

Distribution of phenols [µg/g] in various tissues of crayfish after injection (3 mg/kg)

	4 h				15 h				45 h			
	Ph	NP	DP	AP	Ph	NP	DP	AP	Ph	NP	DP	AP
Antennal gland	11.7	12.6	14.0	15.8	7.7	13.0	4.6	11.3	2.6	6.5	2.2	3.5
Gut	4.1	8.7	2.8	5.4	4.6	3.5	2.1	2.5	2.7	0.9	1.3	2.3
Hemolymph	2.5	3.0	2.6	2.5	1.9	4.6	0.5	3.4	0.9	0.7	0.4	1.3
Heart	4.2	4.3	2.6	4.2	5.0	3.6	0.7	3.1	2.3	1.1	0.6	1.8
Gill	3.9	4.2	3.0	4.4	7.8	4.1	1.3	3.2	6.2	1.4	1.3	1.8
Stomach	4.8	5.4	3.4	6.6	5.7	3.1	1.3	3.8	3.7	0.9	3.9	7.0
Hepatopancreas	6.7	41.4	6.9	18.6	9.0	25.5	3.7	9.2	3.5	1.6	3.1	8.5
Brain	17.1	3.6	4.0	6.7	15.8	4.4	0.8	4.5	5.7	2.1	1.1	3.5
Remainder	2.0	1.7	1.5	2.7	2.7	1.5	0.5	1.8	1.6	0.8	0.4	1.3
Contents of gut	15.2	3.3	5.0	17.7	43.6	10.1	26.7	12.0	15.8	1.2	13.3	16.3

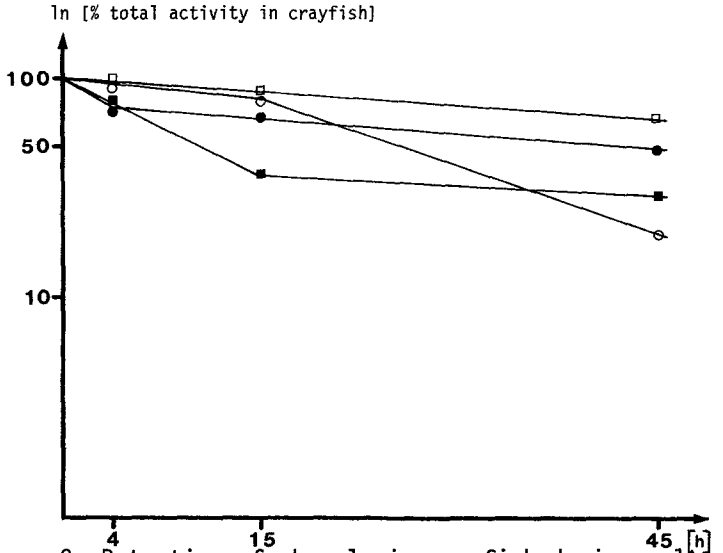

Figure 2. Retention of phenols in crayfish during culture in water after injection (3 mg/kg)

Figure 1 + 2 show the change in amount of phenols in frog and crayfish. Using the two compartment model (NAGEL & URICH 1980) the rate constants of elimination in table 3 are found.

TABLE 3

Rate constants of the elimination of phenols from frog and crayfish [h^{-1}]

	P	frog: k_{10}	k_{20}	crayfish: k_{10}	k_{20}
PH	28,8	1,61	0,11	0,45	0,01
NP	100	0,38	0,12	0,003	0,07
DP	2188	0,16	0,15	0,34	0,02
AP	0,7	0,55	0,09	0,02	0,01

P = Partition coefficient n-octanol/water
(FUJITA et al. 1964)
PH = Phenol, NP = 3-Nitrophenol,
DP = 3,5-diethylphenol, AP = 4-aminophenol

The smaller the molecular weight and the more polar the phenols are the better they can be eliminated by frog from compartment I. The elimination from compartment II shows the reverse correlation. 4-aminophenol represents an exception. The elimination of phenols by crayfish cannot be correlated in this simple way because 3-nitrophenol represents an additional exception.

Routes of excretion:Frog: From the low concentrations of phenols in gallbladder and gut can be deduced that the biliary excretion is the minor route compared with the renal elimination.
Crayfish: Table 4 shows that the excretion via the gills represents the major route of excretion of phenols, contrary to the conclusion of ZABEL (1971) that crayfish eliminate phenol only via antennal gland.

TABLE 4

Elimination of phenols from crayfish within 15 hours
(% activity eliminated)

	gill			antennal gland			gut		
		non			non			non	
	total	polar	polar	total	polar	polar	total	polar	polar
PH	97	97	0	3	2	1	0	0	0
NP	91	73	18	9	1	8	0	0	0
DP	80	0	80	13	3	10	7	4	3
AP	85	27	58	11	3	8	4	1	3

Comparative elimination of phenols by goldfish, frog and crayfish: Goldfish is able to excrete phenol, 3-nitrophenol and 3,5-diethylphenol more rapidly then frog and crayfish. This can be explained by the high permeability of the fish gill. The cuticula of the crustacean gill impedes a quick elimination. Nevertheless the branchial elimination represents the major route of the elimination of phenols by crayfish. The frog ranks between goldfish and crayfish in its ability to excrete phenol, 3-nitrophenol and 3,5-diethylphenol. 4-aminophenol is most rapidly eliminated by frog.

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