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Seasonal Variations in Aldrin Epoxidase (MFO) Activity of Yellow-Legged Herring Gulls: The Relationship to Breeding and PCB Residues

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The hepatic mixed function oxidases (MFO) constitute a defense mechanism which enables the organism to make xenobiotics more polar and thus render them more readily excretable. This is the main line of defense of many organisms against lipophilic chemicals (Lu and West 1980; Wolf 1982). The degree of induction of this system is an expression of its exposure to xenobiotics, but it is also a function of endogenous physiological mechanisms, such as the transformation of steroid hormones (see in De Bruin 1976; Moore 1985). These two forms of induction may lead to mutual interference: foreign compounds may stimulate hepatic hydroxylation and affect the metabolism of steroid hormones; the latter may in turn stimulate the activity of the MFO system favoring the degradation of the xenobiotics (see in De Bruin 1976). Induction and detoxication processes of endogenous and exogenous compounds have been observed in mammals in laboratory experiments. Animals treated with testosterone exhibit an increased detoxication capacity towards certain toxicants; while animals treated with DDT show increased hormone hydroxylation activity (Conney 1967).

Relationships between MFO activity, the reproductive cycle and variations in tissue levels of liposoluble xenobiotics, have been reported for marine organisms (Fossato et al. 1979; Mix et al. 1982; Livingstone 1985; Suteau et al. 1985). In birds, seasonal variations of MFO levels have been observed (Knight and Walker 1982), but the relationship between these enzyme variations and the levels of contaminants in the animal tissues has never been made clear. We aim to clarify this relationship by determining the levels of PCBs residues and aldrin epoxidase activities in Yellow-

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-legged Herring gull (Larus cachinnans)[1] specimens from different areas of Italy collected during two phases of the annual cycle, namely those of reproduction (spring) and of sexual inactivity (autumn). This species was chosen because of its wide distribution, its opportunistic feeding habits and its adaptive capacity in polluted environments.

MATERIALS AND METHODS

Yellow-legged Herring gull (L. cachinnans)[1] specimens were collected in four feeding areas of central and north eastern Italy during the spring and autumn of 1984.

For the determination of hepatic aldrin epoxidase activity (MFO) the liver was homogenized in phosphate buffer at pH 7.4, centrifuged 10 min at 1200 x g, and the microsomal fraction isolated by gel filtration of the supernatant as described by Pyykko (1983). Aldrin epoxidation activity was measured using the method of Krieger and Wilkinson (1969). The enzymatic activities are expressed in nmol substrate mg microsomal protein^{-1} min^{-1}. Microsomal protein was quantified by the Bio-Rad protein assay.

PCBs were determined in the liver of the collected specimens. For analysis a 0.1-1 g aliquot of freeze-dried material was extracted in Soxhlet apparatus with n-hexane. The extract underwent sulphuric acid clean up and Florisil chromatography. The eluates were analyzed with Perkin-Elmer Sigma 3 gas chromatographs equipped with ^{63}Ni electron capture detectors. Glass columns packed with 4% SE-30 + 6% SP-2401 on Supelcoport and a 30-m-long SBP-5 fused silica capillary column were used.

Statistical analysis was performed on an IBM personal computer (AT) using an SPSS program (IBM); in order to determine the differences between groups we used Mann-Whitney non parametric test.

RESULTS AND DISCUSSION

Aldrin epoxidase activity and PCBs concentrations in the liver of Yellow-legged Herring gulls are reported in Table 1. As no

1. The Yellow-legged Herring gull generally considered as sub species (Larus argentatus michaellis) has recently been revised and considered a separate species (Larus cachinnans) (Nicolau-Guillamet, 1977; Glutz and Bauer, 1982).

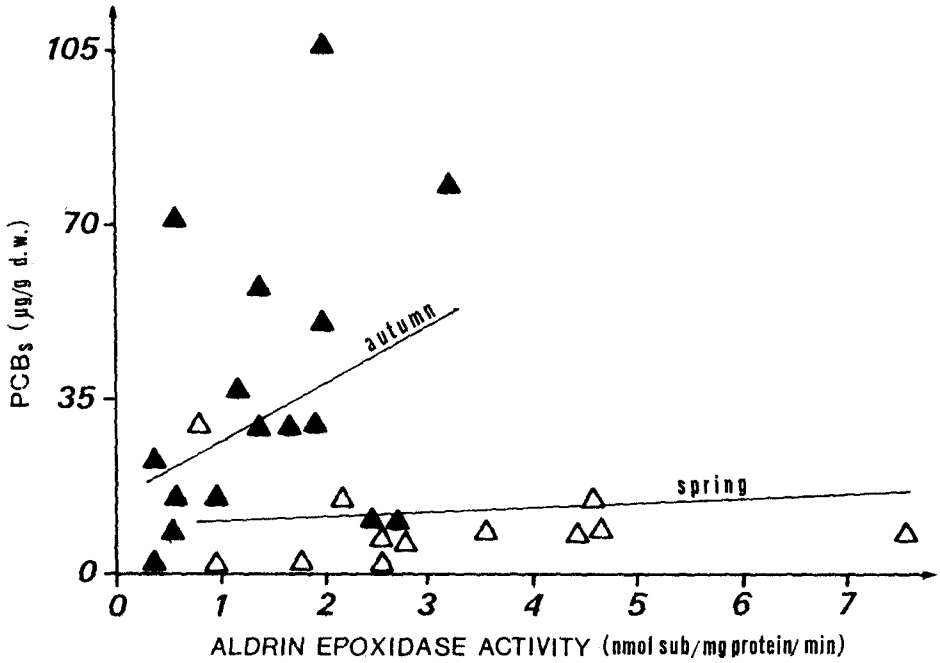

Figure 1. Relationship between PCBs concentrations and aldrin epoxidase activity in liver of Yellow-legged herring gull. (△ = reproductive period (Spring): y=0.731x + 11.23, r= -0.184; ▲ = period of sexual inactivity (Autumn): y=11.73x + 15.94, r= 0.345).

Table 1. Concentrations of PCBs (mg/kg dry weight), aldrin epoxidase activity (nmol substrate·mg microsomal protein^{-1} · min^{-1}) and ratio PCBs/MFO in liver of Yellow-legged herring gull ($\bar{x}$=mean; SD=Standard Deviation).

PERIODS	n	PCBs		ald. epox. act.		PCBs/MFO	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
REPRODUCTIVE (Spring)	12	9.05	7.46	3.18	1.84	5.17	10.24
SEXUAL INACTIVITY (Autumn)	16	33.35	29.14	1.48	0.85	25.88	24.44

significant differences were found between sampling areas and between the sexes, the data was separated only by period.

Enzyme activity was higher during reproduction ($\bar{x}$=3.18) than during the period of sexual inactivity ($\bar{x}$=1.48), and the difference between the mean values was highly significant ($p < 0.005$). PCBs concentrations were also significantly different ($p < 0.005$), with higher levels in birds collected within the period of sexual inactivity. Table 1 also gives the values of the ratio PCBs/MFO, which was much higher in autumn ($p < 0.005$).
By plotting the PCBs concentrations against the enzymatic levels of aldrin epoxidase (Figure 1), we obtained a distribution of data according to the period of collection: one group is characterized by high levels of PCBs and low MFO activity and this corresponds to the period of sexual inactivity; the other has high enzyme activity and low PCBs levels and represents the reproductive period.

The results of this study indicate that variations of aldrin epoxidase (MFO) activity observed in the liver of Yellow-legged herring gull in the two periods do not seem to depend on the inductive effect of PCBs in the tissues. This accords with data which suggests that the capacity of certain PCB congeners to induce MFO activity seems to be more effective towards cytochrome P-448-dependent monooxygenases (3 MC-type inducers), than toward cytochrome P-450-dependent monooxygenases (PB-type inducers), such as aldrin epoxidase (Safe et al. 1985; Clarke 1986). This was confirmed by preliminary experiments with Japanese quails treated with Aroclor 1260 which produced a strong induction of benzo(a)pyrene hydroxylase and of ethoxyresorufin o-deethylase and a lesser induction of aldrin epoxidase (Leonzio et al. in prep.).
The fact that the gulls analyzed came from different and distant areas makes it unlikely that the increase in the aldrin epoxidase activity was due to an input of other chemical inducers during the reproductive period. It therefore seems likely that this increase is linked to the annual cycle and that prior to reproduction there occurs an inductive phenomenon of specific monooxygenases involved in the metabolism of sex hormones. This agrees with the findings of Knight and Walker (1982) for Razorbills and Renzoni et al. (in prep.) for Wood pigeons. In this species, aldrin epoxidase levels increase markedly (about three times) in February with respect to the period October-January.

The condition of the gonads and the circulating levels of steroids as well as the different physiology of males and females are important parameters in determining variability in MFO activity especially when referred to the reproductive period. At the moment

data on these histological and physiological parameters are not available . The non significant difference found between sexes is probably due to the small number of specimens.

Although other factors, such as for example the seasonal change in the lipid content may influence the levels of PCBs in the tissues, it may be supposed that the high levels of aldrin epoxidase activity present in the liver of Yellow-legged herring gull, during the reproductive period, markedly influence the degradation of PCBs in this organ, thus favoring their excretion.
The biotransformation pathway of these xenobiotics in biological systems has been investigated by many authors (Sundstrom et al. 1976; Safe 1984) and there is evidence, in vivo and in vitro, that PCBs are processed by the microsomal enzymes, first by epoxidation of the benzene ring, and then by hydroxylation and further phenol conjugation. Thus it may be affirmed that the biotransformation and excretion of certain foreign compounds, such as PCBs, are fundamentally influenced by the metabolism of endogenous components. These interactions and the global distribution of PCBs make it possible to consider the relationship between PCBs concentrations in the liver and MFO activity (PCBs/MFO) as an index of the detoxication capacity of this species during different periods of the year.

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REFERENCES

Conney AH (1967) Pharmacological implications of microsomal enzyme induction. Pharmacol Rev 19:317-366
De Bruin A (1976) Biochemical toxicology of environmental agents. Elsevier/North-Holland Biochemical Press, Amsterdam
Clarke JU (1986) Structure-activity relationship in PCBs: use of principal components analysis to predict inducers of mixed-function oxidase activity. Chemosphere 15:275-287
Fossato V, Nasci C, Dolci F (1979) 3,4-benzopyrene and perylene in mussels, Mytilus sp., from the Laguna Veneta, north-east Italy. Mar Environ Res 2:47-53
Glutz Von Blotzheim, Bauer UN (1982) Handbuch der Vögel Mitteleuropas. Vol 8/I, Wiesbaden
Knight GC, Walker CH (1982) A study of the hepatic microsomal epoxide hydrolase in sea birds. Comp Biochem Physiol 73C:463-467
Krieger RL, Wilkinson CF (1969) Microsomal mixed function oxidases in insects - 1. Localisation and properties of an enzyme system affecting aldrin epoxidation in larvae of the Southern Armyword

(Prodenia eridania). Biochem Pharmacol 18:1403-1415
Livingstone DR (1985) Responses of the detoxication/toxication enzyme system of molluscs to organic pollutants and xenobiotics. Mar Pollut Bull 16:158-164
Lu AYH, West SB (1980) Multiplicity of mammalian microsomal cytochromes P-450. Pharmacol Rev 31:277-295
Mix MC, Hemingway SJ, Schaffer RL (1982) Benzo(a)pyrene concentrations in somatic and gonad tissues of bay mussels, Mytilus edulis. Bull Environ Contam Toxicol 28:46-51
Moore MN (1985) Cellular responses to pollutants. Mar Pollut Bull 16:134-138
Nicolau-Guillaumet p (1977) Mise au point et réflexions sur la répartition des Goélands argentés Larus argentatus en France. Alauda 45:53-73
Pyykko K (1983) Characterization and stability of rat liver microsomes isolated by rapid gel filtration method. Acta Pharmacol Toxicol 52:39-46
Safe S (1984) Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs): biochemistry, toxicology and mechanism of action. CRC Crit Rev Toxicol 13:319-395
Safe S, Bandiera S, Sawyer T, Robertson L, Safe L, Parkinson T, Thomas PE, Rayan DE, Reik LM, Levien W, Denomme MA, Fujta T (1985) PCBs: structure-function relationship and mechanism of action. Environ Health Perspect 60:47-56
Sundstrom G, Hutzinger O, Safe S (1976) The metabolism of chlorobiphenyls - a review. Chemosphere 5:267-298
Suteau P, Migaud ML, Narbonne JF (1985) Sex and seasonal variations of PAH detoxication/toxication enzyme activities in marine mussels Mytilus galloprovincialis. Mar Environ Res 17:152-153
Wolf CR (1982) Oxidation of foreign compounds at carbon atoms. In: Metabolic basis of detoxication, edited by W.B. Jacoby, J.R. Bend and J. Caldwell, Academic Press, New York

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