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SHORT COMMUNICATIONS

CONJUGATIONS WITH GLUTATHIONE DISTRIBUTION OF GLUTATHIONE S-ARYL TRANSFERASE IN WILD BIRDS

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Enzymes present in the high speed supernatants of wild bird livers (pheasant, coot, great crested grebe, goosander, eider, tufted duck and common gull) conjugate chlorinated nitrobenzenes and 4 nitropyridine N-oxide with glutathione (replacement of Cl atoms or nitro groups).

3 4 dichloro nitrobenzene
2 4 dinitro chlorobenzene
2 3. 5.6 tetrachloro nitrobenzene
4 nitropyridine N-oxide

enzymic replacement of
Cl or nitro groups
wild bird liver supernatant

1 INTRODUCTION

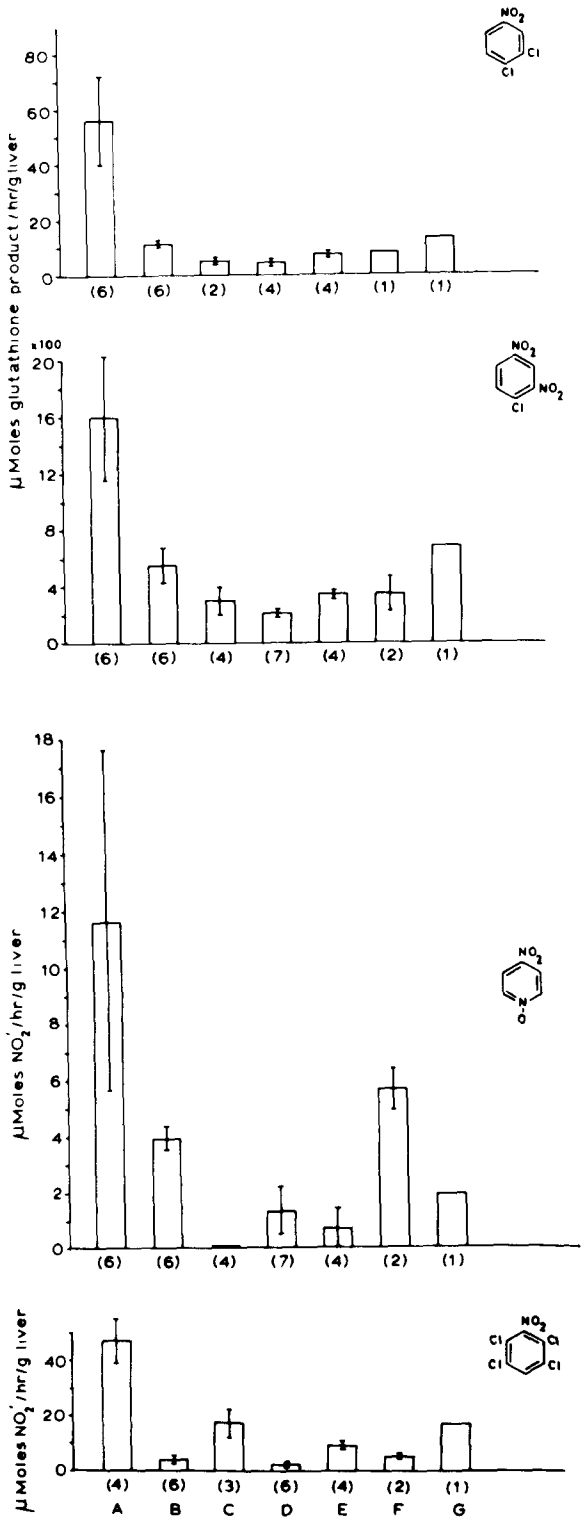
Al-Kassab, Boyland and Williams (1963) and Booth, Boyland and Sims (1961) reported enzymic glutathione conjugations with chlorinated nitrobenzenes by rat liver supernatant Grover and Sims (1964) studied the distribution of glutathione S-aryl transferase in invertebrate species. A similar study was made by Cohen, Smith and Turbert (1964) in locusts and other insects Wit and Snel (1968) demonstrated the presence of enzymes, conjugating glutathione with an epoxyde and an unsaturated compound, in wild bird livers.

The present study is a further examination of enzymes present in wild bird livers which catalyse the replacement of Cl atoms or nitro groups from chlorinated nitrobenzenes and 4 nitropyridine N-oxide.

2 METHODS

Pheasants, coots, great crested grebes, goosanders, tufted ducks and a common gull (juv.) were shot near Lelystad (Flevopolder, The Netherlands) during the winter 1967-1968. The livers were removed 1½–6 hr later and were kept on ice during the night The tissues were homogenized with 4 vol. of 0 1 M Na-K phosphate buffer (pH 7 4) and the homogenates were centrifuged for 1 hr at 70 000 *g* (ave) The clear supernatant was stored at -20^{o} unless used immediately Female eiders were shot on the Vlieland island (The Netherlands) in June 1968 The livers were removed immediately and kept in dry ice for 16 hr. After thawing, parts were taken and homogenized with 5 vol. of 0.25 M sucrose (1 mM diNa EDTA, pH 7 4) The homogenate was centrifuged for 30 min at 480 *g* (ave), 5000 *g* (ave) and 11.000 *g* (ave) to remove cell debris and coarse particles The supernatant was centrifuged for 1 hr at 78 000 *g* (ave) and the clear supernatant of this preparation was stored at -20^{o} when not used immediately

The enzyme activity towards 3 4 dichloro nitrobenzene and towards 2 4 dinitro chlorobenzene was determined according to Booth et al (1961) and Cohen et al. (1964) respectively. The enzymic conjugations of 2.3.5.6 tetrachloro nitrobenzene and of 4 nitro pyridine N-oxide with glutathione were measured by estimating the release of nitrite-ions (Al-Kassab et al., 1963) The concentrations employed were



1 25 mM and 3 75 mM for the substrates 3 4 dichloro nitrobenzene and glutathione, 1 0 mM and 5 0 mM for 2 4 dinitro chloro benzene and glutathione, 0 1 mM and 6 0 mM for 2 3 5.6 tetrachloro nitrobenzene and glutathione, 0 5 mM and 6 0 mM for 4 nitro pyridine N-oxide and glutathione respectively

3 RESULTS

The activities per *g* liver are given in fig 1 (mean ± S D)

4 DISCUSSION

On examination of the activities of wild bird liver preparations towards glutathione conjugations with an epoxyde (2 3 epoxy phenylpropylether) and with an unsaturated compound (diethylmaleate), Wit and Snel (1968) observed a remarkably high activity in pheasant liver in comparison with the livers of coots, great crested grebes, goosanders, tufted ducks and common gull The same feature was found when chlorinated nitrobenzenes and 4 nitro pyridine N-oxide were used as substrates for glutathione S-aryl transferase in wild bird liver preparations *in vitro* (fig 1) 4 Nitropyridine N-oxide, however, was not a suitable substrate for the liver preparation of the great crested grebe.

Al-Kassab et al. (1963) found a close relationship between, if not identity of, the properties of rat liver glutathione S-aryl transferase respecting the re-

Fig 1 Enzyme activities of glutathione S-aryltransferase in wild bird liver preparations. The ordinate represents the products formed per hour per *g* liver (mean ± S.D) On the abscissa the figures between brackets represent the number of bird livers examined The employed substrates are, reading from above 3 4 dichloronitrobenzene (4-Cl replaced), 2 4 dinitrochlorobenzene (Cl replaced), 4 nitro pyridine N-oxide and 2.3.5.6 tetrachloronitrobenzene (replacement of the nitro groups). A = pheasant (*Phasianus colchicus*), B = coot (*Fulica atra*), C = great crested grebe (*Podiceps cristatus*), D = goosander (*Mergus merganser*), E = eider (*Somateria mollissima*), F = tufted duck (*Aythya fuligula*) and G = common gull (juv) (*Larus canus*)

placement of Cl atoms or nitro groups Wit and Leeuwangh (1968) also found a close similarity between the glutathione S-aryl transferase properties in rat liver and pigeon liver preparations Species differences became apparent when competitive substrates were used The absence from liver preparations of the great crested grebe of an enzyme releasing NO_2-ions from 4 nitropyridine N-oxide indicates an additional species difference among birds

Wit and Snel (1968) speculated upon the possible involvement of dietary factors in the evolution of enzymes dealing with the metabolism of foreign compounds, suggesting that carnivorous birds are less likely to encounter foreign substances in their natural diet than are the herbivorous species. An extension of this view might be that birds which mainly eat seeds would be better able to inactivate foreign compounds than birds with a less specialized vegetable diet c f the pheasant and the coot. This aspect needs further attention

Most compounds containing nitro groups are manmade and it was of interest to know if natural nitro compounds were known to exist in the plant kingdom Besides chloramphenicol, references were found in the literature to azomycine (2 nitro imidazol, occurring in *Nocardia mesenterica* and in *S. eurocidicus*, see Umezawa (1967)), β-nitropropionic acid occurring in "Trailing indigo" (*Indigofera endecaphylla* Jacq) and in *Viola odorata* (Sweet violet) and the "Aristolochia acids", occurring in several species of the Aristolochiaceae (see Pailer, 1960) These findings may indicate that organic nitro compounds are met more often by herbivores than by carnivores.

The results of the present investigation demonstrate the presence of glutathione S-Aryltransferase activity in the livers of wild bird species but caution towards an extrapolation of the *in vitro* experiment to metabolic routes *in vivo* is recommended.

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