SHORT COMMUNICATION

DRUG-ENZYMES IN KESTREL LIVER

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Abstract—1. The livers of two kestrels (*Falco tinnunculus*) were examined for the presence of drug-metabolizing enzymes.

2. The supernatant [11,000 g (av.)] contained aniline hydroxylase (E.C. 1.99.1.1), *n*- and *o*-demethylase and nitro-reductase.

INTRODUCTION

ALTHOUGH biologists are concerned with the effect upon wild birdlife of the increasing use of man-made organic chemicals in the natural environment, little is known of the ability of wild birds to metabolize xenobiotic substances. Wit & Snel (1968) and Wit (1968) studied the enzymic glutathione conjugations of wild bird livers with various precursors of mercapturic acids. Wit & Leeuwangh (1969) reported that pigeons form mercapturic acids from chlorinated nitro-benzenes.

This study records the presence of drug-metabolizing enzymes in the liver of kestrels (*Falco tinnunculus*).

MATERIALS AND METHODS

Kestrels (one male and one female), kept for 18 months in this laboratory, were sacrificed by decapitation. The livers were homogenized with 4 vol. of ice-cold 1.15% KCl. The homogenate was centrifuged for 30 min at 480 g (av.), 5000 g (av.) and 11,000 g (av.). The supernatant was used as the enzyme solution. Enzyme solution (0.5 ml) was incubated with Tris-HCl buffer (50 mM, pH 7.5), MgSO₄ (5 mM), EDTA di-Na (5 mM), glucose-6-phosphate (8 mM), NADP (0.33 mM) and glucose-6-phosphate dehydrogenase (2 µg) in a final volume of 6.0 ml. The substrates employed were aniline (7.5 mM), *n*-methyl aniline (5.0 mM) in the presence of semicarbazide (3.3 mM); and *p*-nitroanisole (0.5 mM). For nitroreductase the incubation mixture contained Na-K phosphate buffer (50 mM; pH 7.5), and *p*-nitrobenzoic acid (2 mM) was used as a substrate. The flasks were pre-incubated for 15 min at 37°C to generate NADPH, before adding the enzyme. Enzyme incubation lasted 15 min at 37°C in O₂ (5% CO₂) atmosphere in case of oxidative reactions and in N₂ atmosphere for the nitro reductase assay. *p*-Aminophenol was measured by the indo-phenol blue method (Kato & Gillette, 1965). *N*- and *o*-demethylation were examined by the determination of formaldehyde by the Nash reagent (Cochin & Axelrod, 1959) and by the measurement of *p*-nitrophenol (Kato & Gillette, 1965). The amount of *p*-aminobenzoic acid was estimated after diazotation and coupling with *n*-(1-naphthyl)-ethylenediamine (Bratton & Marshall, 1939).

RESULTS

The results (µmoles product/15 min per g liver) are given in Table 1.

DISCUSSION

As shown in Table 1, kestrel liver contains enzymes which hydroxylate aniline, demethylate *n*-methylaniline and *p*-nitro-anisole and reduce the nitro-group of *p*-nitrobenzoic acid. These findings indicate that this predatory species is able to handle xenobiotic substances. As a consequence, kestrels may detoxify or may increase the toxicity of foreign compounds, depending upon the biological activity of the resulting metabolites. Because of the small number of birds examined, the present preliminary study does not provide a more general indication of the ability of the kestrel to eliminate substances foreign to the body.

TABLE 1—ENZYME ACTIVITY OF THE 11,000 g (AV.) SUPERNATANT OF THE KESTREL LIVER (HYDROXYLATION OF ANILINE; DEMETHYLATION OF *n*-METHYLANILINE AND *p*-NITRO-ANISOLE; REDUCTION OF *p*-NITROBENZOIC ACID)

Substrate	Reaction	Female	Male
Aniline	<i>p</i> -Hydroxylation	250	570
<i>n</i> -Methylaniline	<i>n</i> -Demethylation	1400	2200
<i>p</i> -Nitro-anisole	<i>o</i> -Demethylation	420	480
<i>p</i> -Nitrobenzoic acid	NO ₂ group reduction	120	80

Experimental conditions are given in Materials and Methods. The values represent $\mu\text{moles}/15 \text{ min per g liver}$.

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REFERENCES

- BRATTON A. C. & MARSHALL E. K. (1939) A new coupling component for sulfanilamide determination. *J. biol. Chem.* **128**, 537–550.
- COCHIN J. & AXELROD J. (1959) Biochemical and pharmacological changes in the rat following chronic administration of morphine, nalorphine and normorphine. *J. Pharmac. exp. Ther.* **125**, 105–110.
- KATO R. & GILLETTE J. R. (1965) Effect of starvation on NADPH-dependent enzymes in liver microsomes of male and female rats. *J. Pharmac. exp. Ther.* **150**, 279–284.
- WIT J. G. (1968) Conjugations with glutathione. Distribution of glutathione S-aryltransferase in wild birds. *Eur. J. Pharmac.* **5**, 100–102.
- WIT J. G. & LEEUWANGH P. (1969) Mercapturic acid formation and enzyme-catalyzed conjugations with glutathione in pigeons. *Biochim. biophys. Acta* **177**, 329–335.
- WIT J. G. & SNEL J. (1968) Enzymatic glutathione conjugation with 2,3-epoxyphenyl propylether and diethylmaleate by wild bird liver supernatant. *Eur. J. Pharmac.* **3**, 370–373.

Key Word Index—Liver; *Falco tinnunculus*; enzymes; detoxication; aniline hydroxylase; *n*- and *o*-demethylase; nitro-reductase.