Steroid Sex Hormones as Inhibitors of Aflatoxin Metabolism in Liver Homogenates

In an earlier paper[1] it was demonstrated that rabbit and avian livers contain a soluble NADP-linked enzyme system capable of reducing aflatoxin B_1 to aflatoxicol (Figure 1) and aflatoxin B_2 to dihydro-aflatoxicol. Because of the general structural similarity between aflatoxin and C17-steroid molecules first noted by WILLIAMS and RABIN[2] in their work on the competitive binding of aflatoxin to sex determined sites on the endoplasmic reticulum of hepatocytes, it seemed probable that NADP-linked 17-hydroxy steroid dehydrogenase (E.C.1.1.1.51) was involved in this reaction. The present communication reports that androstenedione, androstenetrione and oestrone are all capable of inhibiting the in vitro reduction of aflatoxin by the 105,000 *g* supernatant fraction of rabbit and avian livers and that these livers also possess $NADPH_2$-linked 17-ketosteroid reductase activity (i.e. the reverse 17-hydroxysteroid dehydrogenase reaction; see Figure 1).

A procedure for the preparation of liver homogenates in 0.15 *M*KCl and the spectrophotometric assay of aflatoxin reduction by 105,000 *g* supernatant fractions of liver from chicken, duck, guinea-pig, mouse, rabbit, rat and turkey, and thin layer chromatographic identification of metabolites were described in the earlier paper[1]. The same assay method has now been applied to livers of sheep and Japanese quail. Δ_4-androstene-3:17-dione, Δ_4-androstene-3:11:17-trione and oestrone used as inhibitors or substrates were added as 0.1 ml methanolic solution to the incubation medium, 5 ml of which contained NADP, 1 µmole; glucose-6-phosphate, 40 µmoles; glucose-6-phosphate dehydrogenase, Boehringer, 2 µl; magnesium chloride ($MgCl_2$), 25 µmoles; sodium phosphate buffer, pH 7.4, 140 µmoles; aflatoxin B_1 approximately 0.13 µmoles. When measuring 17-ketosteroid reductase activity, aflatoxin was omitted from the medium and thin layer chromatography provided the means of determining visually the approximate proportion of steroid converted to an identifiable metabolite. Silica gel plates with or without fluorescent additive were used, chromatograms were developed in acetone: chloroform (1:9 by volume) and testosterone was observed as a UV absorbing spot, while oestradiol was visualized as 2 pink spots after spraying with acetic-anhydride-H_2SO_4[3]. Sometimes $NADPH_2$ was incorporated in the medium in place of the regnerating system (glucose-6-phosphate dehydrogenase), when the rate of $NADPH_2$ oxidation was followed at 340 nm in a recording spectrophotometer (Pye-Unicam SP 800) thermostatically controlled at 37 °C.

As shown in Figure 2, androstenedione, androstenetrione and oestrone inhibited the bio-reduction of aflatoxin by 54.4, 16 and 49% respectively when added to incubation mixtures at approximately the same concentration (0.026 m*M*) as the aflatoxin B_1 substrate. At concentrations above 0.052 m*M* aflatoxin inhibited its own reduction, but by varying substrate concentrations below this value it was determined that the apparent Km for this reaction was approximately 9.3 µ*M*. In the presence of 0.026 m*M* androstenedione, this was increased to 47.6 µ*M*. By inspection of a Lineweaver-Burke plot, the inhibition appeared to be of the simple competitive type.

Livers of species that reduce aflatoxins via this $NADPH_2$-dependent soluble enzyme system also reduce androstenedione and oestrone. In the Table approximate levels of 17-ketosteroid reductase activity are given. Pairs of assays were made using livers from male and female animals but no consistent sex differences were observed.

It had already been shown that $NADH_2$ was a poor substitute for $NADPH_2$ as co-factor for the in vitro reduction of aflatoxin[1] and in the few instances where it was investigated these soluble liver fractions were found to reduce 17-ketosteroids more rapidly in the presence of $NADPH_2$ than $NADH_2$. Rates determined spectrophotometrically for duck liver were 0.580 µmole androstenedione per g/min with $NADPH_2$, and 0.129 µmole per g/min with $NADH_2$.

Androstenedione inhibited aflatoxin reduction by the livers of species capable of this biotransformation but 17-ketosteroid reductase activity was also measurable in mouse, guinea-pig and sheep livers where little or no aflatoxin reductase activity could be detected (Table). It therefore appears that the same soluble $NADPH_2$-linked reductase is probably involved in both sex hormone and aflatoxin reductions but it has a greater affinity for

[1] D. S. P. PATTERSON and B. A. ROBERTS, Fd Cosmet. Toxicol., *9*, 829 (1972).
[2] D. J. WILLIAMS and B. R. RABIN, FEBS Lett. *4*, 103 (1969).
[3] E. WALDI, in *Thin Layer Chromatography: a Laboratory Handbook* (Ed. E. STAHL; Springer-Verlag, Berlin 1965), p. 249.

$NADPH_2$-dependent reduction of 17-ketosteroids by 105,000 *g* supernatant liver fractions

	Aflatoxin [a] reducing activity	Inhibitory effect of 17-ketosteroids	Proportion of substrate metabolized in 30 min at 37 °C [b] (%)	
			Androstenedione [c]	Oestrone [d]
Chicken	Highly active	+	20	10
Duck		+	12.5	11.5
Quail		+	50	11
Rabbit		+	20	3
Turkey		+	40	8
Guinea-pig	Inactive	0	20	4.5
Mouse		0	6	10
Rat		0	2	0
Sheep		0	60	3

[a] Aflatoxin B_1 reduced to aflatoxicol (i.e. cyclopentenone reduction)[1]. [b] Incubation mixture (see methods section) contained one or other hormone (approximately 0.05 m*M* final concn.) and 105,000 *g* liver supernatant equivalent to 150 mg liver tissue. [c] The major metabolite was testosterone. [d] Oestradiol-17α and oestradiol-17β formed (predominantly the latter).

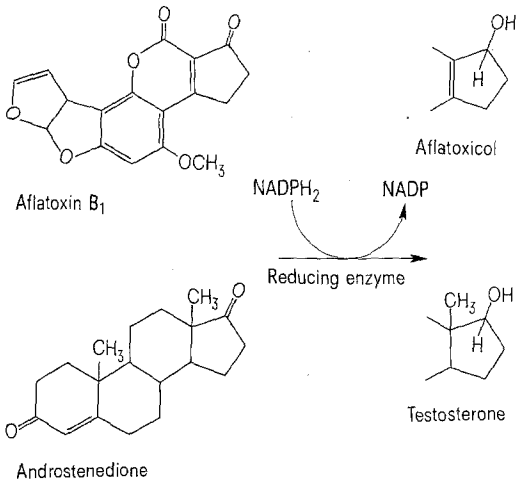

Fig. 1. $NADPH_2$-linked enzymic reduction of aflatoxin B_1 to aflatoxicol and of androstenedione to testosterone. Structural formulae have been drawn to the same scale in order to indicate the broad similarities in substrate configuration.

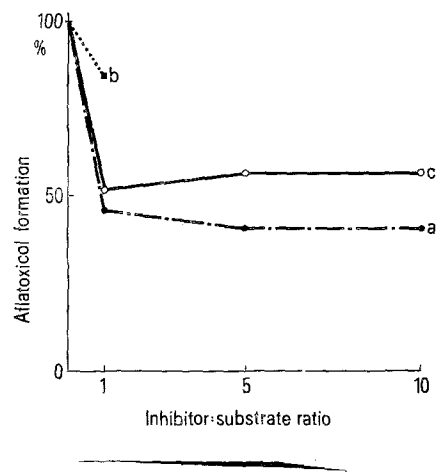

Fig. 2. The inhibition of in vitro aflatoxicol formation by androstenedione (a), and androstenetrione (b), and oestrone (c) in a duck liver homogenate. Substrate concentration: 0.026 mM aflatoxin B_1. 0.05 ml of 105,000 g supernatant liver fraction (10 mg tissue) incubated 15 min at pH 7.4 and 37 °C. Uninhibited rate of aflatoxicol formation: 60 nmoles/g liver/min.

aflatoxin in avian and rabbit livers than in guinea-pig, mouse, and sheep. Each one of the latter species metabolizes the toxin through microsomal pathways (although the major products of metabolism differ[4]) and unlike rabbit and avian livers there is no alternative pathway in the cytosol. Besides having an affinity for microsomal enzymes, aflatoxin appears to compete with steroid sex hormones for binding sites on the endoplasmic reticulum in the rat[2,5] and this may also be true of other species. However, in avian and rabbit livers a soluble enzyme, probably 17-hydroxysteroid dehydrogenase, also competes for aflatoxin. This would tend to modify any toxic effects arising from interaction with the endoplasmic reticulum[6].

Zusammenfassung. Die im Zytoplasma der Vogel- und Kaninchenleber enthaltene NADP-abhängige 17-Hydroxysteroid Dehydrogenase beteiligt sich wahrscheinlich am extramikrosomalen Stoffwechsel des Aflatoxins. Die enzymatische Reduktion des von Aflatoxin B_1 stammenden Cyclopentenons bildet den sekundären Alkohol Aflatoxikol. Diese Reaktion wird durch Androstendion und Oestron gehemmt.

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[4] D. S. P. PATTERSON, Biochem. J. *125*, 19P (1971).
[5] B. R. RABIN, G. H. SUNSHINE and D. J. WILLIAMS (1970), in *Chemical Reactivity and Biological Role of Functional Groups in Enzymes* (Ed. R. M. S. SMELLIE Biochemical Society Symposium No. 31, Academic Press, London 1970).
[6] D. J. WILLIAMS and B. R. RABIN, Nature, Lond. *232*, 102 (1971)

Effect of Lithium Chloride on the Rat's Paw Edema Induced by Serotonin, Histamine and Formalin

Recently it has been reported from this laboratory that lithium chloride has an analgesic and hypothermic effect in rats when given parenterally[1]. It enhances the analgesic effect of morphine but inhibits its hyperthermic action. This effect has been shown to be somehow related to the effect of lithium ion on brain monoamines, which has been taken into consideration by many authors to explain the ameliorative effect of lithium in the treatment of mania and aggressive behavior [2–5].

Since lithium chloride has an analgesic effect in rats, it appeared of interest to investigate whether this ion also has an anti-inflammatory effect. The present paper describes the results of this investigation.

Methods. Experiments were performed on white male rats from a homogenous strain weighing 150 to 250 g. The animals were fed with a standard rat food and allowed to drink ordinary water *ad libitum*. Experiments were carried out at room temperature (21°C). A group of rats were bilaterally adrenalectomized under ether anesthesia 24 h before the experiments. These animals were given physiological saline solution for drinking. The difference in volume of paw was obtained by measuring the displacement of water level in a specially designed graduated

[1] F. C. TULUNAY, B. K. KIRAN and S. KAYMAKÇALAN, Acta Med. turc. *8*, 51 (1971).
[2] S. GERSON and A. YOWILER, J. Neuropsychiat. *1*, 229 (1960).
[3] M. SCHOU, in *Antidepressant Drugs* (Eds. S. GARRATINI and M. N. G. DUKES, Excerpta Medica Foundation, Amsterdam 1967), p. 80.
[4] M. Z. WEISCHER, Psychopharmacologia *15*, 162 (1967).
[5] M. H. SHEARD, Nature, Lond. *228*, 284 (1970).