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A STUDY INTO THE EFFECTS OF 2-ACETYLAMINOFLUORENE ON HEPATIC MONOOXYGENASE ACTIVITIES IN THE CHICK EMBRYO

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Abstract—2AAF is a potent inducer of cytochrome P-450 in the chick embryo liver. The induction has been characterized with respect to a range of monooxygenase activities and the regiospecificity of 2AAF hydroxylation. Similarities to the response elicited by both PB and 3MC were noted. 2AAF was rapidly deacetylated by hepatic microsomes prepared from control animals to 2AF, an inhibitor of monooxygenase activity. Metabolites generated *in vivo* and carried over *in vitro* might have therefore interfered with the subsequent kinetic analysis. In general terms induction of a unique cytochrome P-450 subform(s) could not be attributed to 2AAF in the chick embryo.

The data is discussed with respect to the reported resistance of avian species to the hepatocarcinogenic effects of 2AAF. Two possibilities are highlighted, a diversion of 2AAF to ring hydroxylated metabolites and/or deacetylation of 2AAF. Both effects could reduce carcinogenicity by decreasing the concentration of proximate carcinogen and/or promoter(s).

2AAF is a potent hepatocarcinogen activated by the sequential action of phase 1 and phase 2 xenobiotic metabolizing enzymes. Conversion to the N-hydroxy derivative and sulphoconjugation or transacetylation is thought to produce the proximate carcinogen [1]. In contrast ring hydroxylation is considered to result in detoxification. The spectrum of hepatic cytochrome P-450 subforms may therefore regulate the balance of competing activation/inactivation pathways [2]. Treatment of rats with 3MC prior to challenge with 2AAF, for example, may reduce the carcinogenic potential of 2AAF by enhancing the rate of ring hydroxylation to a greater extent than *N*-hydroxylation. 2AAF is itself reported to induce cytochrome P-450, which might be significant in terms of its toxicity, but the data is inconsistent. Astrom and DePierre found a 1.4-fold induction of cytochrome P-450 with a concomitant increase in the rate of both ring and *N*-hydroxylation [3]. Using the same strain of rats but an altered induction regime Malejka-Giganti *et al.* [4] found no increase while Cameron *et al.* reported induction using a different strain of rat [5]. Others have suggested that enhancement of *N*-hydroxylating activity occurs without an increase in total cytochrome P-450 concentration [6].

In a recent report we showed that administration of 2AAF to chick embryos produced a 3-fold increase in hepatic microsomal cytochrome P-450 concentration [7]. Given the size of change and its reproducibility we have used the chick embryo to characterize the induction of this haemoprotein by 2AAF. In this study we examine the effects of 2AAF administration on a range of monooxygenase activities in chick embryo livers. In comparison with PB, 3MC and AIA. We confirm the cytochrome P-450 induction potential of the compound and discuss the data in terms of the subform/s of cytochrome P-450 induced and its significance to the activation of 2AAF *in vivo*.

MATERIALS AND METHODS

Materials. Fertilized hens eggs were obtained from Orchard Farm Gt. Missenden, Bucks, U.K. Sources of specialist reagents were as follows: Aldrich, 2AF, biphenyl, 4-hydroxybiphenyl, 7-hydroxycoumarin, *N,N*-dimethylnitrosamine; Koch-Light, benzo[*a*] pyrene; Boehringer, 7-ethoxycoumarin; Eastman-Kodak, *p*-nitroanisole; British Drug Houses, phenobarbital. We thank Dr P. Simms (Chester Beatty Institute, Sutton, Surrey) for providing 3-hydroxybenzo[*a*]pyrene; Dr S. Thorgeirsson (National Cancer Institute, Bethesda, U.S.A.) for 2AAF metabolite standards, Ciba-Geigy for desferrioxamine mersylate; Roche Products for AIA and May & Baker for ethylmorphine. (9-C^{14})-2AAF (50 mCi/mmole) and (7(*n*)-H^3)-2AAF (20 Ci/mmole) were obtained from Amersham International. The former was purified, by TLC, prior to use. All other reagents were from the Sigma Chemical Co. and of the highest grade available.

Treatment of animals and microsome preparation. Embryos were derived from a cross between White Leghorn and Rhode Island Red chickens. Seventeen days after commencing incubation, inducing agents were administered in 0.2 ml DMSO, into the fluid surrounding the embryo at the following doses; PB, 10 mg; 2AAF, 8 mg; 3MC, 2 mg; AIA, 3 mg. Controls received the vehicle alone. Twenty-four hours later the livers were removed and microsomes prepared as described by us previously [7].

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† Abbreviations used: 2AAF, 2-acetylaminofluorene; 2AF, 2-aminofluorene; AIA, allylisopropylacetamide; DMSO, dimethylsulphoxide; 3MC, 3-methylcholanthrene; PB, phenobarbital.

Monooxygenase activities. Assays were performed at 37° in a final volume of 0.4 ml. Each incubation contained an NADPH regenerating system (of 0.5 mM NADP, 5 mM magnesium chloride, 5 μM manganese chloride, 5 mM isocitrate and 0.5 units of isocitrate dehydrogenase) and NADH (1 mM) in 20 mM phosphate buffer pH 7.5. Substrates were dissolved in distilled water, acetone or methanol. Total addition of organic solvent to incubations did not exceed 2% of the reaction volume. The final concentration of substrates used and references describing product measurement were: 1 mM aminopyrene [8]; 0.1 mM benzo[*a*]pyrene [9]; 1 mM biphenyl [10]; 1 mM coumarin [11]; 5 mM *N,N*-dimethylaniline [8]; 5 or 200 mM dimethylnitrosamine [8]; 1 mM ethoxycoumarin [12]; 8 mM ethylmorphine [8]; 5 mM hexobarbital [8] and 1 mM *p*-nitroanisole [13]. The amount of microsomal protein added was in the range of 200–400 μg.

Inhibition studies. Monooxygenase activity was measured as described above except that 2AF was added to the final concentration of 250 μM.

2AAF metabolism. The reaction mixture contained NADPH (2 mM), NADH (1 mM), sodium fluoride (100 mM), and microsomes (1 mg) in 20 mM sodium phosphate buffer in a final volume of 1 ml. After 2 min incubation at 35° the assay was initiated by addition of 2AAF dissolved in acetone (0.005 ml). Control incubations received the vehicle alone. Incubation was continued for a further 20 min, the reaction quenched by addition of 1 M sodium acetate (1 ml), pH 6.5 and metabolites extracted with two 5 ml aliquots of (peroxide free) ether. Solvent was removed in a vacuum oven and the residues resuspended in 1 ml buffer A (23% (v/v) propan-1-ol, 0.1% (w/v) desferrioxamine mesylate and 0.15% (v/v) acetic acid). Metabolites were resolved by HPLC analysis (using a 30 cm Waters, Fatty Acid Analysis Column, eluted isocratically with buffer A at a flow rate of 2 ml/min), eluate was monitored at 280 nm and individual metabolites identified on the basis of their retention time.

2AAF deacetylase activity was determined using 100 nmoles of substrate per assay. Product formation was quantified by reference to a calibration curve of peak height versus 2AF concentration. In the radiochemical assay, used to measure the rate of 2AAF hydroxylation, the substrate comprised 12×10^4 dpm (9-C^{14})-2AAF, 7×10^6 dpm (7(*n*)-H^3)-2AAF and unlabelled 2AAF added to a final concentration of 20 nmoles. Prior to HPLC analysis 1-OH, 5-OH and 3-OH-2AAF were added to metabolite extracts. Eluate from the column was collected and fractions containing individual metabolites bulked for scintillation counting. Metabolite concentrations were calculated from activities which had been corrected for quenching effects using the external standard facility available on the instrument.

Liquid scintillation counting. Samples, dissolved in buffer A, were added directly to scintillant (PCS, Amersham International) and counted (Beckman LS 7800 Liquid Scintillation System) using a C^{14}/H^3-dual label program. Counting efficiencies were 92% for C^{14} and 35% for H^3; more than 90% of added activity was routinely recovered.

Cytochrome P-450 *determination*. Total (spectrophotometric) concentrations of cytochrome P-450 were determined as described by Estabrook *et al.* [14].

Protein determination. Microsomal protein concentration was measured according to Lowry *et al.* [15].

RESULTS

Cytochrome P-450 induction regimes were chosen such that a maximal increase in the level of this haemoprotein occurred for each compound over the time course of treatment [7]. We observed a marked induction of hepatic cytochrome P-450 by 2AAF (2.6-fold; Table 1), an effect obtained reproducibly over a period of 2 years using different batches of 2AAF. In a previous study using a Ross 1 strain of embryos a 3.4-fold increase was recorded [7]. The marked induction observed in chick embryos contrasts with the small increases (1.4-fold) reported in rats [3, 16].

Monooxygenase activities

In preliminary experiments conditions for each monooxygenase assay were optimized to ensure that the reaction was linear over the time course of the assay. Microsomes prepared from either control or induced embryos hydroxylated biphenyl only at the 4-position, which is in agreement with the report by Rifkind *et al.* [17]. Since "high" and "low" affinity forms of dimethylnitrosamine *N*-demethylase have been reported this activity was measured at two substrate concentrations [18]. We found no detectable coumarin hydroxylase activity in microsomes prepared from control or induced animals; this activity appears to be species dependent however [11].

The data in Table 1 are pooled values for microsomes prepared from livers of male and female embryos; sex-linked differences were not found in any of the activities measured. It is evident from the data that PB and 3MC have distinct effects on hepatic monooxygenase activities. PB produces the greatest stimulation of ethylmorphine and hexobarbital *N*-demethylation (6.6- and 4.3-fold respectively) and 3MC stimulates primarily benzo[a]pyrene hydroxylation (5.9-fold). AIA corresponds with PB in inducing ethylmorphine and hexobarbital demethylation maximally (8.9- and 4.9-fold respectively). Induction of aminopyrene, dimethylaniline, benzo[*a*]pyrene and biphenyl phase 1 metabolism ($\leq$2 fold) was also noted after PB treatment, while 3MC also stimulated ethylmorphine and dimethylaniline demethylation and biphenyl hydroxylation (1.3–1.4-fold). 2AAF showed a number of features in common with both PB and AIA. Thus like PB there was maximal induction of ethylmorphine and hexobarbital demethylase activity (4- and 2.5-fold respectively) and weaker induction (1.3- and 1.9-fold) of demethylaniline demethylation and benzo[*a*]pyrene hydroxylation.

As with AIA, but not PB or 3MC, 2AAF reduced the rate of dimethylnitrosamine demethylation (0.4-fold) and biphenyl hydroxylation (0.7-fold). Additionally 2AAF treatment reduced the rate of ethoxycoumarin and *p*-nitroanisole demethylation

Table 1. Effects of PB, 2AAF, 3MC and AIA on hepatic monooxygenase activities in the chick embryo

Activity	Control	PB	2AAF	3MC	AIA
Cytochrome P-450*	237 ± 23	1037 ± 170‡	622 ± 100**	477 ± 82	1166 ± 193**
N-Demethylase					
Aminopyrene	5.6 ± 1.4	11.7 ± 2.5§	7.7 ± 1.4	8.0 ± 1.5	nd
Dimethylaniline	15.6 ± 1.5	31.9 ± 2.2‡	20.4 ± 0.9‡	20.8 ± 1.2‡	35.5 ± 1.6‡
Dimethylnitrosamine					
5 mM	2.7 ± 0.4	3.0 ± 0.6	1.1 ± 0.3	2.9 ± 0.2	1.8 ± 0.1§
200 mM	6.4 ± 0.4	5.7 ± 0.6	3.7 ± 0.3‡	7.4 ± 0.2	3.9 ± 0.5§
Ethylmorphine	3.3 ± 0.4	21.8 ± 0.3‡	13.1 ± 1.0‡	5.0 ± 0.1‡	29.4 ± 1.6‡
Hexobarbital	7.7 ± 0.3	32.9 ± 3.1‡	19.3 ± 0.7§	8.8 ± 1.0	37.5 ± 2.8§
O-Dealkylase					
Ethoxycoumarin	2.3 ± 0.3	2.4 ± 0.2	1.0 ± 0.1§	2.1 ± 0.2	nd
p-Nitroanisole	2.0 ± 0.2	2.3 ± 0.1	1.3 ± 1.0‡	2.0 ± 0.1	2.1 ± 0.3
Aromatic Hydroxylase					
Benzo[*a*]pyrene†	91 ± 12	156 ± 18‡	170 ± 18‡	540 ± 44‡	107 ± 12
Biphenyl	3.1 ± 0.3	4.3 ± 0.4‡	2.0 ± 0.1‡	4.3 ± 0.4	2.0 ± 0.3
Coumarin	bd	bd	bd	bd	bd

Methods are described in the text. Each assay was performed on microsomes prepared from at least four different groups of animals.

* pmole/mg protein.

† pmole 3-OH benzo[*a*]pyrene/min/mg protein.

All other activities were nmole product/min/mg.

§ $P \leq 0.05$.

‡ $P \leq 0.01$.

(0.4- and 0.7-fold). We have considered three possible causes for this effect: (i) that administration of 2AAF causes the destruction of cytochrome P-450; (ii) inhibition of activity by 2AAF and/or its metabolites and (iii) a change in the spectrum of cytochrome P-450 subforms unique to 2AAF. We were able to test for two of these possibilities directly (i and ii).

AIA is known to convert the haem moiety of cytochrome P450 to "green pigments" with ensuing reduction in monooxygenase linked activity [17, 19]. Loss of hepatic haem and cytochrome P-450 on AIA administration to chick embryos has also been demonstrated [17]. When embryos were challenged with 2AAF no loss of cytochrome P-450 was observed up to 4 hr after administration. This contrasts with AIA where effects are reported after 0.5–1 hr. We cannot, however, exclude the possibility that minor subforms of cytochrome P-450 were effected, which might not have been detectable by us, or that destruction of haem by 2AAF is slower than by AIA.

Direct metabolite inhibition of mixed function oxidation has been reported for α-naphthoflavone in the rat [20]. In view of the reduction found in some activities on treatment with 2AAF we investigated the possibility of a similar effect by 2AAF. Preliminary experiments established that 2AAF was rapidly deacetylated by chick liver microsomes (4.8 ± 0.7 nmoles 2AF produced/min/mg protein as compared with 0.07 nmoles 2AF produced/min/mg protein for Sprague–Dawley rat liver microsomes [21]). In view of these findings the inhibition potential of both 2AAF and 2AF for monooxygenase linked activities was examined. 2AAF did not inhibit *p*-nitroanisole-*O*-demethylation (Table 2); indeed at low concentrations slight activation (approximately 10–20%) was observed (data not shown). In contrast 2AF reduced this activity by 40%, as compared with 78% by α-napthoflavone and 24% by metyrapone. The degree of inhibition varied with the substrate used, however. Dimethylaniline demethylation was inhibited by 13% and benzo[*a*]pyrene hydroxylation by 75% for example (Table 2). Since primary amines are able to form a complex with the haem moiety of cytochrome P-450, and inhibit mixed function oxidation [22] the apparent inhibition observed could result from 2AAF deacetylation *in vivo* followed by binding of 2AF (or other metabolites) to cytochrome P-450. 2AF added to microsomes, prepared from control embryos, was in fact found to produce a type 2b difference spectrum, with an absorbance maximum at 432 nm. If this were the underlying mechanism it implies a high degree of selectivity in terms of the subforms which are inhibited.

Table 2. Inhibition of monooxygenase activities in hepatic microsomes from control embryos

Activity	% Inhibition (relative to control)
P-Nitroanisole-*O*-demethylase	
+2 AF	40 ± 4
+2 AAF	0
+α-Naphthoflavone	78
+Metyrapone	24
Ethylmorphine-*N*-demethylase	
+2 AF	20 ± 7
Dimethylaniline-*N*-demethylase	
+2 AF	13 ± 2
Benzo[*a*]pyrene hydroxylase	
+2 AF	75 ± 8

Assay methods are described in the text. Test compounds were added to the assays at a final concentration of 250 μM. Each assay was performed on microsomes prepared from at least 3 different groups of animals. The degree of inhibition is expressed as a percentage of the control assay rate.

2AAF Metabolism

Due to the high 2AAF deacetylase activity in chick embryo hepatic microsomes the concentration of 2AAF available for hydroxylation is difficult to regulate. Sodium fluoride can be used to inhibit deacetylase activity, however [21, 23]. Under the conditions we describe 96% inhibition of activity occurred (to 0.2 ± 0.05 nmoles 2AF produced/min/mg protein). Under these conditions the major metabolites of 2AAF produced by microsomes prepared from control animals were 9-OH, 7-OH, 5-OH, 3-OH, 1-OH and N-OH 2AAF (Table 3), with the 9-OH and 7-OH 2AAF predominating. This in agreement with *in vivo* and *in vitro* studies using other species [21, 24].

Treatment with inducers produced differences in the amounts and spectrum of metabolites formed during the time course of the assay. PB only increased 7 plus 9 hydroxylation relative to the control whereas 3-MC increased the rate of production of all metabolites but 5-OH (×28), 1-OH (×10) and N-OH (×17.5) 2AAF in particular. Challenge with 2AAF also increased production of all metabolites particularly 7-OH plus 9-OH (×5), 5-OH (×8) and N-OH (×4) 2AAF. The HPLC separation we used did not resolve 7-OH 2AAF from 9-OH 2AAF, we monitored their relative ratio in metabolite mixtures by dual label counting, however. The substrate comprised 2AAF labelled at C-7 with H^3 mixed with 2AAF labelled with C^{14} at C-9, the ratio of C^{14} to H^3 in the 7-OH plus 9-OH 2AAF peak provides a measure of the relative amounts of 7-OH and 9-OH 2AAF. A change in concentration of 7-OH or 9-OH 2AAF would alter this ratio (assuming the mechanism of hydroxylation remained unaltered). No significant change from the control ratio (0.54) was found on treatment of animals with PB, 2AAF or 3MC which suggests unaltered relative rates of 7 and 9-hydroxylation in microsomes prepared from treated animals.

DISCUSSION

We confirm the induction of cytochrome P-450 in the chick embryo liver by 2AAF. Based on the measurement of (ten) monooxygenase activities (Table 1) it appears to elicit a "PB-type" response. Difference in induction profiles between PB and 2AAF could have been due to (highly selective) inhibitory effects of 2AAF metabolites contaminating microsomes, prepared from treated animals, and/or an altered spectrum of cytochrome P-450 subforms reflecting a unique induction profile for 2AAF. The regioselectivity of 2AAF hydroxylation also reflect these differences (Table 3). Chick microsomes were found to produce a similar range of metabolites and at rates comparable to those reported for rat microsomes in refs 3 and 16, but significantly lower than reported by Hara *et al.* [25] and Shut and Thorgeirsson [21]. Possible reasons for such differences have been discussed by Astrom *et al.* [3]. Based on the amount of cytochrome P-450 present in each assay, all the compounds tested selectively induce 2AAF metabolizing potential. Given that the major PB induced form in rabbit has no catalytic activity with 2AAF (<1% that of other forms) [26] and the report that the 7, 5, 3, 1, and *N*-hydroxylation of 2AAF are catalysed by a unique 3MC induced cytochrome P-450 subform (or several with common antigenic determinants) [27] the increased rate of 2AAF metabolism on PB and 2AAF treatment could relate to induction of benzo[*a*]pyrene hydroxylase activity. Differences between 2AAF and PB in enhancing (2AAF) ring hydroxylating capacity could reflect unique induction features or a limited 3MC-type response by 2AAF. Given the marked inhibition of benzo[*a*]pyrene hydroxylase activity by 2AF and the similarity in 2AAF metabolite profiles generated by animals treated with either 3MC or 2AAF, 2AAF may need to be considered a mixed PB-3MC type of inducer in the chick embryo. In general terms the data does not provide positive evidence for the induction of a unique cytochrome P-450 subform(s) in contrast to studies using a rat model system [3].

Deacetylation and transacetylation of 2AAF is suggested to favour production of the proximate mutagen and sulphoconjugation production of promoter(s) [28]. The marked 2AAF deacetylase activity of chick embryo microsomes, as compared with rat microsomes (see above), may play a significant role in the reported resistance of avian species to 2AAF mediated carcinogenicity [25]. By converting 2AAF to 2AF net flux through promoter production pathway(s) would be reduced. Although net production of mutagen would increase, a reduced concentration of the carcinogenic promoter could

Table 3. Hydroxylation of 2AAF by microsomes prepared from control and induced embryos

	2AAF Hydroxy product (pmoles/20 min/mg protein)					
	7 + 9	5	3	1	N	Total
Treatment						
None	1200 ± 100	160 ± 20	10 ± 4	10 ± 4	40 ± 30	1420
PB	5200 ± 400	90 ± 10	10 ± 10	10 ± 5	30 ± 10	5340
	(4.3)	(0.56)	(1)	(1)	(0.75)	(3.7)
2 AAF	6000 ± 700	1300 ± 200	30 ± 6	30 ± 4	160 ± 30	7520
	(5)	(8.1)	(3)	(3)	(4)	(5.4)
3 MC	6800 ± 1000	4500 ± 900	70 ± 30	100 ± 20	700 ± 20	12200
	(5.7)	(28)	(7)	(10)	(17.5)	(8.7)

Assays were performed in the presence of 100 μM sodium fluoride, products were quantitated by HPLC analysis, each assay was performed with microsomes from at least three sets of animals.

decrease tumorogenesis. In this respect it is interesting to note that hepatic microsomes from Cotton rats, which are also resistant to 2AAF mediated hepatocarcinogenicity, show higher N-OH 2AAF deacetylase activity and lower sulphoconjugation capacity than microsomes from more susceptible Sprague–Dawley rats [21]. Indeed Haug *et al.* have shown that paraoxon, a powerful deacetylase inhibitor, blocks the mutagenic potential of 2AAF and its *N*-hydroxy derivative in the presence of chick liver microsomes [23].

2AAF metabolism by hepatic cytochrome(s) P-450 may also be involved in resisting hepatocarcinogenicity. In discussing the data comment is necessary on the effects of sodium fluoride on cytochrome P-450 linked systems. Seal and Gutman [29] report that it acts by inhibiting deactylation rather than stimulating hydroxylation. A finding consistent with the comparable range of 2AAF metabolites found in studies using sodium fluoride and those which do not (compare for example the data in [21] and [16]). 2AAF is subject to hydroxylation by two competing pathways. Ring hydroxylation favours detoxication while *N*-hydroxylation is the first, and obligatory, step in carcinogenesis. By directing 2AAF to the former at the expense of the latter its carcinogenic potential may be reduced and/or inhibited. The ratio of ring to *N*-hydroxylated metabolites generated using microsomes prepared from control animals was 34:1 (Table 3). In the (highly susceptible) Sprague–Dawley rat we calculate a ratio in the range of 6 to 25:1 from the data in [3, 16, 21, 24]. Cotton rat liver microsomes produce a ratio of only 8:1 however [21]. Equally the specific activity of individual hydroxylating reactions may be important to resistance. In the chick embryo the major metabolites (7 + 9-hydroxy 2AAF) are produced at a rate 300-fold greater than *N*-hydroxy 2AAF. This compares with a 6–19-fold difference in the Sprague–Dawley rat [3, 16, 21, 24]. Again data from a study using Cotton rats are inconsistent with this view; a ratio of only 5:1 is suggested by the data in [21]. The differences between the chick embryo and Cotton rat, in 2AAF metabolising potential, may reflect differences in the underlying protective mechanism or in the spectrum of hepatic cytochrome P-450 isozymes. It is interesting to speculate, however, that the high rate of 2AAF deacetylation (linked to the cytochrome P-450 inhibitory potential of 2AF) may be the key event in protection in both species.

Treatment of animals with 2AAF alters the spectrum of cytochrome P-450 isozymes present in the livers, as evidenced by the changes in monooxygenase activity observed (Table 1). This could be of direct consequence to 2AAF metabolism *in vivo*. On treatment with the compound only small differences were found in individual specific activities (Table 3); cumulatively they produced a 1.4-fold increase in the ratio of ring to *N*-hydroxylated metabolites (to 46:1). Clearly induction of cytochrome P-450 by 2AAF alters the profile of metabolites such as to favour the production of less toxic derivatives of 2AAF. Astrom and De Pierre found a 105-fold increase in the specific activity of (2AAF) 5-hydroxylating capacity for microsomes prepared from 2AAF pre-treated rats [16] which suggests it may also have a protective effect in the rat. Although the ratio of ring to *N*-hydroxylated metabolites remains significantly lower than in the chick (9:1 as compared with 46:1).

To our knowledge there are no reports detailing the effects of pre-treatment of avian species with PB or 3MC in respect to their susceptibility to 2AAF mediated hepatocarcinogenesis. Discussion of 2AAF metabolism profiles by microsomes prepared from PB and 3MC pre-treated animals is therefore possible only in general terms. On the basis of both metabolite ratios and increases in specific activity, pre-treatment with PB is predicted to provide a protective effect. This would contrast with the rat where an increase in mutagenic potential is observed on pre-treatment with PB [30]. Using microsomes prepared from 3MC pre-treated animals there was a 2-fold reduction in the ratio of ring to *N*-hydroxylated metabolites (to 16:1), a similar effect has been reported with microsomes prepared from 3MC pre-treated Sprague–Dawley rats [3, 21, 24]. An activation effect is predicted although in the rat reduced mutagenic potential is observed [30].

In conclusion we show there is a marked induction of hepatic cytochrome P-450 by 2AAF in the chick embryo. The response elicited has features in common with that produced by PB and 3MC. Differences in response may be due to unique features associated with 2AAF induction and/or inhibition effects of 2AAF metabolites. Avian resistance to the hepatocarcinogenic effects may result from an alteration in the net flux through competing pathways for 2AAF activation and inactivation. Two, possibly additive, effects are highlighted; the high rate of ring hydroxylation compared with *N*-hydroxylation and the high deacetylase activity of chick microsomes for 2AAF. Both factors could divert 2AAF to less tumourogenic metabolites and confer resistance to the carcinogenic effects of 2AAF.

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