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ONTOGENY OF THE CHICKEN CYTOCHROME P-450 ENZYME SYSTEM

EXPRESSION AND DEVELOPMENT OF RESPONSIVENESS TO PHENOBARBITAL INDUCTION*

NANCY A. LORR† and STEPHEN E. BLOOM

Department of Poultry and Avian Sciences, and The Institute of Comparative and Environmental Toxicology, Cornell University, Ithaca, NY 14853, U.S.A.

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Abstract—The sensitivity of the developing embryo to toxins and drugs is highly dependent on the state of development of the cytochrome P-450 system. Previous work in this laboratory has demonstrated the genotoxicity of aflatoxin B_1 (AFB_1) to the chicken embryo at 3 days of incubation (DI) and induction of AFB_1 genotoxicity by phenobarbital at 7 DI. In this study, the basal and 24-hr phenobarbital (PB) induced levels of aminopyrine-*N*-demethylase (AMPD) and cytochrome P-450 were assayed in hepatic microsomes from 7 DI to 36 days posthatching (PH) and in microsomes from whole embryos at 5 DI. A dose–response for induction by PB was observed in embryonic hepatic microsomes as early as 7 DI, whereas a low level of cytochrome P-450 was detected in control 7 DI microsomes using the reduced CO vs oxidized CO difference spectrum. Basal levels of AMPD and cytochrome P-450 in hepatic microsomes increased steadily throughout development as did the responsiveness of the embryonic liver to induction with PB. Hepatic microsomes from control and PB-induced chickens had the highest AMPD activities posthatching particularly from 1 to 3 days PH. Maximal induced levels, which were 2- to 3-fold over control throughout development, ranged from 1.22 at 7 DI to 12.72 nmol HCHO/mg protein/min at 2 days PH. The potency of PB as an inducer increased about 1000-fold between 7 DI and hatching. PB induction did not increase the specific activity of AMPD at any period of development. The specific activity of AMPD posthatching increased about 3-fold above embryonic levels, indicating the development of a cytochrome P-450 complex more active toward aminopyrine in the neonatal period.

Exposure of an embryo to toxic chemicals can have serious developmental consequences. Evidence is accumulating that the cellular changes leading to cancer, birth defects and immunosuppression are often induced by exposure to chemicals in the environment prenatally or neonatally [1–7]. The rapidly dividing and differentiating cells of the embryo are highly vulnerable and, if chemical exposure occurs at a particular stage of differentiation, a teratogenic response may be targeted to just one tissue as development proceeds [8].

Most xenobiotics require metabolic activation to be converted to forms which react with DNA and other macromolecules [9, 10], a process which is implicated in mutagenesis, carcinogenesis, teratogenesis and immune suppression [10–12]. The cytochrome P-450 monooxygenase system, located primarily in the endoplasmic reticulum of the cell and most prominent in the liver, oxidizes xenobiotics and endogenous substrates transforming some molecules to reactive, electrophilic intermediates [13]. The cytochromes P-450 exist in multiple forms which are differentially inducible and have distinct but overlapping substrate specificities [14].

The sensitivity of the developing organism to a xenobiotic is highly dependent on a functioning cytochrome P-450 system with the capacity for metabolism of the specific chemical. In most non-primate mammals, the fetal cytochrome P-450 system is present at low levels only, and detoxification pathways are hardly developed [15]. 3-Methylcholanthrene (MC‡) inducible cytochrome P-450 activities are less than 10% of adult levels in rat and mouse liver during mid to late gestation [16–18]. However, MC-type cytochrome P-450 activities have been detected as early as the onset of the blastocyst stage in several strains of mice [19]. The cytochrome P-450 levels are sufficient to activate benzo[*a*]pyrene (BP) *in situ* in the mouse fetus at 12 days post-conception [20]. In MC-pretreated 12-day post-conception rat embryo explants, acetylaminofluorene-induced teratogenesis is enhanced but cyclophosphamide does not induce damage with or without phenobarbital (PB) pretreatment [21]. Correspondingly, PB-inducible cytochrome P-450 activities are undetectable earlier than

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† Address correspondence to: Dr. Nancy A. Lorr, Department of Poultry and Avian Sciences, Cornell University, 213 Rice Hall, Ithaca, NY 14853.

‡ Abbreviations: MC, 3-methylcholanthrene; AMPD, aminopyrine-*N*-demethylase; AFB_1, aflatoxin B_1; PB, phenobarbital; AHH, arylhydrocarbon hydroxylase; TCB, 3,4,3′,4′-tetrachlorobiphenyl; HCHO, formaldehyde; DI, days of incubation; days PH, days posthatching; PCN, pregnenolone 16-alpha-carbonitrile; SCE, sister chromatid exchange; AAF, acetylaminofluorene; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; ER, endoplasmic reticulum; and BP, benzo-[*a*]-pyrene.

the day of birth in rodent liver [22, 23]. Giachelli and Omiecinski [24] did not detect mRNA expression of the PB-inducible isozymes, cytochromes P-450b and e, in the fetal rat, although these mRNAs increase rapidly in the neonatal period.

Human fetal liver cytochrome P-450 levels are about 30–50% of the adult levels by the second trimester [25]. In contrast to the rodents, human fetal liver microsomes have a very low capacity to metabolize polycyclic aromatic hydrocarbons [25, 26]. Most of the cytochrome P-450 constitutes isozymes with catalytic activities characteristic of PB or pregnenolone 16-alpha-carbonitrile (PCN) induced cytochromes P-450 [27, 28].

The competency of the early chicken embryo to activate a wide spectrum of xenobiotics to DNA-damaging metabolites has been demonstrated in this laboratory using a sister chromatid exchange (SCE) assay on tissues of embryos exposed to promutagens *in vivo* [29]. SCE induction as early as 3–4 DI, using promutagens, demonstrates the presence of metabolic capacity. SCE induction at 6 DI by the promutagens, aflatoxin B_1 (AFB_1) and acetylaminofluorene (AAF), was higher than at 3 DI at the same mg dose, implying an increase in basal enzyme levels [30]. Basal aryl hydrocarbon hydroxylase (AHH) is detectable in chick embryo liver as early as the third day of development and is inducible in the liver by 3,4,3′,4′-tetrachlorobiphenyl (TCB), MC and related inducers after day 6. Basal and TCB-induced AHH is equal to or greater than the levels at maturity during most of the embryonic period [31]. PB pretreatment, which induces activation of AFB_1 in the rat [32], increases DNA damage in the liver of the AFB_1-exposed 7 DI chick embryo, whereas TCB pretreatment decreases the damage [33]. Cytochrome P-450 is detectable in homogenates of liver from chicken embryos by 6 to 7 DI and treatment with PB at 11 DI produces induction of cytochrome P-450 and its associated catalytic activities in the homogenates [34]. However, the development of PB-inducible cytochrome P-450 in the chicken liver throughout the embryonic period has yet to be critically examined.

Significant changes in cytochrome P-450 isozyme composition may occur during the embryonic and neonatal period. There is a need for thorough examination of basal levels of different types of cytochrome P-450 and their inducibility patterns during development. In this study, aminopyrine-*N*-demethylase (AMPD), a classical and reliable indicator of PB-induced cytochrome P-450 was optimized for use with chicken hepatic microsomes. Dose–response kinetics for 24-hr PB induction of cytochrome P-450 and AMPD were assayed throughout embryonic development. Basal and 24-hr PB-induced levels of these variables were followed on through the neonatal period. Changes in specific activity (AMPD to cytochrome P-450 ratios) that occurred around hatching suggest distinct changes in the chicken cytochrome P-450 system during this transition.

MATERIALS AND METHODS

Chemicals. Formaldehyde and aminopyrine were purchased from the Aldrich Chemical Co. (Milwaukee, WI). NADP, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were purchased from the Sigma Chemical Co. (St. Louis, MO). Phenobarbital (PB) was purchased from Merck & Co., Inc. (Rahway, NJ). All chemicals were of the highest purity commercially available.

Animals, treatments and microsomes. Fertile eggs were obtained from the Cornell K-MFO line of White Leghorn chickens, which was developed from the Cornell K strain [31]. Eggs were incubated at 37° and 85% humidity and rotated once an hour. Experimental eggs were selected for fertility and normal embryonic development by candling. Chick embryos were pretreated by placement of the solution by pipette onto the inner shell membrane through a 5-mm diameter hole made in the shell of each egg directly over the air cell [35]. The hole was sealed with a piece of adhesive tape and the egg was returned to the incubator. A range of doses was used to establish a dose–response for PB induction of AMPD and cytochrome P-450 and the maximal dose tolerated at different stages of embryonic development. Hatched chickens were injected intraperitoneally with PB at a dose of 100 mg/kg body weight. Twenty-four hours later, embryos or chicks were killed, and their livers were removed. Liver microsomes were prepared by differential centrifugation as previously described [31], and the pellets were stored at −80° until use. Pellets were resuspended in AHH buffer (0.1 M potassium phosphate, 5 mM $MgCl_2$, 0.1 mM EDTA, pH 7.4). Since the livers of 5 DI embryos are extremely small, whole embryos were used at this age to obtain microsomal pellets. The number of livers pooled for one homogenate varied with the age of the embryo from about 85 to 5 livers at 7 and 21 DI respectively.

Enzyme assays. Aminopyrine-*N*-demethylase was modified from the oxidative demethylase protocol of Nash [36] which measures the formation of formaldehyde. Incubations were carried out in 1 ml total volume and included 0.5 to 1.5 mg microsomal protein depending on the level of AMPD activity, 100 mM potassium phosphate, 5 mM $MgCl_2$, 0.1 mM EDTA, 10 mM glucose-6-phosphate, 0·5 mM NADP, 2 units/ml glucose-6-phosphate dehydrogenase and 5 mM aminopyrine. Tubes were preincubated for 2 min at 37°, the reaction was initiated with an NADPH-generating system, and then the tubes were further incubated for 15 min in a shaking water bath. The reactions were terminated by addition of 0.25 ml of cold 10% trichloroacetic acid and immediate chilling on ice. After centrifugation, aliquots of the supernatant fraction were used for formaldehyde determinations using a Nash reagent according to Tu and Yang [37]. Under these assay conditions, the amount of HCHO formed was proportional to incubation time and protein concentration.

Cytochrome P-450 was measured using a DW2 Aminco spectrophotometer according to the procedure of Matsubara *et al.* [38] which employs the reduced CO vs oxidized CO difference spectrum.

RESULTS

In initial experiments, the relationship of amino-

pyrine concentration to activity of AMPD in microsomes from control and PB-induced 1 day posthatch (PH) chicks was examined. The velocity concentration curve (Fig. 1A) indicated that 5 mM aminopyrine was a concentration yielding activity near saturation; this concentration was used for the rest of the study. Double-reciprocal plots showed a distinct difference between microsomes from control and PB-induced 1 day PH chicks (Fig. 1B). With control microsomes, there were at least two K_m values for AMPD, 0.07 and 0.53 mM, whereas the microsomes from PB-induced chicks had one K_m value, 0.25 mM, intermediate to that of the control microsomes. The V_{max} in the PB-induced microsomes was about two times that of the control. The K_m values indicate that in the PB-induced microsomes the reaction reaches substrate saturation at lower concentrations and that PB induction produces changes in the cytochrome P-450 isozyme composition.

The development of basal (control) and PB-induced AMPD activity in the liver from 7 DI through 36 days PH is shown in Tables 1 and 2 and Fig. 2. Activity consistently above the blank was not detected in microsomes from whole 5 DI embryos. The basal value of AMPD was lowest at 7 DI, at which age activity was only marginally detected by this assay. It increased to 0.55 nmol HCHO/mg protein/min by 10 DI and continued to increase throughout embryonic development and until 1 day PH when basal AMPD reached a level of 4.18 nmol HCHO/mg protein/min. There was a short-term drop in basal and PB-induced AMPD around 6 days PH (to about 60% of the level from 1 to 3 days PH) concomitant with the final complete absorption of the contents of the yolk sac. The AMPD activity was not consistently low in microsomes from 6 days PH individual chick livers and may vary with the state of the yolk sac. The peak of AMPD around hatching is

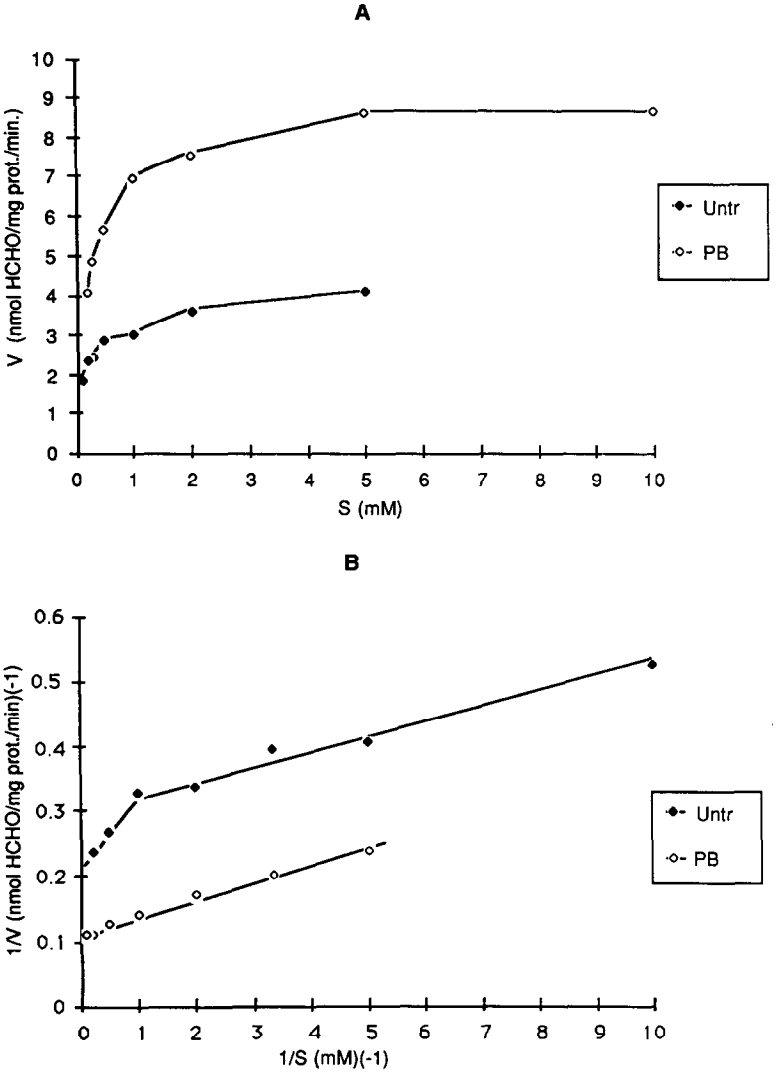

Fig. 1. Substrate dependency of AMPD in 1 day posthatch (PH) liver microsomes. (A) Velocity versus substrate plots. (B) Double-reciprocal plots. For microsomes from untreated neonates the two K_m values were 0.07 and 0.53 mM and the V_{max} values were 3.22 and 4.67 nmol HCHO/mg protein/min, respectively, while for microsomes from PB-treated the K_m and V_{max} were 0.25 mM and 8.83 nmol HCHO/mg protein/min.

Table 1. Effects of 24-hr PB induction on hepatic microsomal AMPD and cytochrome P-450 in embryos from 7 to 21 DI*

Age	Dose (mg/embryo)	PB (mol/kg embryo)	AMPD (nmol HCHO/mg protein/min)	Cytochrome P-450 (nmol/mg protein)	Specific activity AMPD/ cytochrome P-450
7 DI	0		0.07	0.020	3.5
	0.4	5.0×10^{-3}	0.13		
	2.2	2.5×10^{-2}	0.50	0.144	3.5
	4.3	5.0×10^{-2}		0.196	
	8.6	1.0×10^{-1}	1.22		
10 DI	0		0.55	0.084	6.5
	2.0	5.0×10^{-3}	0.70	0.143	4.9
	4.0	1.0×10^{-2}	0.85	0.178	4.8
	9.9	2.5×10^{-2}	1.77	0.322	5.5
14 DI	0		0.91	0.168	5.4
	4.5	2.5×10^{-3}	1.42	0.264	5.4
	9.0	5.0×10^{-3}	2.63	0.370	7.1
	17.9	1.0×10^{-2}	1.91	0.362	5.3
18 DI	0		1.66	0.407	4.1
	6.0	1.3×10^{-3}	3.93	0.536	7.3
	12.0	2.5×10^{-3}	6.02	0.769	7.8
	23.0	5.0×10^{-3}	7.28	0.928	7.8
21 DI	0		3.57	0.244	14.6
	3.6	5.0×10^{-4}	8.14	0.697	11.7
	7.2	1.0×10^{-3}	10.44	0.993	10.5

* Each value is from a microsomal sample of pooled livers. Replicates in a run differed by less than 10%. DI = days of incubation.

associated in time with the accelerated absorption of the yolk sac contents which begins about 19 DI when the yolk sac is retracted into the body; absorption is nearly complete by 6–7 days PH [39]. At 36 days PH, basal values were again equal to that of the neonatal period. Previous work [40] indicates that the sex of the chick does not affect the basal AMPD from 1 to 7 days PH. In this study, we found no sex-dependent differences in basal and PB-induced AMPD in liver microsomes from 10 and 36 days PH chickens.

Table 2. Effects of 24-hr PB induction on hepatic microsomal AMPD and cytochrome P-450 in neonates from 1 to 36 days PH*

Age	Dose (mg/kg)	PB (mol/kg body wt)	AMPD (nmol HCHO/mg protein/min)	Cytochrome P-450 (nmol/mg protein)	Specific activity AMPD/ cytochrome P-450
1 day PH	0	0	4.18 ± 0.42 (3)	0.211	19.9
	100	4.0×10^{-4}	9.62 ± 1.77 (3)	0.730	16.0
2 days PH	0	0	4.14		
	100	4.0×10^{-4}	12.72		
3 days PH	0	0	3.70 ± 0.20 (4)		
	100	4.0×10^{-4}	11.95 ± 1.28 (4)		
4 days PH	0	0	3.57 ± 0.66 (3)	0.225	19.2
	100	4.0×10^{-4}	8.94 ± 0.52 (3)	0.450	19.2
6 days PH	0	0	2.31 ± 0.56 (3)	0.148	17.8
	100	4.0×10^{-4}	6.82 ± 3.23 (3)	0.307	22.2
10 days PH	0	0	4.02	0.243	16.5
	100	4.0×10^{-4}	7.84	0.392	20.0
36 days PH	0	0	4.45 ± 0.64 (4)	0.237	19.5
	100	4.0×10^{-4}	10.12 ± 1.40 (4)	0.512	22.9

* The number of microsomal samples is in parentheses. If no standard deviation is given, the value is from a microsomal sample of pooled livers. Replicates in a run differed by less than 10%. Days PH = days post-hatching.

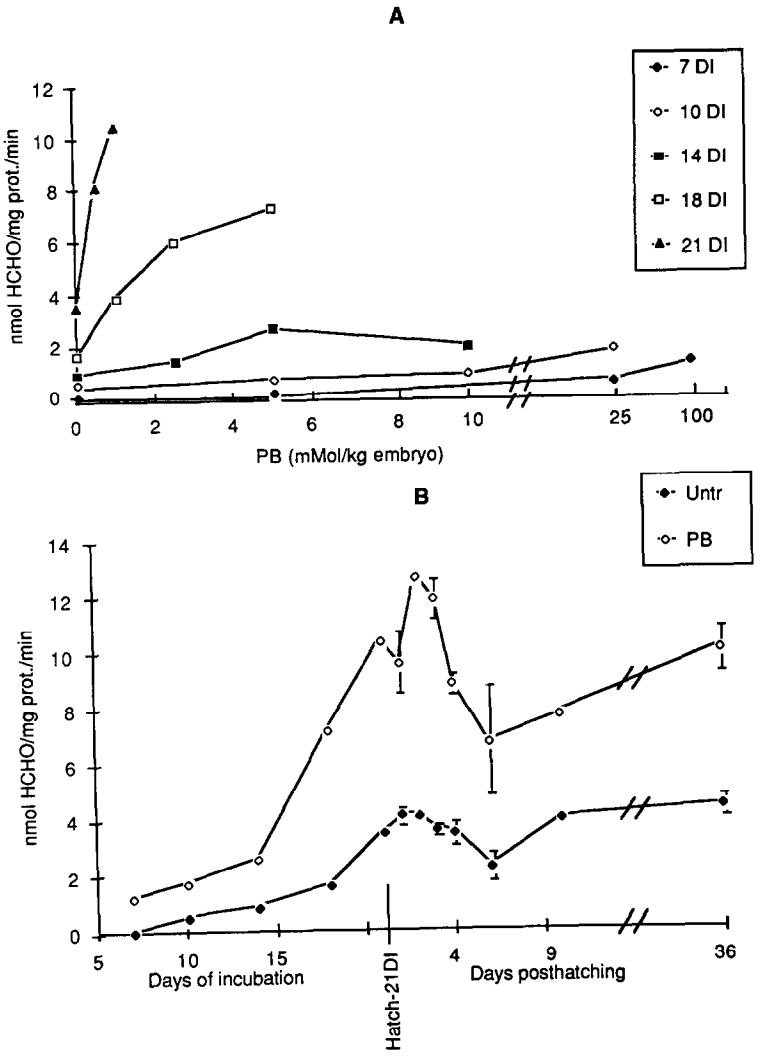

Fig. 2. Hepatic microsomal AMPD: (A) Dose–response curves of AMPD in preparations from embryos at 7, 10, 14, 18 and 21 DI. Embryos were pretreated 24 hr before sacrifice with a range of doses of PB. (B) Developmental profile of AMPD in hepatic microsomes from chicken embryos and neonates from 7 DI to 36 days PH. The AMPD activity associated with the maximally inducing dose of PB at each time point is represented. The standard errors of the means are shown by vertical bars.

A dose–response for induction of AMPD by PB was observed in hepatic microsomes at 7 DI, the earliest age hepatic microsomes were prepared (Fig. 2). The PB-induced levels of AMPD increased throughout embryonic development and reached the highest levels from 1 to 3 days PH. The potency of PB as an inducer based on the mol/kg body weight increased dramatically during development. While 7 DI embryos were maximally induced and tolerated a dose of 100 mmol PB/kg body weight, the maximally effective tolerated dose at 18 DI was 5 mmol and that at 3 days PH was 0.4 mmol (the AMPD values were 1.22, 7.28 and 11.95 nmol HCHO/mg protein/min, respectively). The potency calculated as AMPD/(mol PB/kg body weight) was 2000 times greater at 1 day neonatally than at 7 DI. Since the maximally tolerated dose decreased as development progressed, one dose concentration could not be used for the whole developmental profile. Although the potency of PB increased with age, induction at the maximally effective tolerated dose varied only from 3- to 4.4-fold prenatally excluding the 7 DI induction which appears to be much greater. Postnatally, at the constant dose of 100 mg/kg body weight, induction was 2- to 3-fold. There was no clear pattern ot increasing fold induction; basal and PB-induced AMPD increased nearly in parallel.

The presence of cytochrome P-450 in 7 DI control hepatic microsomes was confirmed by clear detection of a peak at 450 nm using the reduced CO vs oxidized CO difference spectrum. A dose–response for PB induction of cytochrome P-450 was observed at 7 DI and at each embryonic stage examined (Tables 1 and 2, Fig. 3). Basal levels of cytochrome P-450 ranged from 0.02 nmol/mg protein at 7 DI to 0.41 nmol/mg protein at 18 DI, whereas levels maximally

induced by PB ranged from 0.20 nmol/mg protein at 7 DI to 0.99 nmol/mg protein at 21 DI. The posthatching profile of cytochrome P-450 was similar to that of AMPD; the drop in levels around 6 days PH was again observed. While cytochrome P-450 increased steadily during embryonic development, it did not always change in parallel with AMPD. Cytochrome P-450 peaked in the late embryo (Fig. 3), whereas AMPD continued to increase for a few days posthatching (Fig. 2). The ratio of AMPD to cytochrome P-450, the specific activity of AMPD, increased dramatically between 18 DI and the neonatal period (Fig. 4, Tables 1 and 2). From 7 DI to 18 DI, specific activities of control and PB-induced embryonic hepatic microsomes ranged from 3.5 to 7.8; an upward trend was observed in PB-induced microsomes but not in control microsomes. At 21 DI specific activity values were intermediate between that of the embryonic and neonatal period. Posthatch specific activities ranged from 16.0 to 22.9, substantially higher than the embryonic values. PB induction did not increase specific activity of AMPD consistently, and any increases were minor. The increase in specific activity of AMPD in both basal and PB-induced liver microsomes posthatching indicates the development of a cytochrome P-450 complex more active toward aminopyrine.

Hepatic microsomes from individual control and PB-induced 3 days PH chicks derived from several divergent genetic lines (three egg-type lines and a meat-type line) were assayed for AMPD activity to probe for genetic variation in AMPD. All lines had

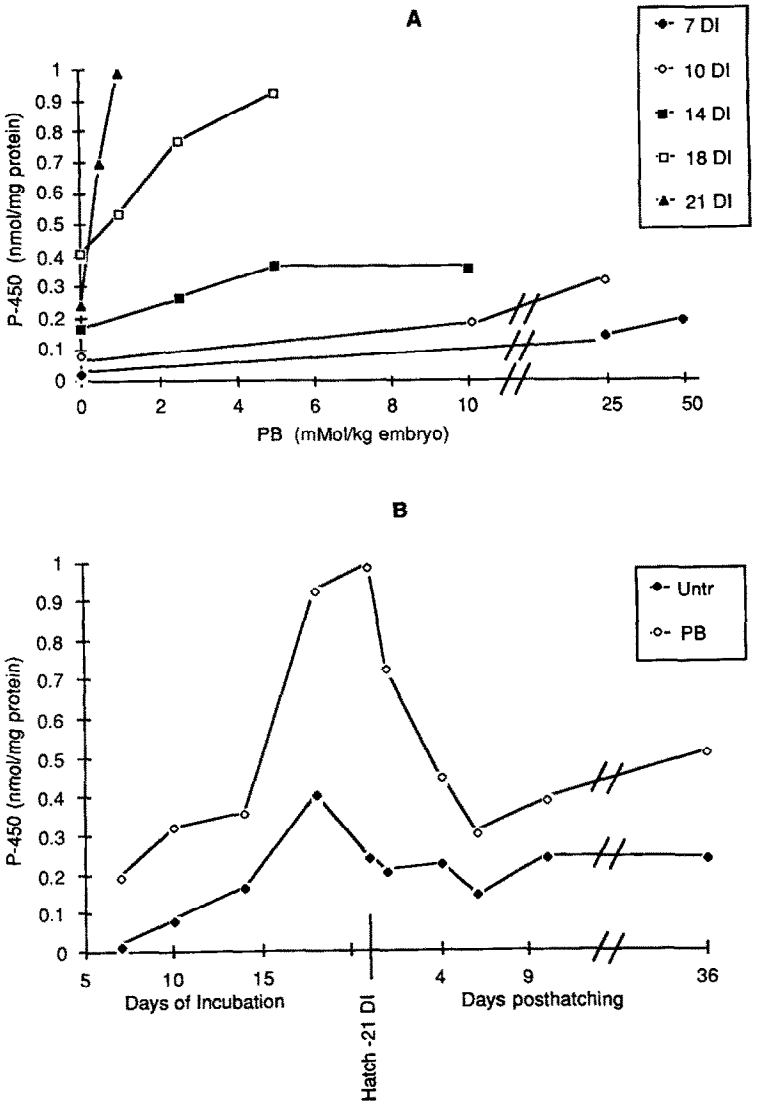

Fig. 3. Hepatic microsomal cytochrome P-450: (A) Dose-response curves of cytochrome P-450 in preparations from embryos at 7, 10, 14, 18 and 21 DI. Embryos were pretreated 24 hr before sacrifice with a range of doses of PB. (B) Developmental profile of cytochrome P-450 in hepatic microsomes from chicken embryos and neonates from 7 DI to 36 days PH. The cytochrome P-450 level associated with the maximally inducing dose of PB at each time point is represented.

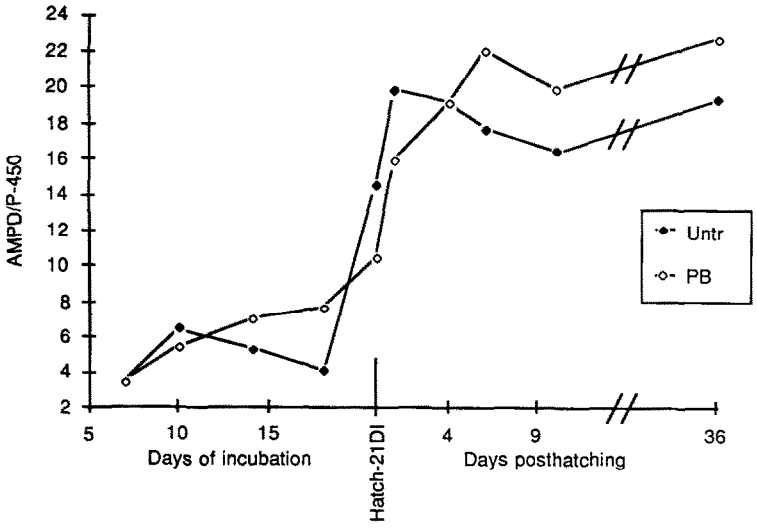

Fig. 4. Developmental profile of the specific activity of AMPD, calculated as AMPD (nmol HCHO/mg protein/min)/cytochrome P-450 (nmol/mg protein), during development of the chicken embryo and neonate. PB values plotted represent the ratio of AMPD/cytochrome P-450 at the maximally inducing dose of PB.

very similar basal activities and were induced 2.2- to 3.3-fold by PB (Table 3); the range of the means of induced AMPD was from 8.2 to 12.2. The hepatic microsomal activity of the K-MFO line was the most inducible by PB, with a level of AMPD significantly higher than that of the white egg line ($P < 0.05$). Individual variation was high at 3 days PH, possibly due to the dynamic state of the yolk sac at this age.

DISCUSSION

Basal and PB-induced AMPD levels in hepatic microsomes of the chicken developed coordinately throughout the embryonic period, steadily rising until 1 day PH. Cytochrome P-450 levels followed a similar pattern. AMPD and cytochrome P-450 were present quite early in embryonic development of the chicken; control and PB-induced activities were detectable as early as 7 DI.

Table 3. AMPD in hepatic microsomes from 3 days PH chickens of several divergent genetic lines*

	AMPD (nmol HCHO/mg protein/min)	
	Sources of microsomes†	
Genetic line	Control	Phenobarbital-induced
White egg	3.73 ± 0.46^{a}	8.24 ± 1.95^{b}
Broiler	3.37 ± 0.24^{a}	$9.17 \pm 1.23^{b,c}$
Brown egg	3.47 ± 1.12^{a}	$11.39 \pm 2.76^{b,c}$
K-MFO	3.68 ± 0.24^{a}	12.23 ± 1.42^{c}

*The "white egg," "brown egg," and "broiler" lines were supplied by Hubbard Farms, while K-MFO is a Cornell line. Determinations were performed on hepatic microsomes from three individual birds for each genetic line and treatment.

†Values are the means ± standard deviations. Values followed by different superscript letters are significantly different ($P < 0.05$), using Student's *t*-test.

Development of AMPD activity in the chicken embryo is quite different from that of arylhydrocarbon hydroxylase (AHH). In contrast to basal and PB-induced AMPD and cytochrome P-450, which increase slowly and steadily during embryonic development, there is a sharp rise in TCB-induced liver AHH between 5 and 10 DI with basal and TCB-induced AHH maxima at 12–14 DI and 1–2 days PH with a drop in induced levels at 17–19 DI [31]. The first rise in TCB inducibility of liver AHH is preceded by the appearance of relatively high levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) receptor; peaks of receptor occur at 7 DI and at hatching [41]. The potency of PB as an inducer of AMPD and cytochrome P-450 increased dramatically during embryogenesis while the induction potency of TCB does not increase during development; maximal induction of AHH by TCB is achieved at about 5 μmol/kg throughout the embryonic period. Induction of AHH by TCB is 15- to 30-fold, whereas induction of AMPD by PB was 2- to 4-fold. Maximal induction by PB in rodents is achieved through multiple dosing over several days or by continuous infusion [42]. The 24-hr induction used in this study allowed us to observe the capability of the embryo or neonate to respond to induction during a limited and defined developmental period.

The peak of basal and PB-induced AMPD and cytochrome P-450 around the time of hatching and the decline within the first week are similar to the pattern observed in basal and TCB-induced AHH activity and confirm previous work with the neonatal chicken [31, 40]. The pattern of activity follows closely the progression of absorption of the contents of the yolk sac. Lipid soluble yolk extracts will induce metabolism in 7 days PH chick embryo liver microsomes [40]. The highly inducible cytochrome P-450 system present in the chicken embryo during the hatching period may have evolved specifically for efficient metabolism of the lipid constituents of the yolk.

The studies of Powis *et al.* [40] using hepatic microsomes from untreated chicken neonates suggest that the highest level of AMPD is at 1 day PH with a return to embryonic levels by 7 days PH. However, in the present study, we observed a rise in AMPD by 10 days PH after a low point at 6 days PH with a return to the high early neonatal levels by 36 days PH. Studies of AMPD later in development may reveal a decline with maturation as observed in some mammalian systems [24].

AMPD has been used extensively to measure the catalytic activity of PB-inducible cytochrome P-450 in rodents and other species because it is more effectively metabolized by these forms than by most other isozymes of cytochrome P-450 [43]. AMPD is more inducible by PB than by TCB, MC, TCDD or AAF in the chick embryo [44, 45]. The constitutive forms of cytochrome P-450 have significant catalytic activity towards the same substrates classically used to measure cytochrome P-450b, including aminopyrine, benzphetamine, ethylmorphine and others, while the cytochrome P-450b isozyme is present in very low concentrations constitutively [43]. With the use of substrates highly specific for cytochrome P-450b such as pentoxyresorufin, higher fold induction after PB has been observed in rodent hepatic microsomes [46]. Preliminary studies in this lab using hepatic microsomes from 1 day PH chickens show very low levels of pentoxyresorufin depentylase (PRD) in both uninduced and PB-induced samples with no induction by PB (N. Lorr and S. Bloom, unpublished). PB-induced rat hepatic microsomes had highly induced PRD activity in our assay. PB and TCDD-induced forms of hepatic cytochrome P-450 in the chicken differ in catalytic activity from rat forms. For example, TCDD induces some AMPD in chicken hepatic microsomes but not in the rat [47]. PRD is highly specific for cytochromes P-450b and e but it is not elevated in several other PB-induced forms of cytochrome P-450 in rat liver [48]. Thus, AMPD is more likely to detect a wider spectrum of PB-induced forms which may differ in substrate specificities from the rat forms. In this study, PB induction of AMPD was about equivalent to induction of cytochrome P-450 during most of development. The ratio of AMPD to cytochrome P-450, the specific activity of AMPD, did not increase after PB induction except during the late embryonic period and after 4 days PH when the specific activity was slightly higher in PB-induced than uninduced hepatic microsomes. The kinetic studies with microsomes from 1 day PH chickens indicate predominance after PB induction of a component with affinity intermediate to those displayed in control microsomes. Thus, it appears that PB does not induce forms of cytochrome P-450 more active toward aminopyrine than the forms in the control microsomes but does increase the amount of cytochrome P-450 capable of AMPD activity.

The specific activity of AMPD is at least doubled postnatally, indicating that the cytochrome P-450 complex is more efficient in the metabolism of aminopyrine posthatching. Possible changes in the cytochrome P-450 system which could be responsible for the observed increase in specific activity of AMPD associated with this important developmental event include (1) a change in the composition of cytochrome P-450 isozymes involved in the metabolism of aminopyrine; (2) changes in NADPH-cytochrome P-450 reductase; or (3) changes in morphology of the liver or the endoplasmic reticulum (ER) containing the cytochrome P-450 complex [15, 22, 49–51]. Like cytochrome P-450 and AMPD, NADPH–cytochrome P-450 reductase peaks at 1 day PH in hepatic microsomes from the chicken [40]. Since the specific activity of AMPD remains elevated for a longer period, the reductase does not appear to be limiting. Studies of pre- vs postnatal cytochrome P-450 isozymes and of the catalytic activities of the representative microsomes in humans, rabbits, rats and mice reveal distinct changes in the cytochrome P-450 isozyme profile around birth [16, 28, 52–54]. Polyclonal antisera against the major form of cytochrome P-450 induced by PB in adult chicken liver immunoprecipitate two polypeptides from cultured chicken embryo (14 DI) hepatocytes, one with the same molecular weight as that purified from the adult chicken [55, 56]. Further characterization of the embryonic and neonatal chicken hepatic cytochrome P-450 isozymes may reveal distinct changes in isozymic composition around hatching.

AFB_1 is highly effective in inducing sister chromatid exchanges in 3–4 DI embryos with increasing potency at 6 DI [30]. If the AMPD and cytochrome P-450 detected in hepatic microsomes from 7 DI chicken embryos represent the enzymes responsible for activation of AFB_1 to its DNA damaging form, then what appears to be minute amounts of cytochrome P-450 can play an important role in the activation of mutagens *in vivo*. BP directly injected into the mouse fetus causes more DNA damage at 15 days post-conception than at 12 or 18 days of postnatally [20], indicating the importance of low level BP metabolism in the fetal mouse.

The chicken may represent a uniquely suitable model for the human fetal cytochrome P-450 system for purposes of the study of activation of mutagens whose metabolism is modulated by PB. Like the chicken embryonic liver, the human fetal liver has substantial levels of cytochrome P-450 with characteristics of the PB- or PCN-induced forms. Findings of substantial basal and induced levels of PB and TCB (MC) induced forms of cytochrome P-450 early in embryonic development give further support to the use of the chicken embryo for studying the development and regulation of the cytochrome P-450 complex and the short- and long-term biological effects of embryonic exposures to environmental mutagens [3, 57].

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