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OXIDATIVE ENZYME COMPONENTS OF AVIAN LIVER MICROSOMES

CHANGES DURING EMBRYONIC DEVELOPMENT AND THE EFFECTS OF PHENOBARBITAL ADMINISTRATION

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SUMMARY

Changes in the levels of oxidative enzyme activities and hemoproteins in chicken liver microsomes were studied during late embryonic and post-hatching development and following administration of phenobarbital at several developmental stages.

1. During the developmental period studied, the oxidative N-demethylation and NADPH–cytochrome *c* reductase activities increased markedly and in parallel fashion, while oxidative O-demethylation and cytochrome b_5 increased only slightly and at a different developmental stage, and the cytochrome P-450 level did not increase. Phenobarbital treatment caused marked increases in the cytochrome P-450 level and N-demethylation activity at each developmental stage, but only minor changes in cytochrome b_5 level, O-demethylation activity or capacity for electron transport from NADH or NADPH to cytochrome *c* or to oxygen.

2. Spectroscopic studies with microsome suspensions indicate that the avian liver microsomal cytochrome P-450 is similar to the mammalian hemoprotein in its spectral properties and lability.

3. The characteristics of the avian microsomal mixed-function oxidase system are considered in the light of the developmental and induced changes in the levels of oxidative components.

INTRODUCTION

The physiological roles and functional interrelations of various microsomal oxidative enzyme components, particularly those associated with cytochrome b_5, remain uncertain[1–3]. In contrast, studies of drug and steroid metabolism indicate that microsomal cytochrome P-450 serves as a terminal oxidase component in hydroxylations, oxidative dealkylations and other mixed-function oxidase reactions of mammalian liver microsomes[2–5]. These studies have been facilitated by the finding that administration of phenobarbital and other inducer drugs to a number of mammalian species produces alterations in the levels of certain drug-metabolizing activities[6–9], in the rate of proliferation of hepatic endoplasmic reticulum and in other aspects of

liver growth[10,11]. Consistent with its postulated function as a terminal oxidase, the level of cytochrome P-450 generally increases in parallel with the level of oxidative drug-metabolizing activity after the administration of such inducer drugs[9,12,13]. Suggestive evidence concerning the organization and functional relations of oxidative components may also be obtained by examining the pattern of differentiation for microsomal components and activities in the course of ontogenetic development; such developmental studies have been made in the chicken[14,15] and the rat[16].

In the present study, the levels of various oxidative activities and hemoproteins in chicken liver microsomes were examined with respect to both the normal patterns of change during embryonic and post-hatching development and the response to phenobarbital administration at various stages of development. Changes in absolute and in relative levels of the microsomal components are considered in terms of the possible functional relationships of these components, particularly with respect to involvement in mixed-function oxidase reactions. Some characteristics of these avian liver microsome systems are compared with those reported for mammalian liver microsomes.

METHODS

Animal development and drug administration

Fertile chicken eggs of the Van Tress-Leghorn and De Kalb 151 Leghorn strains were obtained from a commercial hatchery and incubated at 38° and 65 % humidity in the laboratory. The stage of development of embryos at the time of harvest was determined by the scale of HAMBURGER AND HAMILTON[17], and only embryos of a specific stage were pooled for a preparation. After hatching, the chicks were provided with water and Purina Starter Chow *ad libitum* until sacrificed. The developmental patterns and effects of phenobarbital to be reported here were obtained with the Van Tress-Leghorn strain; results of parallel studies with the De Kalb 151 Leghorn strain were similar, but showed some quantitative differences.

For the induction studies, phenobarbital (50 mg/ml water) was injected beneath the shell membrane and the hole sealed with collodion; phenobarbital in the same concentration was injected intraperitoneally into chicks. The two standard age groups of embryos, 15-days and 18-days old at harvest, were given 5 mg of phenobarbital at 12 and 14 days, and at 15 and 17 days, respectively. Phenobarbital injections for the two standard age groups of chicks were as follows: for the 4-day-old chick, 5 mg at 1 day and 10 mg at 3 days; for the 9-day chick, 5 mg at 1 day, 10 mg at 3 and 6 days, and 15 mg at 8 days. Control groups of embryos and chicks were injected with isotonic saline on the same schedules as the experimental groups.

Preparation of cell fractions

Chicks and embryos were killed by decapitation; livers were quickly excised, trimmed, rinsed with cold 0.25 M sucrose–0.001 M EDTA, pH 7.0 (sucrose–EDTA medium), blotted and weighed. All subsequent steps were carried out at 0–2°. Pooled livers were homogenized in 5 vol. of sucrose–EDTA medium in a glass homogenizer fitted with a Teflon pestle; a moderate speed and duration of homogenization were selected to give about 95 % cell breakage without extensive breakage of mitochondria. Homogenates were diluted with the sucrose-EDTA medium to contain 100 mg liver per ml, and centrifuged for 10 min at 10 000 × g to remove nuclei, debris and mito-

chondria. The supernatant solutions were collected and centrifuged for 60 min at 110000 × *g* to obtain the microsome fractions. These microsome pellets were rinsed by flooding gently with sucrose-EDTA medium, then washed by dispersion in 0.15 M KCl–0.001 M EDTA, and recentrifuged for 60 min at 110000 × *g*. Finally, the washed microsome pellets were dispersed in sucrose–EDTA medium and kept at −15° until assayed, routinely after 1 day for oxidative demethylation and after 2 days for other activities. This storage procedure entailed no significant loss of activities. The O- and N-oxidative demethylation activities of the stored preparations were 0–10% lower than in fresh preparations. Levels of the other oxidative activities of stored preparations were within ±6% of the levels in fresh preparations that were frozen and thawed 2 times before assay; this freezing-and-thawing process, to which preparations were routinely subjected by the storage procedure, was necessary to obtain reproducible maximal levels for certain oxidative activities in fresh preparations.

Assays

Oxidative demethylase activity was determined by assay of the formaldehyde formed, using modifications of the procedure of MAZEL *et al.*[18]. Microsome preparations were incubated at 37° for 1 h in a medium containing $8.5 \cdot 10^{-2}$ M phosphate buffer, pH 7.4, 0.13 μmole $NADP^+$, 10 μmoles nicotinamide, 9 μmoles semicarbazide, 5 μmoles $MgCl_2$, 9 μmoles isocitrate, 2 μmoles $MnCl_2$, 0.25 mg isocitrate dehydrogenase (NADP) (EC 1.1.1.42) and 3.6 μmoles substrate in a total volume of 1.75 ml. The usual substrates were *o*-nitroanisole for O-demethylation and meberal (5-phenyl-5-ethyl-3-methylbarbituric acid) for N-demethylation. The reaction was stopped by addition of saturated barium hydroxide, and the reaction mixture deproteinated by further addition of zinc sulfate. After centrifugation, aliquots of the protein-free supernatant solution were assayed for formaldehyde with the Nash reagent as described by MAZEL, except that after incubation with Nash reagent, the incubation mixtures were centrifuged and the absorbance of the clear supernatant fluids was then determined at 415 nm. Under the conditions routinely used, demethylase activity was linear with time and with amount of tissue preparation added. Activities are expressed as nmoles substrate demethylated/min per mg protein in the tissue preparation assayed.

Electron transport activities were assayed spectrophotometrically at 25° with aerobic microcells of 1 cm light path, essentially as described previously[15]. The reaction mixtures contained 0.10 M phosphate buffer (pH 7.4), with other components as noted. NADH–, NADPH– and succinate–cytochrome *c* reductase activities were determined by the rate of increase of absorbance at 550 nm with $1 \cdot 10^{-4}$ M NADH, $1 \cdot 10^{-4}$ M NADPH or $3 \cdot 10^{-3}$ M sodium succinate, respectively, as electron donor and $5 \cdot 10^{-5}$ M oxidized cytochrome *c* as electron acceptor; $2 \cdot 10^{-3}$ M KCN was added to all cells. NADH oxidase and NADPH oxidase were determined from the rate of decrease in absorbance at 340 nm with $1 \cdot 10^{-4}$ M NADH or NADPH, respectively, as electron donor and atmospheric oxygen as acceptor; the assays were run in both absence and presence of $2 \cdot 10^{-3}$ M KCN. Activities are expressed as nmoles electron donor oxidized/min per mg protein in the tissue preparation assayed, and are corrected for non-enzymatic activity.

The cytochrome b_5 and cytochrome P-450 content of microsome preparations, dispersed in 0.05 M phosphate buffer (pH 7.4), was determined from difference spectra obtained with a Bausch and Lomb 505 spectrophotometer or Cary 14 spectrophoto-

meter at room temperature. Cytochrome b_5 content was determined from the absorbance difference between 424 nm and 409 nm in the NADH-reduced *minus* oxidized difference spectrum, assuming a molar extinction increment of 185 $mM^{-1} \cdot cm^{-1}$ (ref. 19); the experimental cell was equilibrated with nitrogen before addition of NADH. To obtain the cytochrome P-450 content, both the reference and experimental cells were equilibrated with nitrogen and reduced with dithionite, and the experimental cell then equilibrated with carbon monoxide; the absorbance difference between 450 and 490 nm in the reduced-CO *minus* reduced difference spectrum was used to calculate cytochrome P-450 content, assuming a molar extinction increment of 91 $mM^{-1} \cdot cm^{-1}$ (ref. 19). For the study of spectral shifts induced in oxidized microsomes, the microsome suspension in 0.10 M phosphate buffer (pH 7.4), was equilibrated with oxygen and aliquots then diluted with 0.1 vol. of either H_2O (control cell) or a solution of the test substance (experimental cell). The experimental *minus* control difference spectrum was corrected, when necessary, for the absorption spectrum of the test substance.

The protein content of homogenates and fractions was determined by the micromethod of LOWRY *et al.*[20].

The assay data were analyzed by application of the t test for statistical significance[21]. Changes designated as "significant" in the presentation of the data showed P values of < 0.001; those designated as "possibly significant" showed P values of < 0.01 but > 0.001.

Reagents

$NADP^+$, NADH, NADPH, cytochrome *c*, sodium isocitrate, isocitrate dehydrogenase (NADP) and antimycin A were obtained from Sigma Chemical Co.; sodium phenobarbital from Mallinckrodt Chemical Works; CO (99.5 %), N_2 and O_2 from Matheson Co.; and *o*-nitroanisole from Eastman Organic Chemicals. Meberal was the gift of Sterling-Winthrop Research Institute. Other chemicals were of reagent grade.

RESULTS

Electron transport activities

Table I summarizes the electron transport activities of liver microsome preparations from chick embryos and chicks at 4 stages of normal development and from phenobarbital-treated embryos and chicks of the same developmental stages. Some of these enzymatic activities show significant and distinctive changes in the course of normal development or as a result of phenobarbital treatment.

The NADH–cytochrome *c* reductase activity, present at high levels at each stage of development, showed a "possibly significant" decrease in the 18-day embryo and a gradual recovery; at all 4 stages the phenobarbital treatment resulted in a "possibly significant" decrease of activity, compared to the activity in the normal organism at the same stage of development. The NADPH–cytochrome *c* reductase activity also showed a slight "possibly significant" decrease in the normal 18-day embryo, but a "significant" increase above the 15-day embryo levels after hatching. Phenobarbital treatment produced only slight increases of NADPH–cytochrome *c* reductase activity of doubtful significance at each of the 4 developmental stages tested. Both NADH– and NADPH–cytochrome *c* reductases were insensitive to $5 \cdot 10^{-6}$ M anti-

TABLE I

EFFECT OF DEVELOPMENTAL STAGE AND OF PHENOBARBITAL TREATMENT ON ELECTRON TRANSPORT ACTIVITIES, OXIDATIVE DEMETHYLATION, HEMOPROTEIN CONTENT AND PROTEIN CONTENT OF CHICKEN LIVER MICROSOMES

Microsome fractions were prepared from livers of control and phenobarbital-treated chick embryos and chicks as described under METHODS, and were assayed for the components tabulated by the procedures described under METHODS. Enzyme activities and hemoprotein levels are expressed as units per mg microsomal protein; the microsomal protein level is expressed as mg protein in the microsomal fraction obtained from 1 g wet wt. of liver. The values tabulated are the mean values and standard errors of assays on 4–6 preparations of each type.

Assay	*Phenobarbital treatment*	*Developmental stage*			
		15-day embryo	*18-day embryo*	*4-day chick*	*9-day chick*
NADH–cyt. *c* reductase	−	132.0 ± 11.0	77.6 ± 7.7	102.1 ± 9.2	142.6 ± 13.4
(nmoles/min per mg protein)	+	73.0 ± 3.3	48.9 ± 4.8	62.1 ± 5.9	83.5 ± 8.9
NADPH–cyt. *c* reductase	−	16.3 ± 1.2	9.5 ± 0.4	27.3 ± 1.9	42.5 ± 2.5
(nmoles/min per mg protein)	+	17.2 ± 0.7	12.8 ± 0.9	35.1 ± 2.6	47.3 ± 2.3
Succinate–cyt. *c* reductase	−	1.65 ± 0.09	3.72 ± 0.40	5.51 ± 1.24	5.90 ± 1.04
(nmoles/min per mg protein)	+	1.46 ± 0.11	2.71 ± 0.36	4.31 ± 0.48	5.29 ± 0.85
NADH oxidase	−	26.3 ± 2.8	94.6 ± 6.9	201.8 ± 10.6	151.5 ± 12.9
(nmoles/min per mg protein)	+	16.7 ± 1.8	74.0 ± 11.5	132.0 ± 8.7	98.0 ± 13.2
NADPH oxidase	−	4.66 ± 0.19	9.75 ± 0.52	8.74 ± 0.62	8.50 ± 0.36
(nmoles/min per mg protein)	+	7.40 ± 0.27	9.56 ± 0.90	12.93 ± 0.79	11.67 ± 0.64
NADPH oxidase + CN^-	−	4.70 ± 0.29	9.41 ± 0.90	7.40 ± 0.40	6.87 ± 0.54
(nmoles/min per mg protein)	+	6.00 ± 0.32	8.50 ± 1.25	9.89 ± 0.49	8.31 ± 0.27
o-Nitroanisole O-demethylation	−	0.90 ± 0.06	1.13 ± 0.11	1.20 ± 0.10	1.19 ± 0.14
(nmoles/min per mg protein)	+	1.08 ± 0.09	1.11 ± 0.05	1.58 ± 0.20	1.97 ± 0.15
Meberal N-demethylation	−	0.059 ± 0.007	0.058 ± 0.005	0.132 ± 0.012	0.266 ± 0.022
(nmoles/min per mg protein)	+	0.180 ± 0.022	0.198 ± 0.013	0.545 ± 0.038	0.743 ± 0.053
Cytochrome b_5	−	0.252 ± 0.008	0.378 ± 0.021	0.400 ± 0.014	0.361 ± 0.013
(nmoles/mg protein)	+	0.329 ± 0.010	0.412 ± 0.023	0.487 ± 0.012	0.413 ± 0.014
Cytochrome P-450	−	0.160 ± 0.006	0.188 ± 0.004	0.160 ± 0.008	0.126 ± 0.008
(nmoles/mg protein)	+	0.499 ± 0.032	0.648 ± 0.043	0.685 ± 0.009	0.623 ± 0.034
Microsomal protein	−	13.75 ± 0.52	17.93 ± 0.31	20.69 ± 0.87	19.18 ± 0.69
(mg/g liver)	+	15.00 ± 0.66	17.63 ± 0.54	19.32 ± 0.35	16.58 ± 0.83

mycin A (data not tabulated). The low succinate–cytochrome *c* reductase activity present in these microsome preparations was 95 % inhibited by $5 \cdot 10^{-6}$ M antimycin A (data not tabulated), and presumably represents contamination by mitochondrial fragments (see ref. 15). The specific activity of succinate–cytochrome *c* reductase in the microsome preparations was 2–7 % of that in comparable preparations of mitochondria, with the higher levels being found in microsomes from the later stages of development. The increasing level of this activity at later developmental stages paralleled the increased homogenization time required to disrupt liver cells from older

embryos or chicks. Succinate–cytochrome *c* reductase levels in phenobarbital-treated animals were not significantly different from those in control animals.

NADH oxidase activities of the microsome fractions also showed a marked, statistically "significant" increase after the 15-day embryo stage; the decreased activity in phenobarbital-treated animals of each stage is "possibly significant", except for the 18-day embryo values. Addition of $2 \cdot 10^{-3}$ M KCN produced 95–100% inhibition of NADH oxidase activity in preparations from all developmental stages, and in both control and treated animals (data not tabulated). The NADPH oxidase activity was "significantly" higher than the 15-day embryo level in later developmental stages, and, except in the 18-day embryo, the phenobarbital-treated animals showed a "possibly significant" increase above the controls. As is shown in Table I, the major portion of NADPH oxidase activity was insensitive to $2 \cdot 10^{-3}$ M KCN. The NADH and NADPH oxidases also differed in that $5 \cdot 10^{-6}$ M antimycin A produced about 90% inhibition of the former activity but less than 10% inhibition of the latter (data not tabulated). The similar patterns of change seen for NADH oxidase and succinate–cytochrome *c* reductase, and the similar sensitivity of the two activities to antimycin A suggest that the NADH oxidase activity may also largely reflect mitochondrial contamination rather than an intrinsic microsomal activity.

Oxidative demethylation

The effects of developmental age and of phenobarbital treatment on capacity for oxidative O-demethylation of *o*-nitroanisole and oxidative N-demethylation of meberal are summarized in Table I. Activity with *o*-nitroanisole as substrate showed only a slight increase of doubtful significance above the 15-day embryo level in later developmental stages; phenobarbital produced a "possibly significant" increase in the 9-day chick. In contrast, the capacity for N-demethylation of meberal was "significantly" higher in the control 4-day and 9-day chicks than in embryos, and phenobarbital produced a marked, "significant" rise above the control level at each developmental stage.

Michaelis constants for oxidative demethylation substrates were obtained from reciprocal plots of enzyme activity at different substrate concentrations. For *o*-nitroanisole the apparent K_m values were $6.7 \cdot 10^{-5}$ M and $7.4 \cdot 10^{-5}$ M with microsome preparations from control and phenobarbital-treated 9-day chicks, respectively. For meberal the values were $7.8 \cdot 10^{-5}$ M and $8.3 \cdot 10^{-5}$ M with preparations from the control and treated chicks, respectively.

Microsomal hemoproteins

Table I shows that the microsomal cytochrome b_5 level increased "significantly" during normal development between the 15-day and 18-day embryo, and remained at that higher level in later development. Phenobarbital treatment produced no appreciable change in cytochrome b_5 levels, except for a "possibly significant" increase in the 15-day embryo liver. In contrast, cytochrome P-450 levels showed no change in normal development, except for a slight decrease of uncertain significance in the 9-day chick, but phenobarbital produced marked, "significant" increases at each developmental stage.

Difference spectra, obtained as described under METHODS, indicated the presence of typical cytochrome b_5 and cytochrome P-450 components in liver microsomes

from control and phenobarbital-treated embryos and chicks of all 4 standard developmental stages. The NADH-reduced *minus* oxidized curves gave a cytochrome b_5-type spectrum with major absorption peak at 424 nm, lesser peaks at about 528 nm and 556 nm, and a trough at 409 nm. In reduced-CO *minus* reduced difference spectra, the major peak was that of the cytochrome P-450–CO complex, centered at 450 nm in preparations from both control and phenobarbital-treated chicks. The reduced-CO *minus* reduced spectrum showed no significant absorption between 480 and 600 nm, but showed a secondary peak at about 420 nm, lying between troughs at about 408 nm and 430 nm. The height of the 420-nm peak above a baseline drawn between the adjacent troughs was somewhat variable but similar in fresh preparations from both control and phenobarbital-treated chicks, and varied between 10 and 35 % of the height of the 450-nm peak above the 490-nm baseline. Incubation of microsome suspensions with 0.5 % sodium deoxycholate, either aerobically or anaerobically, resulted in rapid loss of a demonstrable cytochrome P-450–CO component; under anaerobic conditions, this decrease of the 450-nm peak was accompanied by an increased absorption at 420 nm.

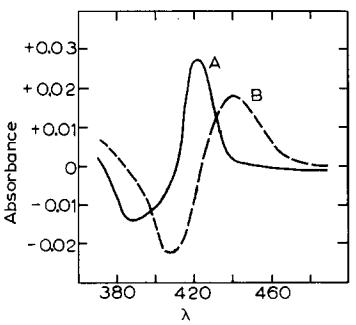

Fig. 1. Effects of nicotinamide and cyanide on oxidized spectrum of microsomes. Difference spectra were obtained as described under METHODS on a suspension of oxidized microsomes from 9-day chick liver whose cytochrome P-450–CO complex peak (absorbance difference 450 nm *minus* 490 nm) was 0.105. Curve A, 0.2 M nicotinamide *minus* control; Curve B, 0.02 M KCN *minus* control.

As is shown in Fig. 1, the addition of 0.2 M nicotinamide to oxidized microsomes produced a spectral shift with a peak at 422 nm and a trough at about 390 nm; with preparations from both control and phenobarbital-treated chicks, the height of this peak was equal to about 25 % that of the reduced cytochrome P-450–CO peak obtained with the same preparation. The presence of 0.02 M KCN elicited a spectral shift with a peak at 440 nm, equal to 15–20 % of the reduced cytochrome P-450–CO peak, and a trough at about 408 nm. The addition of 0.001 M *o*-nitroanisole, 0.02 M meberal or 0.02 M phenobarbital (not shown) did not produce detectable spectral shifts over the range 370–500 nm.

Protein content and liver weight

As is shown in Table I, there was a "possibly significant" increase in the amount of microsomal protein per g wet weight of liver after the 15-day embryo stage during normal development, but phenobarbital caused no significant change at any developmental stage. In assays not summarized in Table I, the total liver protein in 3 prep-

arations from each developmental stage showed a similar but less marked rise from 151 mg/g wet weight of liver in the 15-day embryo to a maximum of 189 mg in the 4-day chicks; phenobarbital treatment caused no significant change in total protein per g wet weight of liver. The proportion of total liver protein recovered in the microsome fraction did not change significantly in the developmental stages studied or as a result of phenobarbital treatment; the mean values fell within the range 9.1–10.9 %. The mean wet weight of the liver increased from 0.31 g in the 15-day embryo to 3.23 g in the 9-day chick, and the mean wet weight of livers from phenobarbital-treated embryos and chicks fell within $\pm$ 5 % of the controls at each developmental stage. Since phenobarbital treatment did not cause appreciable changes in wet weight of liver, total liver protein or microsomal protein/g wet weight of liver, the results of the present study would not be significantly altered by expressing the levels of oxidative components on the basis of total protein or wet weight rather than per mg microsomal protein.

DISCUSSION

The results reported here and in a previous study[15] indicate that liver microsomes from late chick embryos and chicks contain oxidative enzyme components and activities similar to those of mammalian liver microsomes. These activities include a high level of antimycin A-insensitive NADH–cytochrome *c* reductase, a lower level of antimycin A-insensitive NADPH–cytochrome *c* reductase and a significant level of NADPH oxidase that is largely insensitive to both cyanide and antimycin A. The cytochrome b_5 and cytochrome P-450 levels per mg microsomal protein are lower than but of the same order of magnitude as those found in mammalian liver microsomes[12,19]; the normal cytochrome P-450 levels reported here for chick embryo and chick are similar to the level in adult chicken liver microsomes[23]. The avian microsomes show a substantial capacity for oxidative O-demethylation with *o*-nitroanisole as substrate and a lower activity for oxidative N-demethylation of meberal; other studies (C. F. STRITTMATTER, unpublished work) indicate that chick liver microsomes also can carry out S-demethylation with 6-methylmercaptopurine as substrate.

The pattern of enzyme changes during normal development from the 15-day embryo to the 9-day chick (Table I) provides little evidence of a parallel development of all components implicated in microsomal mixed-function oxidase systems. While the overall N-demethylation activity showed a 350 % increase beginning after the 18-day embryo stage, O-demethylation showed only a minor increase between the 15-day and 18-day embryo stage. Cytochrome P-450, which presumably is involved in these activities, did not increase during the developmental period studied; cytochrome b_5, which has not been implicated, showed a minor increase. The NADPH–cytochrome *c* reductase activity showed a significant increase above the 15-day embryo level after the 18-day embryo stage, but NADPH oxidase activity showed only a modest rise that took place between the 15-day and 18-day embryo stages. These developmental patterns in the avian liver microsomes about the time of hatching thus differ from those seen in mammalian liver microsomes about the time of birth: in rat liver microsomes[16], the NADPH–cytochrome *c* reductase activity showed a 400 % rise within 2 days to a maximum at 1 day after birth; cytochrome b_5 and P-450 showed a parallel 200 % rise during the first week after birth, while N-demethylation, which

doubled about the time of birth, showed a slower rise in the first week after birth.

The mixed-function oxidase systems of avian liver can respond to phenobarbital administration at the embryonic stages studied here as well as after hatching. As was shown in Table I, both oxidative N-demethylation activity and cytochrome P-450 levels showed several-fold increases at each developmental stage studied. However, there was no definite rise in capacity for electron flow from NADPH to either cytochrome *c* or oxygen after phenobarbital treatment. The pattern of response to phenobarbital administration in the avian liver is therefore similar to that in mammalian liver in showing selective increases of cytochrome P-450 and some mixed-function oxidations[9,12,13], but differs in that mammalian liver microsomes also show a definite rise in NADPH–cytochrome *c* reductase[9,13] and of NADPH oxidase activities[13].

The spectral studies reported here indicate that avian liver microsomes contain cytochrome b_5 and cytochrome P-450 with properties similar to the analogous hemoproteins of mammalian liver microsomes[19,22,24,25]. Like the mammalian component, the avian cytochrome P-450 is labile to deoxycholate treatment and appears to undergo conversion to "P-420" (refs. 19, 25, 26). The secondary peak at 420 nm seen in reduced-CO *minus* reduced difference spectra of avian liver microsomes may reflect the presence of "P-420" or other altered form of cytochrome P-450, or of some other CO-combining component, either intrinsic or contaminant. Analogous two-banded difference spectra with peaks at about 450 nm and 420 nm have been reported for some mammalian microsome preparations[27–29]. The spectral shifts seen with oxidized avian liver microsomes upon addition of nicotinamide or cyanide (Fig. 1) are similar in magnitude and position to the effects of these substances on the spectrum of oxidized mammalian microsomes[22], and may reflect binding with cytochrome P-450, as is postulated for the shifts with mammalian microsomes[22,24].

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