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# EFFECT OF MICROSOMAL PREPARATIONS AND INDUCTION ON CYTOCHROME P-450-DEPENDENT MONOOXYGENASES IN FETAL AND NEONATAL RAT LIVER

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**Abstract**— A study of the sedimentation behavior of fetal and neonatal rat liver microsomes allowed a better recovery of a less contaminated microsomal fraction, especially by the use of an EDTA-containing buffer. The specific cytochrome P-450 content and related catalytic activities in the 105,000 *g* pellet of fetal and neonatal liver were thus much higher than usually reported, while molecular activities were comparable to adult ones.

The transplacental inducing effects of phenobarbital and 3-methylcholanthrene on the monooxygenase system were studied in microsomes prepared by the modified procedure and compared to results obtained in crude liver homogenate: 3-methylcholanthrene induces a net biosynthesis of cytochrome P-450 in fetal liver, whereas phenobarbital produces only a premature transformation of rough into smooth endoplasmic reticulum, which decreases the 'contamination' of the 105,000 *g* pellet by ribosomal protein. As a result, the specific cytochrome P-450 content of the microsomal fraction appears to be increased by phenobarbital, though there is no true induction of the monooxygenase system in near-term rat fetus.

Liver drug-metabolizing activities are generally low at birth and reach values close to adult level in 3–4-week-old animals [1–4]. However, large variations relative to sex [3], animal species [5] and substrate [1, 3, 6, 7] are observed during development.

Rat is largely immature at birth and exhibits very low cytochrome P-450-dependent activities in 105,000 *g* pellets of near-term fetal or neonatal liver [3, 8–11]. However, Chatterjee *et al.* [12] reported that, in fetal rat liver, ascorbic acid synthesis was mainly associated with the 200–8000 *g* fraction, and observed a poor microsomal recovery in high speed pellets. This prompted us to reinvestigate the sedimentation behavior of fetal and neonatal liver microsomes and describe a more efficient preparation procedure.

Guenthner and Mannering have shown that, when injected into the mother, 3-MC * but not PB appeared to promote a transplacental induction of the monooxygenase system in crude homogenates of fetal liver [13]. The aim of the present study was to compare such results with determinations performed in microsomal fractions prepared by the described improved procedure. Thus, 'specific' activities in microsomal pellets could be compared with 'total' activities in crude homogenates. Moreover, the transplacental effects of PB and 3-MC on fetal liver endoplasmic reticulum were correlated with the observed modifications of the monooxygenase system.

## MATERIALS AND METHODS

### *Animals*

Pregnant rats (Sprague–Dawley strain) were purchased from C.E.R. Janvier. Females were bred overnight and vaginal smears were performed to date mating: the presence of spermatozoa determined day 1 of gestation. Controls and pregnant females were housed separately and fed a stock pellet diet (UAR) *ad lib.*

Phenobarbital (80 mg/kg in saline) or 3-methylcholanthrene (20 mg/kg in triolein) were injected intraperitoneally once a day during the 3 days preceding death. Adult males were similarly pretreated and fasted overnight before death. Controls were injected with vehicle only.

### *Liver preparation*

Fetuses were removed *in utero* on day 20 or 22 of gestation and killed by decapitation; livers were immediately excised, pooled by litters, washed twice with ice-cold isotonic NaCl and homogenized as follows. Three procedures were tested:

*Procedure* 1. A 10% homogenate in 0.25 M sucrose was successively centrifuged: 20 min at 15,000 *g*, the supernatant 60 min at 105,000 *g* and the washed pellet 30 min at 105,000 *g*. The resulting pellet was resuspended in 50 mM Tris, 150 mM KCl and 10 mM $MgCl_2$, pH 7.4 to give a final protein concentration of 10–15 mg/ml.

*Procedure* 2. After homogenization in 0.25 M sucrose and 5 mM Tris, pH 7.4, the 10% homogenate was centrifuged for 10 min at 600 *g*. The supernatant was centrifuged at 10,000 *g* for 15 min. Supernatant and a mixed layer composed of mitochondria and microsomes were carefully removed from the mitochondrial pellet and centrifuged for 15 min at 10,000 *g*. Mitochondria were prepared from the combined pellets and resuspended in the Tris buffer. Supernatants were pooled and microsomes prepared as in Procedure 1.

*Procedure* 3. Livers were homogenized in 0.25 M

* Abbreviations: PB, phenobarbital; 3-MC, 3-methylcholanthrene; G-6-Pase, glucose-6-phosphatase.

sucrose, 1 mM Tris and 1 mM EDTA, pH 7.4. Centrifugations were carried out as in procedure 2. Mitochondria and microsomes were resuspended in 10 mM $Na_2HPO_4$ and 20% glycerol buffer, pH 7.5.

Livers from neonates (12-hr-old or 5-day-old animals after spontaneous delivery) were similarly processed. For comparison with fetal and neonatal preparations, microsomes from adult males were prepared according to Procedure 3. When rough and smooth microsomes were separated, 10,000 *g* supernatants (2–3 nmoles cytochrome P-450 in 0.25 M sucrose, 1 mM Tris and 1mM EDTA) were layered on an equal volume of 1.3 M sucrose, 1 mM Tris and 1 mM EDTA [14]; after a 4 hr centrifugation at 170,000 *g*, 1.2 ml fractions were collected from the top of the tubes and the cytochrome P-450 content determined.

Proteins were determined according to Lowry *et al.* [15] and RNA by the diphenylamine reaction [16]. Cytochrome P-450 determinations were carried out as described by Greim [17]; baseline was established after bubbling CO in both cuvettes, spectra were recorded after addition of dithionite in assay and NADH in reference cuvette. This method allowed us to measure cytochrome P-450 concentration in homogenate, mitochondrial and microsomal fractions without appreciable interference with other hemoproteins.

*Enzyme assays*

All experiments were performed within 36 hr following microsomal preparation. Microsomal suspensions were stored in liquid nitrogen without appreciable loss of catalytic activities. Cytochrome oxidase [18], glucose-6-phosphatase [19], NADPH-cytochrome *c* reductase [20], *p*-nitroanisole-*O*-demethylase [21] and aniline hydroxylase [20] were determined by described methods with 0.2–1 mg microsomal protein per assay for fetal and neonatal rat and 0.1–0.5 mg with adult preparations.

*Electron microscopy*

Mitochondrial and microsomal pellets were fixed for 1 hr with 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4 and postfixed in 2% osmium tetroxide. Materials were embedded in Epon and cut on an LKB Ultratome. Sections were contrasted by both 3% uranyl acetate and alkaline lead citrate, and examined in a Philips EM 300 electron microscope at 80 kV.

*Statistical analysis*

Statistical significance of differences between groups was estimated by the Student's *t* test.

## RESULTS

*Effect of centrifugation forces and buffer composition on microsomal preparation.* G-6-Pase activity (as marker of endoplasmic reticulum membranes) and cytochrome P-450 concentration were determined in hepatic microsomes prepared by different procedures from 22-day-old fetuses and 12-hr-old newborns.

In microsomes prepared by Procedure 1 from untreated fetuses, cytochrome P-450 remained below detection limits, but a measurable cytochrome P-450 concentration was observed in the mitochondrial pellet (0.087 ± 0.014 nmoles/mg protein). Pretreatment of pregnant females with PB or 3-MC resulted in the occurrence of cytochrome P-450 in 105,000 *g* pellets from fetal livers.

By modifying centrifugation forces (Procedure 2), the recovery of microsomes in 105,000 *g* pellets was increased but remained very low with preparations of untreated fetuses: the major part of cytochrome P-450 was present in 10,000 *g* pellets, which indicates that microsomes cosedimented with mitochondria (Table 1). This conclusion was verified by electron microscopic examination of 10,000 *g* and 105,000 *g* pellets

Table 1. Effect of different preparation procedures on cytochrome P-450 concentration and G-6-Pase activity in mitochondrial and microsomal pellets

| | Mitochondrial pellet | | Microsomal pellet | |
|---|---|---|---|---|
| | Cytochrome P-450* | G-6-Pase† | Cytochrome P-450* | G-6-Pase† |
| Procedure 2 | | | | |
| 22-Day-old fetuses | | | | |
| Non-induced | 0.084 ± 0.013 | 0.164 ± 0.018 | 0.035 ± 0.035 | 0.012 ± 0.005 |
| PB-treated | 0.075 ± 0.020 | 0.081 ± 0.022 | 0.196 ± 0.028 | 0.081 ± 0.012 |
| 3-MC-treated | 0.121 ± 0.026 | 0.203 ± 0.016 | 0.112 ± 0.065 | 0.385 ± 0.090 |
| 12-Hr-old-newborns | | | | |
| Non-induced | 0.084 ± 0.026 | 0.310 ± 0.033 | 0.122 ± 0.061 | 0.69 ± 0.21 |
| PB-treated | 0.127 ± 0.035 | 0.269 ± 0.027 | 0.227 ± 0.013 | 0.96 ± 0.16 |
| 3-MC-treated | 0.118 ± 0.039 | 0.373 ± 0.147 | 0.188 ± 0.072 | 1.11 ± 0.54 |
| Procedure 3 | | | | |
| 22-Day-old fetuses | | | | |
| Non-induced | 0.04 ± 0.03 | 0.25 ± 0.16 | 0.112 ± 0.019 | 2.87 ± 0.57 |
| PB-treated | 0.02 ± 0.02 | 0.64 ± 0.35 | 0.202 ± 0.037 | 4.07 ± 0.39 |
| 3-MC-treated | 0.07 ± 0.01 | 0.75 ± 0.21 | 0.198 ± 0.045 | 3.51 ± 0.39 |
| 12-Hr-old newborns | | | | |
| Non-induced | 0.06 ± 0.01 | 0.92 ± 0.32 | 0.322 ± 0.063 | 15.3 ± 2.9 |
| PB-treated | 0.05 ± 0.01 | 1.01 ± 0.62 | 0.460 ± 0.090 | 14.2 ± 3.5 |
| 3-MC-treated | 0.05 ± 0.02 | 1.10 ± 0.59 | 0.417 ± 0.053 | 17.0 ± 3.9 |

For preparation procedures see Materials and Methods. Results are mean ± S.D. for 4–6 preparations and expressed as: *, nmoles/mg protein; †, μmoles $P_i$ liberated in 20 min per mg protein.

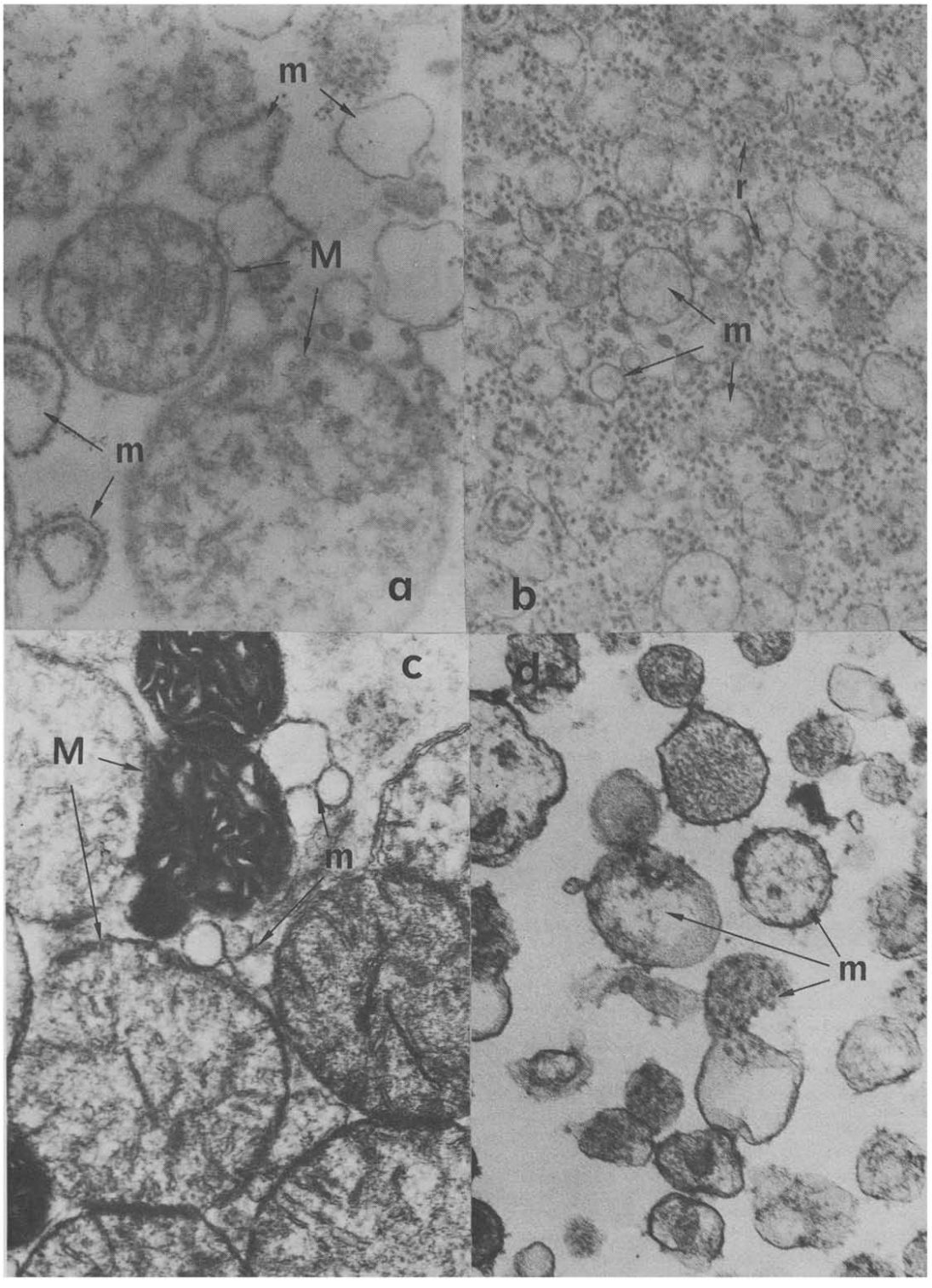


Fig. 1. Electron micrographs of 10,000 and 105,000 *g* pellets prepared with or without EDTA from liver of non-induced 22-day-old fetuses.
Without EDTA: a, 10,000 *g* pellet (×55,000); b, 105,000 *g* pellet (×55,000).
With 1 mM EDTA: c, 10,000 *g* pellet (×66,000); d, 105,000 *g* pellet (×73,000).
M, mitochondria; m, microsomal vesicles; r, ribosomes.

(Fig. 1). In the 10,000 *g* pellet, swollen and condensed mitochondria but also numerous microsomal vesicles were present, whereas the 105,000 *g* pellet was constituted by microsomal vesicles, ribosomes and polysomes. Pretreatment with PB or 3-MC and spontaneous delivery enhanced microsome recovery in the 105,000 *g* pellet but ribosomal contamination remained important.

The aggregation of ribosomes in polysomes, or the aggregation of microsomes among themselves or with mitochondria may be due to the presence of endogenous divalent cations in homogenates. The addition of 1 mM EDTA to sucrose–Tris buffer (Procedure 3) prevented the propensity to aggregation: G-6-Pase activity and cytochrome P-450 concentration were predominantly located in 105,000 *g* pellets, irrespective of age and pretreatment. With this procedure, the recovery of microsomes in 105,000 *g* pellets was about 50 per cent (40–55 per cent) of the microsomal content of total homogenate.

On the other hand, the degree of contamination of microsomes by mitochondria was determined by measuring the cytochrome oxidase activity (marker of mitochondrial inner membranes): less than 3 per cent of the activity measured in the 10,000 *g* pellet was recovered in the 105,000 *g* pellet. Electron microscopy confirmed this biochemical assumption (Fig. 1): the 105,000 *g* pellet was essentially constituted by microsomal vesicles with a small number of free ribosomes, while only few microsomes were present in 10,000 *g* pellets.

This procedure was thought correct for the preparation of microsomes from fetal and neonatal rat liver, without inconvenient contamination by polysomes or mitochondria, and was run for all further preparations.

*Perinatal development of the cytochrome P-450 content of microsomes.* The perinatal development of microsomal cytochrome P-450, expressed as nmoles per mg microsomal protein, is shown in Table 2. The cytochrome P-450 content, very low in 20-day-old fetuses (5 per cent of adult value in controls), increased during the last days of gestation. It rose rapidly and reached a third of male adult value as soon as 12 hr after birth, and a half in 5-day-old newborns. G-6-Pase activity exhibited a similar pattern of evolution, and reached higher values in newborn than in adult animals.

Pretreatment of pregnant females with 3-MC resulted in a significant increase of the microsomal content of cytochrome P-450 (expressed as nmoles/mg protein) but not of G-6-Pase activity in fetal and neonatal rats. Similarly administrated to pregnant females, PB produced different effects: in 20-day-old fetuses, no modifications were observed, but in 22-day-old fetuses both G-6-Pase activity and cytochrome P-450 content were significantly increased. In 12-hr-old newborns, G-6-Pase activity was not affected but the cytochrome P-450 content of microsomal membranes was enhanced by PB pretreatment of pregnant females.

*Perinatal development of total cytochrome P-450.* The cytochrome P-450 concentration in total liver homogenate rose gradually from 1 nmole/g liver in 20-day-old fetuses to adult level (Table 3). PB failed to increase the cytochrome P-450 concentration during fetal life, but its inducing action was observed in 12-hr-old newborns. Fetal liver showed a different response to 3 MC injection: a significant increase occurred from the 20th day of gestation and remained effective in all stages of development studied.

In regard to the inducing capacity of PB and 3-MC, such data were apparently conflicting with those obtained in isolated microsomes. The presence of variable amounts of ribosomes in microsomal fractions might explain this discrepancy; ribosomal proteins artificially decrease the cytochrome P-450 content of microsomes

Table 2. Modifications of microsomal enzyme activities in rat liver according to age and pretreatment

| | G-6-Pase* | Cytochrome P-450† | NADPH-cytochrome *c* reductase‡ | Aniline hydroxylase‡ | *p*-Nitroanisole-O-demethylase‡ |
|---|---|---|---|---|---|
| 20-Day-old fetuses | | | | | |
| Non-induced | 0.23 ± 0.03 | 0.049 ± 0.002 | 29.8 ± 5.6 | < 0.010 | < 0.010 |
| PB-treated | 0.28 ± 0.14 | 0.054 ± 0.010 | 36.4 ± 7.3 | < 0.010 | < 0.010 |
| 3-MC-treated | 0.19 ± 0.04 | 0.040 ± 0.014 | 28.2 ± 1.4 | < 0.010 | < 0.010 |
| 22-Day-old fetuses | | | | | |
| Non-induced | 2.87 ± 0.57 | 0.112 ± 0.019 | 41.8 ± 10.5 | 0.042 ± 0.009 | 0.257 ± 0.057 |
| PB-treated | 4.07 ± 0.39§ | 0.202 ± 0.037‖ | 58.0 ± 12.0 | 0.060 ± 0.012§ | 0.583 ± 0.101‖ |
| 3-MC-treated | 3.51 ± 0.39 | 0.198 ± 0.045‖ | 61.8 ± 19.0 | 0.064 ± 0.007§ | 0.571 ± 0.117‖ |
| 12-Hr-old newborns | | | | | |
| Non-induced | 15.3 ± 2.9 | 0.322 ± 0.063 | 135 ± 17 | 0.137 ± 0.037 | 0.72 ± 0.16 |
| PB-treated | 14.2 ± 3.5 | 0.460 ± 0.090§ | 134 ± 12 | 0.265 ± 0.070§ | 1.70 ± 0.40§ |
| 3-MC-treated | 17.0 ± 3.9 | 0.417 ± 0.059§ | 190 ± 4‖ | 0.330 ± 0.070‖ | 2.16 ± 0.41‖ |
| 5-Day-old newborns | | | | | |
| Non-induced | 11.1 ± 0.7 | 0.472 ± 0.056 | 172 ± 12 | 0.520 ± 0.055 | 3.20 ± 0.43 |
| Male adults | | | | | |
| Non-induced | 6.46 ± 0.68 | 1.02 ± 0.06 | 212 ± 33 | 0.368 ± 0.046 | 1.85 ± 0.60 |
| PB-treated | 5.91 ± 0.29 | 2.62 ± 0.10‖ | 360 ± 67‖ | 0.748 ± 0.127‖ | 13.2 ± 1.78‖ |
| 3-MC-treated | 4.75 ± 0.54 | 1.97 ± 0.11‖ | 273 ± 22‖ | 0.787 ± 0.033‖ | 6.31 ± 0.89‖ |

Microsomes were prepared according to Procedure 3 (see Materials and Methods). Results are mean ± S.D. for 4–6 preparations and expressed as: *μmoles $P_i$ liberated in 20 min per mg protein; †nmoles/mg protein; ‡nmoles substrate transformed per mg protein in 1 min; §$P < 0.05$; ‖$P < 0.001$ as compared with controls.

Table 3. Effect of age and pretreatment on cytochrome P 450 content of rat liver homogenate

| Age | Controls | Phenobarbital-treated | 3-Methylcholanthrene-treated |
|---|---|---|---|
| 20-Day-old fetuses | 1.04 ± 0.34 | 1.21 ± 0.27 | 1.79 ± 0.20* |
| 22-Day-old fetuses | 2.25 ± 0.43 | 2.30 ± 0.46 | 3.16 ± 0.52* |
| 12-Hr-old newborns | 4.76 ± 0.78 | 6.13 ± 1.04* | 5.90 ± 0.44* |
| 5-Day-old newborns | 10.4 ± 1.0 | n.d. | n.d. |
| Male adults | 32.6 ± 0.6 | 109 ± 12† | 62.3 ± 7.8† |

Animals (males or pregnant females) were pretreated with phenobarbital (80 mg/kg) or 3-methylcholanthrene (20 mg/kg) on the 3 days preceding death. Controls were injected with vehicle only. Results are mean ± S.D. for 4–6 homogenates, and are expressed as nmoles cytochrome P 450 per g of liver (wet wt). *$P < 0.05$; †$P < 0.001$ as compared with controls; n.d.: not determined.

when it is expressed per mg of total proteins in the pellet. This effect of ribosomes on microsomal specific activities will be conventionally referred to as 'contamination'. In order to estimate the relative content in ribosomes, the RNA/protein ratio was measured in 22-day-old fetuses: 0.35 ± 0.02 in microsomes prepared from control fetuses, 0.21 ± 0.04 in PB-treated fetuses and 0.61 ± 0.02 in 3-MC-treated fetuses. Moreover, the distribution of cytochrome P-450 between rough and smooth endoplasmic reticulum was determined on a 2 layer sucrose discontinuous gradient as described in Materials and Methods. Percentages of total cytochrome P-450 recovered in rough and smooth endoplasmic reticulum fractions were, respectively: 86 and 10 in control fetuses; 76 and 20 in PB-treated fetuses; 92 and 5 in 3-MC-treated fetuses. Thus, PB apparently increased the fraction of total cytochrome P-450 present in smooth endoplasmic reticulum, whereas 3-MC decreased it.

Another method of estimating the relative cytochrome P-450 contents of different microsomal fractions was the measurement of the ratio between a cytochrome P-450-independent enzyme (G-6-Pase) and the cytochrome P-450 concentration for each stage of development (Table 4). This ratio was slightly but not significantly decreased by PB pretreatment of fetuses; but it was significantly lowered by PB in newborn and adult animals and by 3-MC in all ages studied, a result which indicates a true induction of cytochrome P-450 in endoplasmic membranes.

*Perinatal development of microsomal monooxygenase activities.* Aniline hydroxylase and *p*-nitroanisole demethylase were assayed during the perinatal period (Table 2). These enzymatic activities showed an evolution parallel to cytochrome P-450 development and were increased by PB and 3-MC pretreatment of pregnant females. When these activities were referred to cytochrome P-450 content (Table 4), turn over numbers towards both aniline and *p*-nitroanisole were comparable to adult values, except in 5-day-old newborns which exhibited higher activities. Pretreatment modified catalytic activity for *p*-nitroanisole but aniline hydroxylase was unaffected, except in 12-hr-old newborns by 3-MC pretreatment.

Table 4. Catalytic activities of microsomal cytochrome P-450 and G-6-Pase/Cyt P-450 ratio in rat liver according to age and pretreatment

| | Aniline hydroxylase | *p*-Nitroanisole demethylase | G-6-Pase / cytochrome P-450 |
|---|---|---|---|
| 22-Day-old fetuses | | | |
| Non-induced | 0.36 ± 0.10 | 2.21 ± 0.70 | 25.6 ± 6.1 |
| PB-treated | 0.30 ± 0.04 | 2.89 ± 0.28 | 20.1 ± 1.5 |
| 3-MC-treated | 0.32 ± 0.11 | 2.88 ± 0.54 | 18.2 ± 3.0* |
| 12-Hr-old newborns | | | |
| Non-induced | 0.40 ± 0.09 | 1.96 ± 0.36 | 47.5 ± 1.85 |
| PB-treated | 0.54 ± 0.14 | 3.65 ± 0.29† | 33.2 ± 15.3* |
| 3-MC-treated | 0.83 ± 0.07† | 5.40 ± 0.27† | 42.5 ± 2.65* |
| 5-Day-old newborns | | | |
| Non-induced | 1.11 ± 0.08 | 6.82 ± 0.26 | 23.7 ± 1.8 |
| Male adults | | | |
| Non-induced | 0.35 ± 0.06 | 1.80 ± 0.48 | 6.81 ± 0.62 |
| PB-treated | 0.29 ± 0.04 | 5.04 ± 0.64† | 2.25 ± 0.05† |
| 3-MC-treated | 0.40 ± 0.03 | 3.18 ± 0.27† | 2.41 ± 0.41† |

Microsomes were prepared according to procedure 3 (see Materials and Methods). Results are mean ± S.D. for 4–6 preparations and expressed as nmoles substrate transformed per nmole cytochrome P-450 in 1 min. *$P < 0.05$; †$P < 0.001$ as compared with controls.

## DISCUSSION

When measured in the microsomal pellet, hepatic cytochrome P-450 and related monooxygenase activities are generally found to be very low in end-term fetuses and newborn rats [3, 8–11], and exhibit a rapid rise after birth [1–3]. This may result from either a low microsomal cytochrome P-450 content or a poor recovery of microsomes prepared from fetal liver. An analogous developmental behaviour of microsomal ascorbic acid synthesis was ascribed to a sedimentation pattern of fetal different from adult microsomes [12]. Similarly, microsomal activities (G-6-Pase, cytochrome P-450 and related metabolizing enzymes) of human fetal liver are recovered in 200 and 9,000 *g* pellets [22–24]. Such data led us to reinvestigate the sedimentation behavior of fetal and neonatal liver microsomes. When microsomes were prepared by the usual procedure using EDTA-free buffers (Procedures 1 and 2), no or very low cytochrome P-450 and related activities were recovered in 105,000 *g* pellets, but they were present in 10,000 *g* pellets (Table 1). A similar distribution of G-6-Pase, an enzyme localized in endoplasmic reticulum, was observed. The cytochrome P-450 content of fetal and neonatal microsomes prepared by these procedures was close to results previously reported in 105,000 *g* pellets or supernatants of 10,000 *g* or 12,000 *g* centrifugations [3, 8–11]. Electron microscopic examination of pellets was in agreement with biochemical conclusions and confirmed the assumed cosedimentation of microsomal vesicles with mitochondria (Fig. 1). Divalent cations are known to aggregate microsomes, a property used for their rapid preparation [25] and due to the high negative charge of microsomal membranes [26]. Thus, the presence of EDTA should reduce the spontaneous aggregation of fetal microsomes. Actually, by addition of 1 mM EDTA to the homogenization medium (Procedure 3), microsomal vesicles were mainly recovered in the 105,000 *g* pellet and mitochondria in the 10,000 *g* pellet. Furthermore, the amount of free ribosomes and polysomes was greatly reduced in the 105,000 *g* pellet, which partly eliminates the 'contamination' of microsomes by proteins from unattached ribosomes. A better recovery of microsomes and a low contamination by protein-rich components increased the cytochrome P-450 content and drug metabolizing activities (expressed per mg protein) of the 105,000 *g* pellet, in agreement with a recent report of Negishi and Kreibich [27], whereas no significant differences were observed between adult liver microsomes prepared by Procedures 2 and 3 (unpublished results).

Surprisingly, G-6-Pase specific activity was increased much more by this preparation procedure than was the cytochrome P-450 content of microsomes (Table 1). This may be explained by a better recovery of rough microsomes in 105,000 *g* pellets and by the heterogeneous distribution of G-6-Pase in 1-day-old rats [28]: the latter enzyme is predominantly located in rough microsomes which sediment at 10,000 *g* in Procedure 2. In microsomes prepared with EDTA according to Procedure 3, cytochrome P-450 was measurable as soon as the 20th day of gestation, but catalytic activities were not; this may be due to either an insufficient sensitivity of assay methods or an inability of fetal microsomes to bind and/or to oxidize aniline and *p*-nitroanisole. Cytochrome P-450 and drug metabolizing activities then appeared to increase during fetal life and reached a third of adult values in 12-hr-old newborns from untreated females. During this period, except on the 5th day following birth, molecular catalytic activities remained nearly constant at the adult level. Thus, in end-term fetus and early newborn, hepatic cytochrome P-450 is present under an active form which possesses about the same molecular catalytic activities as adult cytochrome P-450. Surprisingly, in 5-day-old newborns, drug metabolizing molecular activities were higher than in adults, a result in agreement with aniline hydroxylase molecular activities calculated from data of Iba *et al.* [29].

When the inducing capacity of PB and 3-MC was investigated, apparently conflicting results were observed according to the experimental procedure. When estimated in crude homogenates, cytochrome P-450 expressed as nmoles/g liver (wet wt) was increased by 3-MC pretreatment of pregnant females in all studied ages of fetus or newborn. In the same conditions, PB failed to increase the cytochrome P-450 concentration of liver from 20 and 22-day-old fetuses, but induction became efficient in 12-hr-old newborns. Similar results were previously reported by Guenthner and Mannering on liver homogenates [13]: PB treatment appeared to have no effect on fetal hepatocytes.

When cytochrome P-450 measurements were performed in isolated microsomes (results expressed per mg protein), quite different data were obtained: PB produced a marked increase of cytochrome P-450, drug metabolizing and G-6-Pase activities in the 22-day-old fetus. However, the cytochrome P-450 to G-6-Pase activity ratio was not modified by PB pretreatment. A net biosynthesis of the hemoprotein may then be ruled out and a modification of endoplasmic reticulum structure hypothesized: ribosome attachment was lowered by PB pretreatment as indicated by the RNA/protein ratio and the distribution of cytochrome P-450 between rough and smooth endoplasmic reticulum in end-term fetuses. The decreased 'contamination' by ribosomal proteins under PB influence artificially increased the apparent cytochrome P-450 content referred to total proteins in 105,000 *g* pellets. Thus, PB triggers a premature transformation of rough to smooth endoplasmic reticulum, which naturally occurs in the early extrauterine life [30–33]. Our data extend to the end-term fetus the previously observed proliferation of smooth endoplasmic reticulum in PB-treated adult rats [34, 35] and the modification of sedimentation properties caused by inducers [36]. In newborn and adult rats, PB also produces a net biosynthesis of cytochrome P-450 which is not observed in fetal liver. 3-MC neither modifies the ultrastructure of adult liver [37], nor promotes a detachment of ribosomes from endoplasmic reticulum membranes; on the contrary, in microsomes prepared from fetuses of 3-MC-pretreated females, the RNA to protein ratio was higher than in controls and a less important fraction of cytochrome P-450 was associated with smooth endoplasmic reticulum. Thus, 3-MC really induces a net cytochrome P-450 biosynthesis; it increases the concentration of cytochrome P-450 in total homogenates and the cytochrome P-450/G-6-Pase ratio in microsomes. Moreover, in spite of higher 'contamination' by ribosomal protein, 3-MC increased cytochrome P-450 content and related mono-

oxygenase activities referred to total protein of the 105,000 *g* pellet, as already described with various polycyclic hydrocarbons [38–40].

The ribosomal content of microsomes may also explain the variable microsomal contamination of 10,000 *g* pellets (Table 1); smaller in PB-treated animals with lighter microsomes, higher in 3-MC-treated animals with heavier microsomes.

These observations allow us to clearly distinguish the transplacental effects of PB and 3-MC in near-term fetal liver: 3-MC produces a net biosynthesis of cytochrome P-450 and PB a premature transformation of rough into smooth endoplasmic reticulum which mimics an increased cytochrome P-450 content of microsomes without true induction. The inability of PB to promote a true induction of cytochrome P-450 in fetal liver, in spite of its placental transfer [13, 30] and its effect on endoplasmic reticulum, clearly shows that the transformation of rough into smooth endoplasmic reticulum is insufficient to explain the induction observed after birth. Our results do not rule out the existence, postulated by Guenthner and Mannering [13], of a feto–maternal endogenous factor able to suppress PB induction, but they prove that this factor should not interfere with the described effects of PB on endoplasmic reticulum structure.

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## REFERENCES

1. T. E. Gram, A. M. Guarino, D. H. Schroeder and J. R. Gillette, *Biochem. J.* **113**, 681 (1969).
2. P. Th. Henderson, *Biochem. Pharmac.* **20**, 1225 (1971).
3. S. M. McLeod, K. W. Renton and N. R. Eade, *J. Pharmac. exp. Ther.* **183**, 489 (1972).
4. E. Bresnick and J. G. Stevenson, *Biochem. Pharmac.* **17**, 1815 (1968).
5. W. Kuenzig, J. J. Kamm, M. Boublik, F. Jenkins and J. J. Burns, *J. Pharmac. exp. Ther.* **191**, 32 (1974).
6. J. R. Fouts and R. H. Adamson, *Science* **129**, 897 (1959).
7. T. K. Basu, J. W. T. Dickerson and D. V. Parke, *Biochem. J.* **124**, 19 (1971).
8. P. Arvela, N. T. Karki and R. O. Pelkonen, *Experientia* **32**, 1311 (1976).
9. C. Benedetto, T. F. Slater and M. U. Dianzani, *Biochem. Soc. Trans.* **4**, 1094 (1976).
10. G. Dallner, P. Siekevitz and G. E. Palade, *Biochem. biophys. Res. Commun.* **20**, 135 (1965).
11. P. Siekevitz, *J. Supramolec. Struct.* **1**, 471 (1973).
12. I. B. Chatterjee, Z. H. Price and R. W. MacKee, *Nature, Lond.* **207**, 1168 (1965).
13. T. M. Guenthner and G. J. Mannering, *Biochem. Pharmac.* **26**, 567 (1977).
14. K. Kawajiri, T. Harano and T. Omura, *J. Biochem., Tokyo* **82**, 1403 (1977).
15. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
16. W. C. Schneider, *Meth. Enzym.* **3**, 680 (1957).
17. H. Greim, *Naunyn-Schmiedebergs Arch. Pharmac.* **266**, 261 (1970).
18. D. C. Wharton and A. Tzagoloff, *Meth. Enzym.* **10**, 245 (1967).
19. M. A. Swanson, *J. biol. Chem.* **184**, 647 (1950).
20. P. Mazel, in *Fundamentals of Drug Metabolism and Drug Disposition* (Eds B. N. La Du, H. G. Mandel and E. L. Way) p. 546. Williams and Wilkins, Baltimore (1971).
21. V. G. Zannoni, in *Fundamentals of Drug Metabolism and Drug Disposition.* (Eds B. N. La Du, H. G. Mandel and E. L. Way) p. 566. Williams and Wilkins, Baltimore (1971).
22. E. Ackermann and A. Rane, *Chem-Biol. Interact.* **3**, 233 (1971).
23. E. Ackermann, A. Rane and J. L. E. Ericsson, *Clin. Pharmac. Ther.* **13**, 652 (1972).
24. A. Rane and E. Ackermann, *Clin. Pharmac. Ther.* **13**, 663 (1972).
25. S. A. Kamath, F. A. Kummerow and K. A. Narayan, *FEBS Lett.* **17**, 90 (1971).
26. G. Dallner and A. Azzi, *Biochim. biophys. Acta* **255**, 589 (1972).
27. M. Negishi and G. Kreibich, *J. biol. Chem.* **253**, 4791 (1978).
28. G. Dallner, P. Siekevitz and G. E. Palade, *J. Cell Biol.* **30**, 97 (1966).
29. M. M. Iba, L. F. Soyka and M. P. Schulman, *Molec. Pharmac.* **13**, 1092 (1977).
30. A. Chedid and V. Nair, *Devl Biol.* **39**, 49 (1974).
31. H. P. Rohr, A. Wirz, L. C. Henning, V. N. Riede and L. Bianchi, *Lab. Invest.* **24**, 128 (1971).
32. A. Herzfeld, M. Federman and O. Greengard, *J. Cell Biol.* **57**, 475 (1973).
33. A. Leskes, P. Siekevitz and G. E. Palade, *J. Cell Biol.* **49**, 264 (1971).
34. L. Ernster and S. Orrenius, *Fedn Procs Fedn Am. Socs exp. Biol.* **24**, 1190 (1965).
35. P. C. Burger and P. B. Herdson, *Ann. J. Pathol.* **48**, 793 (1966).
36. P. J. Murphy, R. M. Van Frank and T. L. Williams, *Biochem. biophys. Res. Commun.* **37**, 697 (1969).
37. J. R. Fouts and L. A. Rogers, *J. Pharmac. exp. Ther.* **147**, 112 (1965).
38. R. M. Welch, B. Gommi, A. P. Alvares and A. H. Conney, *Cancer Res.* **32**, 973 (1972).
39. E. Schlede and H. J. Merker, *Naunyn-Schmiedeberg's Arch. Pharmac.* **272**, 89 (1972).
40. F. Oesch, *J. biol. Chem.* **251**, 79 (1976).