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DISPOSITION OF MATERNALLY-ADMINISTERED METHADONE AND ITS EFFECTS ON HEPATIC DRUG-METABOLIZING FUNCTIONS IN PERINATAL GUINEA PIGS

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Abstract—Racemic methadone HCl (25 mg/kg given every 12 hr for 2 consecutive days) was administered orally to pregnant (60–65 days of gestation) or nursing (0–8 days post-partum) albino guinea pigs. The dams, fetuses and pups were killed 12 hr after the last dose for the anaysis of maternal and perinatal plasma, brain, hepatic and renal methadone residues by GLC and the *in vitro* measurement of maternal and perinatal microsomal mono-oxygenase [*p*-nitroanisole *O*-demethylase (OD) and aniline hydroxylase (AH)] and glucuronosyltransferase (GT) activities. Methadone was acquired by the perinatal guinea pig both transplacentally and via the milk, more agent being obtained by the former route. Fetal plasma levels of methadone were consistently lower than those detected in the mother due, largely, to differential plasma protein binding. The perinatal pattern of tissue distribution of methadone was markedly different from that observed in the dam, exceedingly high levels being found in fetal brain and kidney. Maternally-administered methadone did not affect the ontogenesis of perinatal OD, AH and GT but it did reduce significantly the levels of hepatic GT activity in the pups and in the dams.

Maintenance therapy, as well as extensive street use, has dramatically increased the number of physically dependent babies born to methadone-exposed mothers [1–4]. In addition to the well-documented withdrawal syndrome and low birth weight, these babies have low Apgar scores and frequent seizures, as well as a high incidence of severe hyperbilirubinemia and hyaline membrane disease [2, 5–7]. Approximately 10% of the methadone-dependent babies show delayed onset of the withdrawal syndrome up to 2–4 weeks after birth [8].

Studies of the disposition of methadone in pregnant rats, mice and monkeys have revealed that the mammalian placenta is not a physical barrier to the drug [9–11]. In the human, methadone is detected in amniotic fluid and cord blood as early as the sixteenth week of pregnancy [12–15]. The transfer of methadone from mother to infant does not end with parturition. In one study, lactating women, maintained on 80–100 mg of methadone per day, had milk levels in the range of 0.17 to 5.6 μg/ml, suggesting that considerable amounts of the agent could be acquired by the nursing infant [15]. To date, the actual amounts of methadone absorbed by the newborn during nursing and the possible untoward consequences of absorption have not been reported.

Experiments in our laboratory and in others have revealed that methadone exerts a subtle influence on mammalian hepatic microsomal drug-metabolizing enzymes. Depending on the species and sex as well as the route and duration of drug administration, induction [16–21], no effect [22–24] or inhibition [17, 25] of hepatic microsomal mono-oxygenases have all been observed. We have reported that methadone exerts a marked depressant or inhibitory influence on the microsomal UDP glucuronate β-glucuronosyltransferase activity in adult guinea pig liver [24]. In the present study, we investigated the placental and milk transfer of methadone as independent routes of perinatal exposure using the guinea pig as a model because it has a well-defined, trimester-type pregnancy and has morphologically and functionally more "mature" young than those of other rodent species [26]. In addition, a study of methadone binding to maternal and perinatal plasma proteins was conducted since differential plasma protein binding has been implicated as an important factor affecting the transplacental diffusion rate of a chemical [27].

MATERIALS AND METHODS

d,l-Methadone hydrochloride (analytical grade) was purchased from May & Baker (Canada) Ltd., Montreal, Canada. Stock solutions of 25 mg/ml were prepared in distilled water. SKF-525A was obtained from Smith, Kline & French, Montreal, Canada. All chemicals and solvents used were of the highest purity available (Fisher Scientific Co., Ltd., Montreal, Canada). The biochemicals required for hepatic drug-metabolizing enzyme assays were purchased from the Sigma Chemical Co., St. Louis, MO.

Timed-pregnancy, albino, Hartley strain female

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guinea pigs at 43–48 days of gestation and weighing 900–1100 g were obtained from Canadian Breeding Farms and Laboratories (St. Constant, Quebec) and were housed individually in stainless steel cages on softwood bedding. All animals were acclimatized to a room temperature of 22° with a controlled light cycle of 12 hr darkness and 12 hr light. Purina guinea pig chow and water were provided *ad lib.* The gestation period of a sample of forty-five pregnant animals were observed to be 68 ± 6 days.

The daily dosage of *d,l*-methadone (25 mg/kg *per os* every 12 hr for 48 hr) was chosen on the basis of previous experiments in which signs of toxicity were observed in adult guinea pigs treated with single daily oral doses of the order of 50 mg/kg. In addition, the plasma β $T_{0.5}$ values of *d,l*-methadone in non-pregnant, pregnant, and lactating female guinea pigs treated with single oral doses of 25 mg/kg were 8.2 ± 1.1, 14.6 ± 4.1, and 10.5 ± 1.4 hr respectively [28]. For the study of placental transfer of methadone, timed-pregnant guinea pigs of 60–65 days gestation received 25 mg/kg on the regimen described above (constant volume of 1.0 ml/kg body wt, 25 mg/ml) via a syringe attached to a curved animal feeding needle. Twelve hours after the last dose, the dams and their caesarean-derived fetuses were anesthetized with chloroform and killed by exsanguination by cardiac puncture. For the study of the milk transfer of methadone, lactating guinea pig dams at 0, 2, 5 and 8 days after parturition received oral methadone, 25 mg/kg, every 12 hr for 48 hr. During this period of time, their suckling pups were allowed to obtain milk freely. Litter size was adjusted to two pups in an attempt to reduce nutritional variation between litters. Twelve hours after the last dose (i.e. at 2, 4, 7 and 10 days post-partum), groups of dams and their pups were killed and studied as described below.

Following anesthesia, blood samples collected in heparinized syringes from treated animals were immediately centrifuged for 20 min, the plasma being removed by Pasteur pipette and stored for methadone residue analysis. The livers, kidneys, and brains were quickly excised, rinsed with saline, blotted, and weighed. In studies using fetal or very young neonatal tissues, it was necessary to pool the tissues of two littermates to provide adequate amounts of tissues for study.

A 6.0-g sample of liver was minced finely with scissors and washed thoroughly with 30 ml of cold 0.9% (w/v) sodium chloride solution to remove excess blood. A homogenate was prepared in a Potter–Elvehjem glass homogenizer with a motor-driven Teflon pestle, using sufficient ice-cold medium composed of sucrose (0.25 M), Tris–HCl buffer (0.05 M, pH = 7.4), $MgCl_2$ (0.005 M), KCl (0.025 M) and $CaCl_2$ (0.008 M) to produce a final homogenate concentration of 20% (w/v). Following the removal of aliquots of homogenate for methadone extraction, the balance of the homogenate was used for the preparation of microsomes by a Ca^{2+}-aggregation method [29]. The kidneys and brains were homogenized with sufficient cold 0.067 M phosphate buffer (pH = 7.4) to produce 50% (w/v) homogenates and were extracted immediately for methadone residue analysis.

The protein concentration of the final microsomal suspension was determined by the Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, CA) using an aliquot of the protein sample (0.1 ml) mixed rapidly with 5.0 ml of the filtered dye reagent on a Vortex mixer.

The hepatic microsomal mono-oxygenase, *p*-nitroanisole *O*-demethylase (OD) and aniline hydroxylase (AH), were assayed using aliquots of the final resuspended microsomes as described previously [30]. Initial rates of produce formation were calculated from the results of 10- and 20-min incubation periods at 37° in an oscillating metabolic shaker. The enzymatic activities were presented as nmoles of product formed $\cdot$ min^{-1} $\cdot$ (mg microsomal protein)$^{-1}$. Glucuronosyltransferase [uridine diphosphoglucuronate β-glucuronosyltransferase (GT), acceptor unspecific] activity was determined by the method of Lucier *et al.* [31], using α-naphthol as the aglycone acceptor. The details of the procedure for analysis have been published [29]. The enzymatic activity was calculated in terms of the nmoles of α-naphthol conjugated $\cdot$ min^{-1} $\cdot$ (mg protein)$^{-1}$.

d,l-Methadone was extracted from homogenates of brain, liver and kidney and from plasma and was quantitated by a method modified from those of Rickards *et al.* [32] and Inturrisi and Verebely [33] and described in a recent paper [28]. Briefly, following digestion of aliquots of tissue homogenates with 30% KOH in a boiling water bath, 0.5 ml of internal standard solution (SKF-525A, 625 ng) was added to the mixture which was subsequently extracted with 10.0 ml of *n*-butylchloride, the organic phase subsequently being extracted with 5.0 ml of 0.5 M H_2SO_4. The sulfuric acid fraction was removed, washed with *n*-heptane, made alkaline with 1.0 ml of 30% (w/w) KOH solution, and extracted with 50 μl of chloroform. An aliquot of 4.0 μl of the chloroform extract was analyzed by GLC, using a Varien Aerograph model 2100 gas chromatograph equipped with a hydrogen flame ionization detector and a glass column (2 m × 4 mm i.d.) packed with 3% SE-30 on Gas-Chrom Q 80–100 mesh [32, 33]. The limit of detection of *d,l*-methadone by the technique used was of the order of 2.5 ng/ml of homogenate. The percent recovery of methadone varied in different biological tissues, i.e. plasma (98%), liver (93%), brain (50%) and kidney (74%), though the relative percent recovery of methadone and the added internal standard (SKF-525A) in any one sample was similar. The extraction deficiencies were adjusted by using peak height ratios for the agent and the known amount of extracted internal standard.

The extent of plasma protein binding of methadone was assayed by an equilibrium dialysis method modified from that of Olsen [34]. An aliquot of plasma (1.0 ml) in a dialysis sac was incubated with constant agitation at 37° for 8 hr in a test tube containing 5.0 ml of a buffered (0.67 M phosphate, pH 7.4) solution containing *d,l*-methadone (500 ng/ml). Following extraction of aliquots of both the plasma and the external aqueous solution, the methadone levels were quantitated by the GLC method described above and the percentage of protein-bound methadone was calculated.

Statistical significance was determined by Student *t*-test for the means of the two independent samples. A probability of less than 0.05% was considered significant.

RESULTS

The daily oral administration of methadone (25 mg/kg) every 12 hr resulted in a significant decrease in the body weights of the animals, the reduction in body weight being more marked in nursing dams (10–15%) than in the pregnant and non-pregnant animals (5–7%). While methadone treatment did not influence significantly the ratios of liver wt/body wt in pregnant and non-pregnant adult females, the ratio was reduced significantly ($P < 0.05$) in the nursing dams at 4 days post-parturition.

The body weights and liver weights of guinea pig pups at different pre- and post-natal ages were not affected significantly by methadone administration to the pregnant or lactating dams except at 4 and 7 days of age where body weight reductions of the order of 10–20 g were recorded. This particular age coincides with the period of maximum milk ingestion by the young animals. The liver wt/body wt ratios of methadone-exposed nursing neonatal pups were not different from those of control pups. In contrast, the liver wt/body wt ratio (5.48 ± 0.51) of treated fetuses was significantly ($P < 0.05$) larger than that (4.34 ± 0.63) of control fetuses.

Figure 1 shows the distribution of methadone residues in plasma, brain, liver and kidney of non-pregnant, pregnant and nursing dams and their fetuses and pups 12 hr following a 2-day period of treatment of the dams with 25 mg methadone/kg every 12 hr.

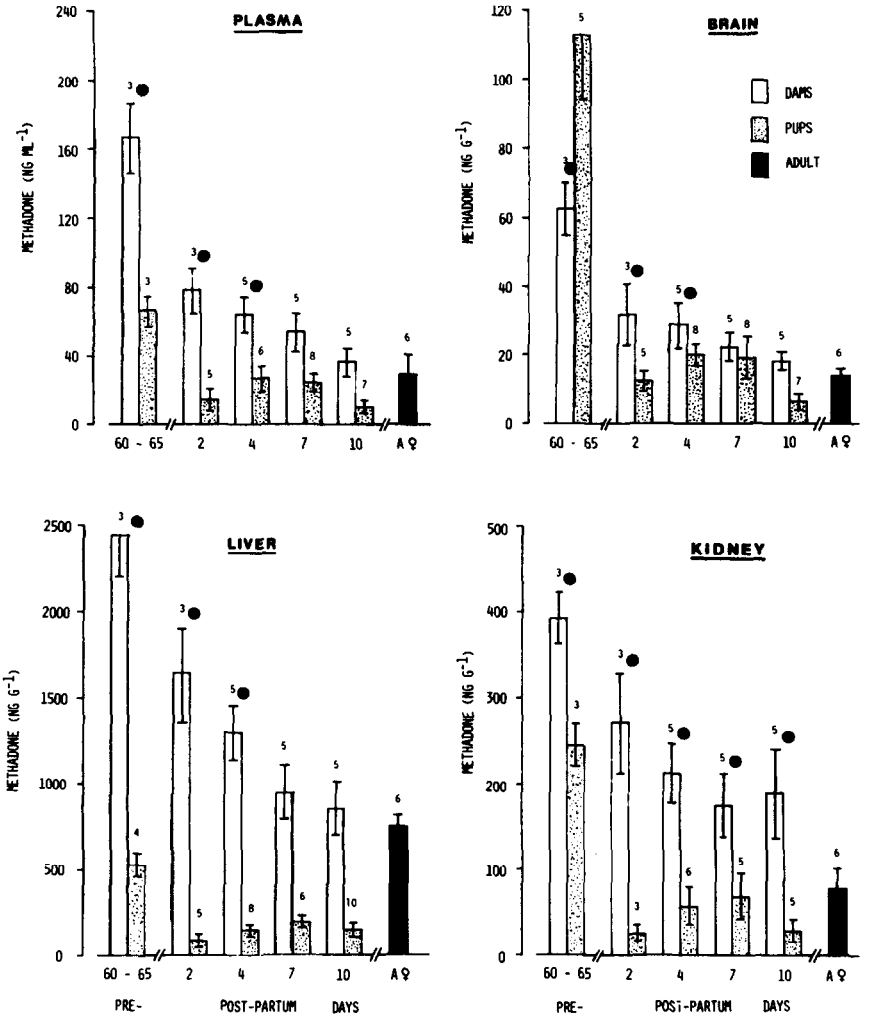

Fig. 1. Methadone residues in the plasma, brains, livers and kidneys of non-pregnant females (black bars) and of pregnant and nursing guinea pigs (open bars) and their fetuses and pups (stippled bars). At selected pre- and post-natal intervals, the dams received oral doses of methadone HCl (25 mg/kg body wt every 12 hr) for 2 consecutive days and the dams, fetuses and pups were killed 12 hr after the final doses. The results presented are mean tissue concentrations ± S.E.M. of the number of experiments shown at the top of each bar. The dot (●) signifies residue levels in the dams which were significantly different from similarly treated non-pregnant guinea pigs ($P < 0.05$). See text for details of analysis.

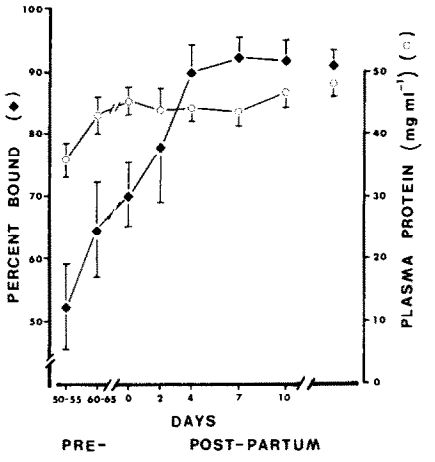

Fig. 2. Total protein content of perinatal guinea pig blood plasma (○) and the extent of plasma protein binding of methadone HCl (◆) in guinea pigs of different pre- and post-partum ages as measured by an equilibrium dialysis method. Each point and bar represents the mean value ± S.D. of a minimum of five determinations from individual samples.

Compared to the residue levels in treated non-pregnant animals, plasma concentrations of methadone in pregnant guinea pigs were exceptionally high. After parturition, the plasma levels dropped sharply but remained significantly higher at 2 and 4 days post-partum. A similar pattern of distribution was observed in maternal brain, liver and kidney, the residues being much higher than those measured in tissues from non-pregnant treated animals.

Fetuses of 60–65 days gestation acquired methadone via placental transfer as can be seen by the presence of the drug in the tissues. The concentrations of methadone in the fetal tissues were lower than those measured in the pregnant dams with the exception of fetal brain, a tissue in which the methadone levels were almost double those in the brain of the dams. Viable methadone-treated fetal animals younger than 60–65 days of gestation could not be obtained since, at 50–55 days of gestation, the fetuses examined had died *in utero* following treatment.

Newborn guinea pigs acquired methadone from their mothers via the breast milk. The post-natal tissue concentrations were never as high as those measured at the pre-natal stage. In all post-natal tissues analyzed, the methadone residues at days 4 and 7 after birth were higher than those measured at 2 and 10 days of age, results which correlated well with the pattern of maximum lactation in the dam and maximum milk consumption by the nursing pups.

An examination of the tissue/plasma ratio of methadone concentrations revealed that distribution of the drug in the pup was different from that in the mother. The brain/plasma ratio for fetuses at 60–65 days of gestation was 4- to 5-fold larger than that of the pregnant mothers. Following partiturition, the ratio dropped sharply to a 2-fold difference from that calculated for the corresponding dams and then declined slowly to approach the value measured for non-pregnant adult guinea pigs. In contrast, the liver/plasma ratio in the pups was approximately 2- to 3-fold smaller during late gestation and the first week of life compared to values calculated for the mothers. The ratio increased markedly in pups by 10 days of age. For the kidney/plasma ratio, values measured in fetuses were higher than those measured in the dams but, after birth, the kidney/plasma ratio became somewhat lower.

The binding of methadone to total plasma protein of adult female guinea pigs was studied *in vivo* over concentrations ranging from 26.1 to 6505.3 ng/ml which covered the concentration range measured in the plasma of methadone-treated animals (Fig. 1). The total plasma protein binding of methadone at concentrations ranging from 26.1 to 1427.0 ng/ml was approximately 90%. As the plasma concentration of methadone increased (1956.1 to 6505.3 ng/ml), a decrease in the binding of methadone from 83.4 to 73.7% was observed.

With an average plasma protein concentration of 48.0 ± 2.1 ng/ml, groups of non-pregnant guinea pigs showed a relatively constant level of binding of methadone *in vitro* of approximately 90.0%. As shown in Fig. 2, blood plasma from fetal guinea pigs at 50–55 and 60–65 days of gestation bound significantly less methadone than did older pups. After parturition, the percent bound increased markedly and, by 2 days of age, the amount bound was comparable to that measured in adult plasma. While the quantity of bound methadone changed dramatically with

Table 1. Effects of methadone on hepatic microsomal OD and AH in perinatal guinea pigs*

Age (days)	OD (nmoles·min^{-1}·mg^{-1})		AH (nmoles·min^{-1}·mg^{-1})	
	Control (N)	Treated (N)	Control (N)	Treated (N)
Gestational 60–65	0.02 ± 0.01 (5)	0.03 ± 0.01 (4)	0.005 ± 0.002 (5)	0.002 ± 0.001 (4)
Post-partum +2	0.48 ± 0.16 (6)	0.52 ± 0.06 (5)	0.024 ± 0.004 (6)	0.020 ± 0.003 (5)
+4	0.47 ± 0.16 (6)	0.49 ± 0.10 (8)	0.012 ± 0.003 (6)	0.019 ± 0.003 (8)
+7	0.47 ± 0.13 (6)	0.43 ± 0.08 (6)	0.019 ± 0.004 (6)	0.015 ± 0.008 (6)
+10	0.59 ± 0.20 (10)	0.48 ± 0.24 (10)	0.021 ± 0.007 (10)	0.016 ± 0.003 (10)
Adult♀	0.67 ± 0.13 (7)		0.024 ± 0.007 (7)	

* The treated animals acquired methadone via placental or milk transfer from their mothers who received oral methadone, 25 mg/kg every 12 hr for 2 days. OD (*p*-nitroanisole *O*-demethylase) and AH (aniline hydroxylase) activities are expressed as nmoles of produce formed per min per mg of microsomal protein. The data are mean values ± S.D. from the number of animals shown (N).

gestational and post-parturitional age, it did not reflect concomitant changes in the plasma protein concentrations between 50–55 days of gestation (35.8 ± 2.7 mg/ml) and 10 days of age (46.5 ± 2.4 mg/ml), the latter levels being comparable to those found in adult animals.

The enzymatic activities of hepatic microsomal OD and AH from pregnant and lactating dams and from pre- and post-natal pups of control and methadone-treated groups were measured and compared. While acute oral methadone treatment did not alter significantly the mono-oxygenase activities in the dams, levels were reduced significantly during the terminal stages of pregnancy and throughout the first post-parturition week [28].

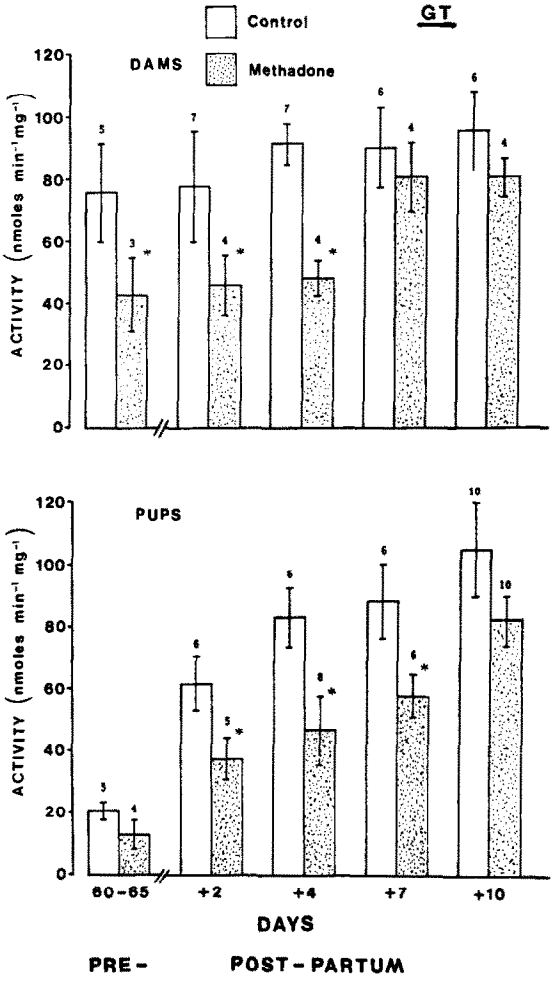

Fig. 3. UDP-glucuronosyltransferase activities (α-naphthol as aglycone acceptor) of control (open bars) and methadone-treated (stippled bars) pregnant or nursing guinea pigs and their fetuses and pups. Methadone HCl was administered orally (25 mg/kg body wt every 12 hr) for 2 consecutive days to the dams at pre-selected intervals. The females and their fetuses and pups were killed 12 hr after the last dose for study. The values presented are mean activities ± S.D. of the number of individual analyses shown at the top of each bar. The asterisk (*) signifies enzymatic activities in treated animals that are significantly different from those of comparable control values ($P < 0.05$).

The activities of fetal (60–65 days of gestation) hepatic microsomal OD and AH were very low (Table 1). At 2 days after birth, an approximate 20- to 25-fold increase in OD activities was observed which continued to increase slowly, attaining the level of activity measured in adult non-pregnant females by 10 days of age. Aniline hydroxylase (AH) activities were somewhat slower to develop, a 2-fold increase over that observed in the fetuses being measured at 2 days of age. After this stage, AH activities appeared to decline somewhat and then gradually increased to approach adult levels. The methadone, administered to the pregnant or nursing dams and consequently acquired by the fetuses and pups via placental or milk transfer, did not alter significantly the hepatic microsomal OD and AH when these enzymatic activities were compared with those determined in control perinatal animals of comparable age.

Figure 3 depicts the enzymatic activities of GT in control and methadone-treated pregnant and nursing dams and their fetuses and pups. The GT activites in control dams were somewhat reduced during pregnancy and at 2 days post-parturition, althought the differences were not statatistically different from values of control non-pregnant animals (80–100 nmoles · min^{-1} · mg^{-1}). At 4 days after parturition and at later time intervals, the GT activities were comparable to control levels. Treatment with methadone caused a significant further reduction ($P < 0.05$) in hepatic microsomal GT activities at 60–65 days of gestation and at 2 and 4 days after delivery. At 7 and 10 days after parturition, GT activities in control and methadone-treated dams were not significantly different ($P > 0.05$).

In control pups, GT activities measured approximately 20 nmoles · min^{-1} · (mg protein)$^{-1}$ at 60–65 days of gestational age. An approximate 3-fold increase in activity was observed 2 days after birth followed by a gradual increase to reach the adult non-pregnant female activity range. While a pattern of development similar to that observed in control pups was found in the methadone-exposed pups, markedly lower GT activities were detected during the stages of perinatal development investigated, significantly reduced ($P < 0.05$) activities being observed at 2, 4 and 7 days after birth. Limited residue analysis of milk samples taken from the stomachs of recently-nursed 4-day-old pups revealed methadone levels of the order of 2.5 to 5.5 μg/g.

DISCUSSION

The acute administration of methadone resulted in a slight but significant ($P < 0.5$) decrease in body weights of both dams and perinatal pups, and observation confirming earlier findings in adult animals [24, 28]. Reduced body and liver weights have been observed in 21-day-old rats born to dams treated with methadone throughout gestation and lactation [35]. Such effects in rats have been reported to be related to drug treatment rather than to drug-related changes in the nutritional status of the dams [36]. In humans, the association between methadone treatment and low birth weights has been well documented [2, 6, 7].

Maternally-administered methadone readily crossed the plancental structure to the fetal guinea pigs, most probably by a process of simple diffusion of the nonionized, unbound molecule. A combination of maternal and perinatal physiological and biochemical factors may have contributed to the high fetal tissue levels of methadone detected (Fig. 1). The high tissue levels in the pregnant dams suggested a reduced biotransformation and elimination of methadone, an observation confirmed by the low hepatic mono-oxygenase activities found during pregnancy as well as a prolonged plasma half-life (mean ± S.D.) in pregnant (β $T_{0.5}$ = 14.6 ± 4.1 hr at 50–55 days gestation) compared to that in non-pregnant female guinea pigs (β $T_{0.5}$ = 7.7 ± 0.3 hr). The higher levels of methadone in maternal blood coupled with the reduced binding of the drug to fetal plasma proteins (Fig. 2) would ensure greater uptake of free drug by fetal tissues. The calculated fetal/maternal plasma methadone concentration ratio was 0.4. Similar findings have been reported in other species [9–11]. In the human, the fetal/maternal ratio of methadone was reported to be 0.5 to 0.6 [12–14]. The high brain levels in fetal guinea pigs may also reflect a deficient or incompletely formed "blood–brain" barrier and the increased penetration of several endogenous and exogenous agents into fetal brain has been reported [37]. High levels of methadone have been detected in the brains of fetal rats and mice [9, 10].

The fetal liver acquired less methadone than did the other organs (Fig. 1). The lack of drug sequestration by the liver may be attributed to an inability of the tissue to absorb drugs from the blood. Gartner and Arias [38] showed a reduced absorption of bilirubin by perinatal guinea pigs, adult rates of uptake being attained only by 15 days of age. In neonatal rats, a low hepatic concentration of ouabain was related to a reduced uptake of this agent [39].

The nursing pups received only a small fraction of the methadone acquired transplacentally, an observation in agreement with studies using other chemicals [40]. The slightly higher tissue levels at 4 and 7 days post-partum agree with known patterns of milk production and consumption in guinea pigs [41]. The decreasing tissue level of methadone in the lactating dam probably reflect a gradual improvement of the drug-metabolizing capabilities of the dam, a fact confirmed by a marked reduction in the plasma half-life in lactating guinea pigs [28]. The lower tissue levels in the post-natal pups may also reflect the rapid development of hepatic drug-metabolizing enzymes (Table 1) and excretory mechanisms in the young animals [30].

The absence of an effect of methadone on hepatic microsomal mono-oxygenase in the dams and post-partum young was consistent with our earlier observations in adult animals [24, 42]. Recently, Roberts and Franklin [43] reported that methadone, unlike structurally-similar agents (SKF 525-A, acetylmethadol, propoxyphene), did not inhibit mono-oxygenase catalyzed reactions *in vitro*, being unable to form inactive cytochrome P-450–metabolite complexes *in vivo*. Equivocal results for the effects of methadone on hepatic microsomal mono-oxygenases have been obtained in studies with other species [16–25]. In contrast, the glucuronidation of α-naphthol by hepatic microsomal GT was markedly affected by methadone treatment, confirming earlier observations obtained for adult animals [24, 42]. While significant reductions in hepatic GT activities were observed up to 7 days of post-natal age in the pups, the rate of ontogenesis of this enzyme activity in perinatal liver was unchanged (Fig. 3). The mechanism by which methadone affects hepatic GT is not known, but the impairment of GT activity might result in elevated bilirubin levels in the blood. While this aspect was not studied in the present experiments, it is of interest to note that human infants born to methadone-treated mothers suffer from a high incidence of severe hyperbilirubinemia compared to those born to heroin-addicted mothers [5, 6].

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