MECONIUM DRUG ANALYSIS

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INTRODUCTION

According to a national survey in 1990, about 4.8 million women used some form of illicit drugs (Khalsa & Gfroerer, 1991). A sizable portion of this population are women of childbearing age or pregnant women. It has been reported that the prevalence of drug abuse among pregnant women ranges between 0.4% to 27% (Chasnoff, 1989). These figures are probably underestimated, because recently, we found in a large survey of infants delivered at our hospital, that 42% of the infants were exposed to cocaine, heroin, or cannabinoids in contrast to the 10.5% rate of illicit drug use in the same population obtained by maternal self report (Ostrea, Brady, Gause, Raymundo, & Stevens, 1992).

Drug abuse during pregnancy is a major health problem because the associated perinatal complications are high. These include a high incidence of stillbirths, meconium-stained fluid, premature rupture of the membranes, maternal hemorrhage (abruptio placenta or placenta praevia), and fetal distress (Chasnoff, Burns, Schnoll, & Burns, 1985; McGregor *et al*., 1987; Oro & Dixon, 1987; Ostrea & Chavez, 1979). For the newborn infant, the mortality rate, as well as morbidity (e.g., asphyxia, prematurity, low birth weight, hyaline membrane disease, infections, aspiration pneumonia, congenital malformations, cerebral infraction, abnormal heart rate and breathing patterns, drug withdrawal, and risk to acquired immunodeficiency disease) are also increased (Chasnoff, Hunt, Kleter, & Kaplan, 1989; Chasnoff, Bussy, Savich, & Stack, 1986; Fulroth, Phillips, & Durand, 1989; Oleske *et al*., 1983; Ostrea, Chavez, & Strauss, 1976; Ostrea, Kresbach, Knapp, & Simkowski, 1987; Ryan, Ehrlich, & Finnegan, 1987; Zelson, Rubio, & Wasserman, 1971; Zuckerman *et al.*, 1989). Long-term sequelae are not uncommon and include delays in physical growth and mental development, sudden infant death syndrome, and learning disabilities (Chasnoff, Hatcher, & Burns, 1982; Chavez, Ostrea, Stryker, & Smialek, 1979; Chavez, Ostrea, Stryker, & Strauss, 1979; Wilson, 1989; Wilson, McCrary, Kean, & Baxter, 1979). Because of these immediate and long-term problems, infants born to women who have abused drugs during pregnancy should be identified soon after birth so that appropriate intervention and follow-up with the infants can be done. For other reasons, an accurate identification of neonates exposed to drugs, in utero, is important. For instance, the data is vital for epidemiologic surveys, for the identification of women who need postnatal support, to assess the effectiveness of programs designed to reduce the incidence of drug abuse among pregnant women, and so on.

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Unfortunately, the identification of the drug-exposed neonate is not easy. Many of the drugs to which the fetus is exposed, in utero, do not produce immediate or recognizable effects in the neonates (Kandall & Gartner, 1974). Maternal admission of drug use is often inaccurate because of fear of the consequences stemming from such admission. Even with maternal cooperation, such information regarding the type and extent of drug use is often inaccurate (Ostrea *et al.*, 1992). One alternative is to test the infant's urine for drugs, but this procedure has its limitations, because successful detection of drug metabolites in the infant's urine is dependent on time of the last drug intake by the mother, or if obtained after birth, when the infant's urine was collected (Halstead, Godolphin, Lockitch, & Segal, 1988). The high rate of false negative results in neonatal urine tests often arise from the mother's abstention from the use of the drug a few days before she delivers or to the inability to obtain a sample of the infant's urine soon after birth. Recently, analysis of the infant's hair for drugs has been used (Graham, Koren, Klein, Schneiderman, & Greenwald, 1989). Technical problems in analysis and sample collection make this method impractical at the moment (Bailey, 1989).

In the last two years, we have successfully developed a new method for identifying the exposure of infants to drugs during pregnancy by the detection of drug metabolites in their meconium. Meconium is the green stool of the newborn formed in utero and excreted within a few days after birth. This narrative highlights the chronology of the studies that have demonstrated the usefulness and sensitivity of the meconium drug test.

ANIMAL STUDIES

The concept behind meconium drug testing was based on our initial studies in animals, which showed that a high concentration of the metabolites of drugs that the pregnant animal was exposed to during pregnancy were found in the gastro-intestinal tract of their fetuses. The index study (Ostrea, Lynn, Wayne, & Stryker, 1980) was designed to determine the distribution of morphine in the various organs of the fetuses of pregnant Rhesus monkeys made addicted to morphine. Unexpectedly, the highest concentrations of morphine and its metabolites were found in the fetal gastrointestines (see Table 10.1).

The high concentration of morphine metabolites in the gastrointestines of the fetus was postulated to occur as a consequence of the excretion of the morphine metabolites through the bile or through the urine, the latter swallowed by the fetus via the amniotic fluid (the morphine concentration of amniotic fluid was found to be 1.9 ± 1.0/dl).

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TABLE 10.1
Distribution of Morphine in the Tissues of Addicted Newborn Monkeys

	Monkey Fetus					
	**1*	**2*	**3*	**4*	**5*	**6*
Gestational age (days)	118	125	135	147	155	161
Total Maternal Morphine(g)	11.9	14.8	13.6	17.9	19.4	15.7
Fetal Tissue Concentration of Morphine (_g/g tissue)						
Intestines	15.8	128.9	108.4	53.7	68.4	42.1
Liver	0	0	0	47.6	169.5	0
Cerebellum	-	-	-	17.2	46.2	-
Heart	15.7	37.9	-	73.9	9.8	6.8
Spleen	16.2	72.5	-	0	0	53.3
Thymus	0	0	69.7	0	31.9	16.2
Lungs	0	0	35.5	0	13.2	0
Kidneys	0	0	0	0	24.5	3.0
Cerebrum	0	0	15.4	0	0	0
Brain stem	-	-	-	0	0	-

Note: (-) = Tissue not available for determination of morphine content.

Subsequent studies were conducted in rat models. In four, timed pregnant Wistar rats, three were given daily doses of cocaine, morphine, or cannabinoid at the schedule shown in Table 10.2. On the 20th day of gestation, the pups were delivered by cesarean section and their meconium (intestines) was collected, pooled, and analyzed for drugs. The drugs the dams received during pregnancy were found in the intestines of their pups (E. M. Ostrea, Brady, Parks, Asensio, & Naluz, 1989).

TABLE 10.2
Recovery of Drug Metabolites in the Intestines of Rat Pups Whose Dams Received Drugs During Pregnancy

Drug	*Dose per Day*	*Rat Weight*	*Number of Pups*	*Drug in Pups (_g/gm)*
Control	0	212	15	0.00
Cocaine HCI	50 mg/kg x 10 d	198	11	0.47
Morphine sulfate	50 mg/kg x 12 d	216	13	1.36
Cannabinoid	25 mg/kg x 12d	223	12	2.50

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TABLE 10.3
Cocaine Concentration in the Intestines of Rat Pups Based on Dose and Time of Cocaine Administration to the Mother

	Mean Cocaine Concentration (_g/gm intestine)					
Group *Dose* *Gestation*	*Group A* *Control* *Day 7-20*	*Group B* *20 mg/kg* *Day 7-20*	*Group C* *40 mg/kg* *Day 7-20*	*Group D* *40 mg/kg* *Day 1-6*	*Group E* *40 mg/kg* *Day 7-13*	*Group F* *40 mg/kg* *Day 14-20*
X	0.362	6.064	20.040	0.942	0.416	18.177
s.d	0.227	5.750	9.956	0.985	0.370	11.842

Note: A vs. B, C, F ($p < .001$); B vs. C ($p < .001$); D, E, vs. F ($p < .001$) by *t* test with Bonferroni correction.

The relationship of the dose and timing of cocaine administration to the pregnant animal and the concentration of cocaine in meconium was further studied (Lucena, Silvestre, Raymundo, Roxas, & Ostrea, 1991). Cocaine was injected daily to timed, pregnant Wistar rats (five per group) at doses and gestational periods shown in Table 10.3. On Day 20, the meconium (intestines) of the pups was analyzed for cocaine by radioimmunoassay. The amount of cocaine in meconium was related to the cocaine dose given to the dams (B vs. C) and the period in gestation when cocaine was administered (F vs. D and E).

Another subgroup of pregnant rats ($N = 5$ per group) were given cocaine (40 mg/kg) on Days 12-14, 15-17, or 18-20 to further characterize the third-week gestation of the fetus. Cocaine concentration in meconium was significantly higher than control only on Days 18-20 ($p < .001$). Thus, the amount of cocaine in rat meconium is influenced by the gestational age of the fetus.

Similarly, the relationship of the dose and timing of morphine administration to the pregnant animal and the concentration of morphine in meconium was studied (Silvester, Lucena, & Ostrea, 1991). Morphine was injected daily to timed pregnant Wistar rats (five per group) at doses and gestational periods shown in Table 10.4. On Day 20, the meconium (intestines) of the pups was analyzed for morphine by radioimmunoassay. The amount of morphine in meconium was related to the morphine dose given to the dams (B vs. C) and the time during gestation when morphine was administered (F vs. D and E).

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TABLE 10.4
Morphine Concentration in the Intestines of Rat Pups Based on Dose and Time of Morphine Administration to the Mother

	Mean Morphine Concentration (_g/gm intestine)					
Group *Dose* *Gestation*	*Group A* *Control* *Day 7-20*	*Group B* *5 mg/kg* *Day 7-20*	*Group C* *10 mg/kg* *Day 7-20*	*Group D* *10 mg/kg* *Day 1-6*	*Group E* *10 mg/kg* *Day 7-13*	*Group F* *140 mg/kg* *Day 14-20*
X	0.400	0.900	1.578	0.435	0.602	2.263
s.d	0.172	0.342	1.022	0.371	0.467	1.198

Note: A vs. B, C, F ($p < .01$); B vs. C ($p < .04$); F vs. D, E ($p < .001$).

A subgroup of pregnant rats ($N = 5$ per group) were given morphine (10 mg/kg) but only on Days 12-14, 15-17, or 18-20 of pregnancy. Morphine in meconium was higher than control only on Days 18-20 ($p < .001$). Thus, gestational age of the fetus significantly affects the amount of morphine in meconium.

From these observations evolved the hypothesis that meconium contains the metabolites of drugs the fetus was exposed to during gestation and the amount of drugs in meconium was related to the dose and period in gestation when the drug was administered during pregnancy.

DEVELOPMENT OF THE MECONIUM ASSAY

Meconium drug testing has ben adapted to various analytical methods, including radioimmunoassay (Ostrea, Parks, & Brady, 1988), enzyme immunoassay (Ostrea, Romero, & Yee, 1993), fluorescence polarization immunoassay (Ostrea, Romero, & Yee, 1993), and gas chromatography/mass spectroscopy (Bandstra, Steele, & Chitwood, 1992; Callahan *et al*., 1992; Clark, Rosenweig, & Raisys, 1990; Montes, Romero, Ostrea, & Ostrea, 1993; Ostrea, Yee, & Thrasher, 1991).

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Radioimmunoassay

The initial assay of meconium for drugs was by radioimmunoassay and was reported in 1988 (Ostrea *et al.*, 1988). The percentage recovery of morphine and cocaine from meconium was determined by spiking meconium with known amounts of morphine glucuronide and benzoylecgonine and having it analyzed by Abuscreen radioimmunoassay. Recovery of drugs was 84% to 97% for morphine and 70% to 100% for benzoylecgonine.

A comparison of two commercially available radioimmunoassays (DPC Coat-a-Count and Roche Abuscreen) was also done to determine which assay was best suited for meconium analysis. Twenty meconium samples from control and drug-dependent infants were tested for cocaine, opiate, and cannabinoid. For cocaine, a high correlation ($r = 0.9$) between the two methods was observed. However, the amount of cocaine detected by DPC was substantially higher than by Abuscreen, because DPC detects both cocaine and its metabolites. All meconium samples except one tested negative for opiate and cannabinoid by both methods. The one sample was positive for opiate by DPC but negative by Abuscreen. The results demonstrate the advantage of DPC radioimmunoassy for total drug analysis in meconium.

We also analyzed meconium for methamphetamine by radioimmunoassay (Gervasio & Ostrea, 1991). Drug-free meconium was spiked with methamphetamine to achieve concentrations from 250 to 1000 ng/ml. Methamphetamine was analyzed by Abuscreen radioimmunoassay, Meconium from 21 infants of drug-dependent mothers was also analyzed for cocaine, opiate, and methamphetamine. The recovery of methamphetamine from spiked meconium samples was high (96.8%). Meconium from 21 infants of drug-dependent mothers was 90% positive for cocaine. 10% positive for opiate, and 5% positive for methamphetamine.

Studies were also conducted to determine the sensitivity, specificity, precision, and drug cross-reactivity of the radioimmunoassay analysis of drugs in meconium (Romero, Mac, Knapp, & Ostrea, 1993).

Sensitivity/Specificity. Eight drug-free meconium samples were spiked with known amounts of morphine 3 glucuronide, benzoylecgonine, and 11-nor, delta-9-tetrahydrocannabinol-9-carboxylic acid to achieve drug concentrations ranging from 0 to 500 ng/ml. These were analyzed by DPC radioimmunoassay. As shown in Table 10.5, radioimmunoassay showed 100% sensitivity and specificity for cocaine, opiate, and cannabinoid detection and a high recovery rate for the three drugs: 122.2% for cocaine, 114.5% for morphine, and 73.4% for cannabinoid.

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Intra-assay Precision. Eight meconium samples from drug-dependent infants were analyzed in triplicate for cocaine, cannabinoid, and morphine by DPC radioimmunoassay. The mean coefficient of variation for the triplicate analysis was 12.6% for cocaine, 4.8% for morphine, and 11.9% for cannabinoid.

Drug Interferrence. Meconium contains significant amounts of bilirubin, blood, and some protein. These endogenous compounds were not found to interfere with the recovery of cocaine, morphine, and cannabinoid in meconium. Som common drugs were also tested for drug interference. The following drugs, added at high concentrations (100,000 ng/ml) to meconium, only had 0% to 0.2% cross-reactivity with the radioimmunoassay for cocaine, opiate, or cannabinoid: acetaminophen, phenobarbital, acetylsalicylic acid, propoxyphene, pentazocine, chlorpromazine, ibuprofen, meperidine, diazepam, lidocaine, and caffeine.

TABLE 10.5
Recovery of Cocaine, Morphine, and Cannabinoid from Spiked Meconium
by DPC Radioimmunoassay

	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Recovery Rate
Cocaine	100% (8/8)	100% (3/3)	100% (3/3)	100% (8/8)	122.2 ± 31.5%
Morphine	100% (10/10)	100% (3/3)	100% (10/10)	100% (3/3)	114.5± 21.9%
Cannabinoid	100% (7/7)	100% (3/3)	100% (7/7)	100% (3/3)	73.5 ± 13.0%

Studies were further conducted to determine the appropriate method of collection and storage of meconium. The stability of cocaine, morphine, and cannabinoid in meconium were studied under three storage conditions: (a) at room temperature for 24 hours, (b) emulsified in meconium solvent for 72 hours at room temperature, and (c) frozen at -15_ C for 12 weeks.

Meconium kept at room temperature without refrigeration for 24 hours resulted in a 25% decrease in cocaine concentration, a 62% increase in morphine concentration, and a 30% decrease in cannabinoid concentration. The increase in morphine concentration represents the hydrolysis of morphine glucuronide into morphine due to the action of beta glucuronidase in meconium and the 40 times higher sensitivity of DPC radioimmunoassay for morphine compared to its glucuronide. Meconium should therefore be sampled and processed within 12 hours after its excretion by the infant to avoid loss of drugs, specifically cocaine and cannabinoid.

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Meconium emulsified in the meconium solvent (buffered methanol) and kept at room temperature for 72 hours did not show a significant decrease in cocaine, morphine, or cannabinoid concentration. Drugs are therefore stable in meconium solvent for at least 72 hours at room temperature. For transport purposes, meconium in buffered methanol can be transported, without the need for refrigeration, for at least 72 hours.

Freezing of meconium for at least 16 weeks (109 days) at -15_ C did not cause a significant change in the concentration of cocaine, opiate, and cannabinoid.

Enzyme Immunoassay/Fluorescence Polarization Immunoassay

The original analysis of drugs in meconium was by radioimmunoassay. For wide-scale clinical application, meconium analysis was adapted to enzyme immunoassay (EIA) or flurescence polarization immunoassay, two methods commonly used in clinical laboratories (Gervasio & Ostrea, 1991; Ostrea *et al.*, 1993). Drug-free meconium was spiked with cocaine, morphine, or cannabinoid at concentrations ranging from 0 to 450 ng/ml and analyzed by enzyme immunoassay (enzyme multiplied immunoassay technique; EMIT), fluorescence polarization immunoassay (ADX), and DPC radioimmunoassay (Table 10.6). By radioimmunoassay, the sensitivity and specificity of cocaine, morphine, and cannabinoid analyses were 100%. By EMIT and ADx, the sensitivity for cocaine, morphine, and cannabinoid detection was 75%, 40%, and 100%, respectively; however the specificity for the three drugs was 100%. The lower sensitivity of EMIT versus radioimmunoassay was due to non-detection of drugs at low concentrations. Thus cutoff concentrations are 50 ng/ml for cocaine, 100 ng/ml for morphine, and 25 ng/ml for cannabinoid (Romero, Mac, Knapp, & Ostrea, 1993).

Using the previously given cutoff concentrations, meconium was obtained from 61 newborn infants and analyzed for cocaine, opiate (morphine), and cannabinoid by radioimmunoassay and enzyme immunoassay (EMIT). Opiate was detected in eight infants (13%) by radioimmunoassay and in nine (15%) by EMIT; cocaine was detected in 39 infants (64%) by radioimmunoassay and in 39 (64%) by EMIT. The concordance between the negative or positive results of the radioimmunoassay versus EMIT were 95% and 98%, respectively, for cocaine, and 98% and 100%, respectively, for opiate.

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TABLE 10.6
Sensitivity/Specificity of RIA, EIA, and FPI for the Detection of Cocaine, Opiate, and Cannabinoid in Spiked Meconium

		RIA	*EIA*	*FPI*
1.	*Cocaine*			
	Sensitivity (%)	100 (8/8)	75 (6/8)	75 (6/8)
	Specificity (%)	100 (3/3)	100 (3/3)	100 (3/3)
	Recovery Rate (%)	104.5 ± 14.1		
	Cutoff conc (ng/ml)	25	50	50
2.	*Opiate*			
	Sensitivity (%)	100 (10/10)	40 (4/10)	40 (4/10)
	Specificity (%)	100 (3/3)	100 (3/3)	100 (3/3)
	Recovery rate (%)	135.6 ± 9.6		
	Cutoff conc (ng/ml)	25	100	100
3.	*Cannabinoid*			
	Sensitivity (%)	100 (7/7)	100 (8/8)	100 (8/8)
	Specificity (%)	100 (3/3)	100 (3/3)	100 (3/3)
	Recovery rate (%)	96.6 ± 6.2		
	Cutoff conc (ng/ml)	15	25	25

Gas Chromatography/Mass Spectroscopy (GC/MS)

GC/MS analysis has been successfully applied to meconium drug testing (Bandstra *et al.*, 1992; Callahan *et al.*, 1992; Clark *et al.*, 1990; Montes *et al.*, 1993; Ostrea *et al.*, 1991). The mass spectrum (M/Z = 98, 119, and 176) of cotinine (metabolite of nicotine) in meconium and deuterated cotinine (M/Z = 101, 122, 179) as internal standard are shown in Fig. 10.1.

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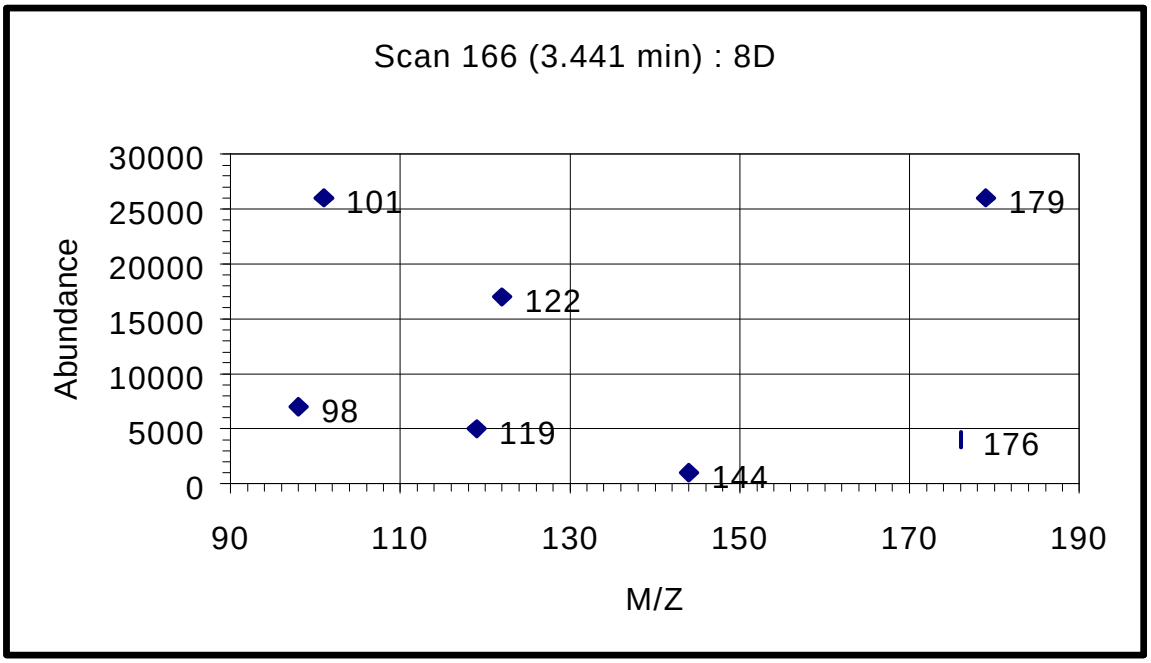

FIG. 10.1. Mass spectrum of cotinine (M/Z = 98, 119 + 176) and deuterated cotinine M/Z = 101, 122, 179) in meconium.

However, analysis by GC/MS is difficult because each drug requires a separate method for analysis, and analysis for opiate or cannabinoids requires preliminary hydrolysis of their metabolites prior to GC/MS analyses. We recently developed an improved method of GC/MS analysis of meconium that features the simultaneous detection of cocaine, morphine, and cannabinoid and the omission of preliminary hydrolysis of drug metabolites of morphine and cannabinoid (Montes *et al*., 1993). Twenty meconium samples from drug-exposed and control infants were analyzed for cocaine, opiate, and cannabinoid by RIA, EMIT, ADX and the improved GC/MS method. Table 10.7 shows the total number of samples positive for the drugs using the four methods of analysis.

The GC/MS method confirmed 100% of cocaine and 78% of morphine positive samples. GC/MS was more sensitive for cannabinoid detection than the three immunoassays.

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CLINICAL STUDIES

We conducted a number of clinical studies with the meconium drug test. Meconium from 20 infants of drug-dependent mothers and 5 control infants was analyzed by radioimmunoassay for morphine, cocaine, and cannabinoid.

As shown in Table 10.8, control stools showed no drug. Meconium from the infants of drug-dependent mothers showed the presence of at least one drug metabolite: 80% of the infants of drug-dependent mothers showed cocaine (range 0.14 to 19.91 _g/g stool), 55% showed morphine (range 0.41 to 14.97 _g/g stool), and 60% showed cannabinoid (range 0.05 to 0.67 _g/g stool). The concentrations of metabolites were highest during the first two days; some stools tested positive up to the third day. In contrast, only 37% of the infants had a positive urine drug screen by fluorescence polarization immunoassay (Ostrea *et al.*, 1989).

TABLE 10.7
Analysis of Meconium for Cocaine, Opiate, and Cannabinoid by
GC/MS ADX, Radioimmunoassay (RIA), Enzyme Immunoassay (EMIT),
and Fluorescence Polarization Immunoassay (ADX)

	GC/MS	*RIA*	*EMIT*	*ADx*
Cocaine (+)	18/20	18/20	18/20	18/20
Opiate (+)	7/20	9/20	6/20	6/20
Cannabinoid (+)	5/20	3/20	1/20	1/20

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TABLE 10.8
Recovery of Drug Metabolites in Meconium of Drug-Dependent Infants

Cocaine (_g/gm stool)			*Morphine (_g/gm stool)*			*Cannabinoid (_g/gm stool)*			
Day 1	*Day 2*	*Day 3*	*Day 1*	*Day 2*	*Day 3*	*Day 1*	*Day 2*	*Day 3*	*Urine[a] Screen*
6.35	3.23	(-)	3.28	1.72	0.56	(-)	(-)	(-)	(-)
2.34	2.17	1.17	1.19	1.17	(-)	(-)	(-)	(-)	(-)
1.77	9.68	3.67	(-)	(-)	(-)	(-)	(-)	(-)	(-)
10.86	11.29	(-)	(-)	(-)	(-)	0.13	0.29	(-)	(-)
(-)	(-)	(-)	5.38	12.11	(-)	0.05	(-)	(-)	Opiates
4.54	17.78	1.03	(-)	(-)	(-)	0.34	0.66	(-)	Cocaine
(-)	(-)	(-)	0.69	0.97	0.54	(-)	(-)	(-)	(-)
2.39	2.16	1.07	3.75	2.43	2.31	(-)	(-)	(-)	(-)
5.40	8.41	0.41	(-)	(-)	(-)	(-)	0.09	(-)	Cocaine
(-)	(-)	NS	11.74	14.97	NS	(-)	(-)	NS	Opiates
(-)	(-)	(-)	(-)	(-)	(-)	0.06	0.09	(-)	(-)
11.48	0.41	(-)	(-)	(-)	(-)	0.13	(-)	(-)	Cocaine
7.40	6.70	NS	5.36	5.73	NS	0.48	0.37	NS	Cocaine
11.42	0.29	NS	6.95	0.73	NS	0.67	(-)	NS	(-)
3.29	19.91	6.10	(-)	(-)	(-)	(-)	(-)	(-)	NS
0.26	(-)	NS	2.26	0.77	NS	0.14	(-)	NS	(-)
1.76	3.52	2.42	1.24	1.21	1.24	(-)	(-)	0.12	(-)
NS	16.23	13.15	NS	0.41	(-)	NS	0.22	0.09	Cocaine
0.95	0.14	(-)	(-)	(-)	(-)	0.07	(-)	(-)	(-)
0.06	0.03	(-)	(-)	(-)	(-)	0.19	0.17	0.05	(-)

With the successful adaptation of the meconium analysis to EMIT and ADX analyses, mass drug screening of newborn infants was initiated (Ostrea *et al*., 1993). A total of 4,409 infants from our institution (Hutzel Hospital) and from three other neonatal centers were tested (see Table 10.9). Hutzel Hospital showed the highest percentage of positive tests among the four centers; 38% positive samples, of which 90% were positive for cocaine. The high prevalence of drug use in the pregnant population at Hutzel Hospital corroborates the findings of an earlier report on the same population. In the remaining three centers, the prevalence of drug abuse was low and ranged between 1% to 4%. These centers represent low-risk rural or middle-class communities. Furthermore, in Center C, which represents a rural community, the principal drug found was cannabinoid, which contrasts sharply to the predominance of cocaine at Hutzel Hospital, which serves an urban population. The disparate results in the prevalence and types of drug abuse between the four centers are consistent with the low- or high-risk characteristics of the population tested.

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TABLE 10.9
Meconium Drug Analysis in Four Neonatal Centers

	Center	*No. Tested*	*No. Positive*	*Cocaine +*	*Opiate +*	*Connabinoid +*
1	Hutzel	2,032	773 (38.0%)	693 (90%)	117 (15%)	31(4%)
2	Center A	269	10 (3.7%)	4 (40%)	4 (40%)	3 (30%)
3	Center B	1,329	39 (2.9%)	22 (56%)	6 (15%)	15 (39%)
4	Center C	779	14 (1.8%)	1 (7%)	4 (29%)	10 (71%)

Routine meconium drug screening of all newborn infants is not practical because of cost, even in high drug prevalence areas. Thus, criteria were established to select the appropriate infants for testing (Ostrea & Romero, 1992). An infant was tested if (a) the mother admitted to the use of illicit drugs during the current (i) or past (ii) (denies current) pregnancy, (b) the mother admitted only to the use of marijuana or alcohol (iii), (c) the mother was a "walk-in" mother with some prenatal care elsewhere (iv), (d) the mother was a "walk-in" mother without prenatal care (v), (e) the infant manifested withdrawal and the mother denied drug use (vi), or (f) there was a social service request (e.g., infant for adoption/home placement, etc.) (vii). A total of 1,036 infants at Hutzel Hospital were tested using these screening criteria (Table 10.10).

Of 1,036 infants screened, 46% were positive for drugs, principally cocaine (90%); 83% of infants in Group 1 tested positive for drugs, which confirmed the high sensitivity of the meconium test; 38% to 47% in Groups 2 and 3 were drug positive, indicative of the high denial rate and the high illicit drug use associated with alcohol or marijuana; 16% to 20% in Groups 4 and 5 were drug positive, which indicates that women with poor prenatal care are at high risk for illicit drug use. Finally, 75% of the drug-positive infants were clinically normal at birth; thus drug screening was the only method to identify these high-risk infants. We conclude that selection criteria for routine drug screening of newborns can be established to efficiently identify the drug-exposed group.

A large-scale, prospective drug screening of newborn infants by meconium analysis was done to determine the prevalence and epidemiologic characteristics of drug use in a high-risk urban obstetric population (Ostrea, Brady, *et al.*, 1992). Every other infant that was delivered in our high-risk perinatal center was enrolled from November 1988 to September 1989 and their meconium was analyzed for the metabolites of the three commonly abused drugs (cocaine, morphine [opiates], and cannabinoid) by radioimmunoassay. Of 3,010 subjects studied (Table 10.11), 44% were positive for cocaine, morphine, or cannabinoid; 31% positive for cocaine, 21% positive for morphine, and

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12% for cannabinoid. Only 11% of the mothers admitted to illicit drug use: 52% of their infants had a positive urine drug screen, whereas 88% had a positive meconium drug screen. Prevalence of drug use among the pregnant women varied per month. A profile of the pregnant addict in the population studied was noted ($p < .001$): service patient, single multigravid (> 3) and little or no prenatal care. The major problems associated with drug use during pregnancy were principally noted in the group that was exposed to cocaine and opiates and in the group where the mothers admitted to the use of illicit drugs. On the other hand, a large number of infants who have been exposed to drugs in utero, but whose mothers denied the use of drugs, may appear normal at birth and go unrecognized. Improved detection of these infants at risk can be achieved with a high index of suspicion and meconium drug analysis.

In the same population, the prevalence of illicit drug exposure among infants admitted to the neonatal intensive care unit was also determined. Fifty percent of the infants were positive for drugs: 44% positive for cocaine, 11% for opiates, and none for cannabinoids (Ostrea, Brady, *et al.*, 1992).

TABLE 10.10
Drug Screening of Infants Based on Selection Criteria

Groups	*i*	*ii*	*iii*	*iv*	*v*	*vi*	*vii*
of cases	416	39	78	120	253	20	110
+ any drug	83%	38%	47%	16%	20%	10%	8%
Cocaine +	79%	28%	33%	12%	18%	10%	4%
Opiate +	12%	8%	5%	4%	2%	0%	4%
Cannabin +	2%	5%	14%	2%	1%	0%	1%

TABLE 10.11
Meconium Drug Screen of 3,010 Infants for the Metabolites of Cocaine, Opiate, and Cannabinoid

Total stools (meconium) analyzed	3,010 (100.0%)
Positive for drugs	1,333 (44.3%)[a]
1. Positive for cocaine	923 (30.7%)
2. Positive for opiate	617 (20.5%)
3. Positive for cannabinoid	346 (11.5%)
Negative for drug	1,677 (55.7%)

Note:[a] Prevalence of drug exposure based on maternal self-report = 335/3,010 (11.1%).

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RECENT DEVELOPMENTS IN MECONIUM DRUG TESTING

Serial meconium drug analysis can estimate the chronology and degree of the infant's in utero drug exposure (Ostrea, Knapp, Ostrea, Tannenbaum, & Saleri, 1994). We enrolled 58 pregnant drug users early in gestation (18-20 weeks) and prospectively monitored their drug use by: (a) in-depth maternal interview every two weeks; (b) maternal hair analysis of samples obtained at enrollment, midgestation, and delivery; and (c) serial meconium analysis by examination of every stool passed by the infant after birth for three days. Hair and meconium were quantitated by RIA and GC/MS for cocaine, opiate, and cannabinoid. Sensitivity, specificity, and correlations between interview, hair, and meconium analyses for type, amount, and timing of drug use were made.

TABLE 10.12
Incidence of Cocaine, Opiate, and Cannabinoid Use as Determined by Maternal Interview, Hair Analysis, and Meconium Analysis

Drug Use Determined by	*Cocaine +*	*Opiate +*	*Cannabinoid +*
Maternal interview	30 (50.8%)	16 (27.1%)	18 (30.5%)
Meconium analysis	40 (67.8%)	19 (32.2%)	7 (11.8%)
Hair analysis	46 (78.0%)	21 (35.6%)	10 (16.9%)

The incidence of cocaine, opiate, and cannabinoid use in the study population as determined by comprehensive maternal interview (excludes drug use during the periconception period), maternal hair analysis, and meconium analysis are shown in Table 10.12. For each method, a positive specific drug use was defined as the presence of a single positive test for that drug within the study period. Hair analysis showed the highest incidence of cocaine (78%) and opiate (35.6%) use, whereas maternal history showed the highest incidence of cannabinoid use (30.5%). Drugs were detected mostly in the drug-abusing group.

The sensitivity and specificity of each method of drug detection was analyzed in the following manner: For each subject, the result of the test for a drug by one method (e.g., meconium analysis), whether positive or negative, was compared to the combined test results of the other two methods (i.e., history and hair analysis). Concordance in test results between the combined methods was *required* to allow for a valid comparison. The reason for this is that the concordance in the test results of the two tests increased the reliability of their results and the validity of serving as the "gold

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standard" for comparison. As shown in Table 10.13, meconium analysis had the highest sensitivity and specificity for cocaine and opiate detection (97%-100%), followed by hair analysis, which also had high sensitivity but lower specificity (87%). For cannabinoid detection, maternal interview had the highest sensitivity (100%) compared to meconium (60%) and hair (38%) analyses.

TABLE 10.13
Sensitivity and Specificity of Maternal Interview, Meconium Analysis, and Hair Analysis in Detecting Gestational Use of Cocaine, Opiate, or Cannabinoid

	Cocaine Detection			*Opiate Detection*			*Cannabinoid Detection*		
	N^a	*Sens*	*Spec*	N^a	*Sens*	*Spec*	N^a	*Sens*	*Spec*
Maternal interview	54	75%	100%	50	94%	97%	39	100%	75%
Meconium analysis	44	97%	100%	52	100%	97%	40	60%	94%
Hair analysis	43	100%	87%	54	100%	87%	42	38%	91%

Note: [a]Indicates number of valid comparisons (see text for definition).

By calculating the positive and negative predictive values (*p*, in percent) of each test, their rates of false positive and false negative tests were determined (100 - *p*), and these are shown in Table 10.14. Maternal interview had the highest false negative rate for cocaine detection (42%) and hair analysis had the highest false positive rates for cocaine (13%) and opiate (24%) detection. Due to the low sensitivity of meconium and hair analysis for cannabinoid detection, each showed a 6% and 14% false negative rate, respectively, for the detection of that drug.

The comparatively low specificity of hair analysis that resulted in a high rate of false positive tests for cocaine (13%) and opiate (24%) was probably due to hair contamination as a result of passive exposure of hair to drugs in the environment. Human hair can be passively exposed to cocaine either from vaporized cocaine freebase (smoking of "crack" cocaine) or from cocaine hydrochloride dust particles in the air or clothes. In the former, it has been shown that even with prewashing of contaminated hair prior to drug analysis, cocaine cannot completely be removed from the hair and enough cocaine residue is left to produce false positive results.

Both meconium and hair analysis showed lower sensitivity to cannabinoid detection (60% and 38%, respectively) than maternal interview. This is because in 54% of the mothers who admitted to cannabinoid use, use of cannabinoid during pregnancy was sparse and episodic, which resulted in mild exposure of the fetus to the drug and drug levels below the detection limits of the two tests. For its clinical relevance, however, this small amount of exposure of the fetus to cannabinoid probably has little or no consequence on ultimate outcome (Fried & Watkinson, 1990). Meconium analysis, like hair analysis, had a high false positive rate for cannabinoid detection (40%). This value

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may be artificially high, because the calculation was based on a very small sample size of positive results (n = 5). Furthermore, in one case, the cannabinoid could be further adjusted down to 20%.

Safeguards were instituted to ensure the accuracy of the maternal interview, which was the most subjective of the methods used. These included a prospective design in the study, with the maternal interview starting at early gestation and at bimonthly intervals, plus the institution of use of a "bogus pipeline" technique. This technique consisted of informing the patient prior to the interview that their drug use would be confirmed by a sensitive laboratory test. Despite these safeguards, the false negative rate of maternal interview for cocaine use was high (42%). Thus, even under ideal conditions, maternal interview is subject to significant denial by the mother, particularly for cocaine use. On the other hand, if a mother admits to the use of a drug, there seems to be no valid reason to doubt it. Thus, the high false positive rate (75%) of maternal interview for cannabinoid use (Table 10.14) is erroneous and is the result of its comparison to meconium and hair analyses, both of which are not sensitive methods to detect small amounts of cannabinoid use by the mother.

In summary, a comparison of the sensitivity and specificity of maternal interview, meconium analysis, and hair analysis for detecting gestational exposure to cocaine, opiate, and cannabinoid reveal that comprehensive maternal interview is a tedious and time-consuming process, impractical for clinical use. Even in research settings, one may question its overall usefulness because of its high false negative rate for detecting cocaine use. Meconium and hair analyses are drug detection methods more applicable for clinical use. However, meconium analysis shows a clear advantage over hair analysis because the former is non-invasive and it is more sensitive and specific. In particular, the high false positive rates of hair analysis for cocaine and opiate detection due to passive exposure, pose a serious limitation to the use of the test.

One of the objectives in meconium drug testing was to determine whether serial, quantitative analysis of meconium for drugs can estimate the amount and the period(s) in gestation when the fetus was exposed to the drug(s). The assumption is that as meconium is deposited in the fetal intestines throughout gestation, the earliest formed meconium would be located in the most distal portion of the colon, whereas the most recently formed meconium would be in the proximal segments. Thus, as the infant excretes meconium after birth, the order in which meconium is passed should correspond to the time order of its formation in utero, and the quantity of drugs in meconium will also reflect the degree of fetal drug exposure during that period. Unfortunately, no data exist that establish this specific time sequence. Thus, for the purpose of the study, we set the following approximations: (a) meconium excreted between 0 and 10 hours after birth represents early gestation meconium, (b) meconium excreted between 11 and 20 hours after birth represents midgestation meconium, and (c) meconium excreted at more than 21 hours after birth represents late gestation meconium. Our reason for choosing this time sequence stems from our observation that by 36 hours after birth, the majority of the infant's stools have become transitional, which indicates mixture of meconium with milk stools. For the purpose of correlating meconium data with hair analysis and

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maternal interview data, the periods we outlined for meconium were set to correspond to the following gestation: early gestation = less than 20 weeks, midgestation = 21 to 30 weeks, and late gestation = more than 30 weeks. The mean concentrations of cocaine, opiate, and cannabinoid in meconium and hair, and the amount of drug use by maternal history corresponding to these time periods were determined. By multiple regression analysis, the correlations between the concentration of drugs in meconium and hair and drug use by maternal interview are shown in Table 10.15.

TABLE 10.14
Rates of False Positive and False Negative for Maternal Interview, Meconium Analysis, and Maternal Hair Analysis in Detecting Gestational Exposure to Cocaine, Opiate, and Cannabinoid

	Cocaine Detection		*Opiate Detection*		*Cannaboid Detection*	
	False +	*False -*	*False +*	*False -*	*False +*	*False -*
Maternal interview	0%	42%	6%	3%	75%	0%
Meconium analysis	0%	7%	6%	0%	40%	6%
Hair analysis	13%	0%	24%	0%	50%	14%

TABLE 10.15
Correlation Between the Amount of Drug Use by the Mother Based on Maternal Interview, Meconium Analysis, and Hair Analysis

Correlation Coefficient	*Cocaine*	*Opiate*	*Cannabinoid*
Meconium vs. Hair	0.471*	0.150	0.446*
Meconium vs. Interview	0.530*	0.132	0.386*
Hair vs. Interview	0.452*	0.418*	0.561*

*$p < .01$.

The concentrations of cocaine and cannabinoid in meconium correlated well to the amount of cocaine and cannabinoid found in maternal hair, and drug use based on maternal interview. For opiate use, however, the correlation was only significant between hair analysis and interview. The reason for the latter is probably overestimation of opiate in meconium by radioimmunoassay. There is almost a 40-fold greater sensitivity of the radioimmunoassay to morphine compared to morphine glucuronide. The conversion of morphine glucuronide to morphine can occur in meconium due to the presence in meconium of intestinal beta glucuronidase; thus the presence of morphine will substantially increase the opiate concentration in meconium by a factor of 40.

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Since the advent of meconium drug testing, one of the frequent questions asked is how early in gestation drugs are detected in meconium. We studied three pregnant women who had early interruptions of their pregnancies (Ostrea, Knapp, Romero, & A. R. Ostrea, 1994). Autopsies were done on their fetuses: two delivered by spontaneous abortions at 16 and 20 weeks of gestation and 1 stillborn delivered at 32 weeks who had jejunal atresia (Case 3). Meconium was obtained from the fetal small (SI) and large (LI) intestines and analyzed for cocaine, opiate, and cannabinoid by DPC radioimmunoassay. The mothers of all three fetuses used cocaine during pregnancy. The three fetuses were all positive for cocaine but negative for opiate and cannabinoid. Cocaine concentration in maternal hair and meconium are shown in Table 10.16.

TABLE 10.16
Concentration of Cocaine in the Meconium of Three Preterm Fetuses

Fetus			*Cocaine (ng/ml)*		
#	*Gest Age*	*Weight*	*Meconium (SI)*	*Meconium (LI)*	*Hair*
1	16 weeks	110 g	84	33	480
2	21 weeks	400 g	2,665	3,426	570
3	32 weeks	2,029 g	0	21	128

Cocaine was detected in meconium as early as the 16th week of gestation. The amount of cocaine in meconium was proportioned to the cocaine concentration in maternal hair. Drugs were deposited in meconium either from bile secretion or from swallowed amniotic fluid (fetal urine); thus, intestinal obstruction in the fetus can affect the deposition of drugs in meconium due to the interruption of flow of the intestinal contents. This was demonstrated in Case 3. High jejunal obstruction in this fetus prevented the passage of cocaine through the intestines. Because cocaine was detected only in the distal segment of the large intestine and not in the small intestine, jejunal obstruction in the fetus must have occurred early in gestation.

We studied fetal exposure to nicotine in passive and active maternal smoking by meconium analysis and for the first time provided evidence that nicotine metabolites (cotinine and trans 3'-hydroxycotinine) can be detected in meconium (Ostrea, Knapp, Robero, Montes, & Ostrea, 1994). There is also a correlation between the concentration of nicotine metabolites in meconium and degree of maternal active and passive smoking. Meconium was collected from 55 infants whose mothers were nonsmokers (N = 10), passive smokers (N = 25), light (< 1 pack per day) active smokers (N = 13), and heavy (≥ 1 pack per day) active smokers (N = 7), Meconium was analyzed for nicotine metabolites (cotinine and 3-OH cotinine) by radioimmunoassay (see Table 10.17).

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TABLE 10.17
Mean (SD) Meconium Concentration of Nicotine Metabolites in Infants of Control, Passive, and Active Smokers, as Analyzed by Radioimmunoassay

Category	*N*	*Concentration (ng/ml)*
Nonsmoker	10	10.9 ± 7.6
Passive smoker	25	31.6 ± 17.2*
Light, active smoker (< 1 pack per day)	13	34.7 ± 22.3*
Heavy, active smoker (≥ 1 pack per day)	7	54.6 ± 19.9**

*p < .05 compared to non-smoker (one way ANOVA).
** P < .05 compared to light, active smoker, passive smoker and non-smoker (one way ANOVA).

The mean concentration of nicotine metabolites in meconium in the passive and active smokers was significantly higher compared to nonsmokers (p < .05, ANOVA). However, the nicotine concentrations in meconium from passive smokers were *not* significantly different from the light active smokers (t = 0.65, p > .05). The correlation coefficient between nicotine metabolites in meconium and the degree of maternal smoking graded as 1 for control, 2 for passive, 3 for light active, and 4 for heavy active smoking was 0.54 (p < .001). Thus, gestational exposure of the fetus to nicotine in both active and passive maternal smoking can be quantitatively measured in the fetus by analysis of meconium for nicotine metabolites. Of significance, in utero exposure to tobacco smoke in infants of passive smokers was as high as among infants whose mothers actively smoked less than 1 pack per day during pregnancy.

We previously reported on the analysis of meconium for cocaine and its metabolites by GC/MS in drug-dependent infants (Ostrea *et al.*, 1991). In nine infants studied, we found that 67% of the cocaine in meconium was present as the parent compound, cocaine. This was unusual, because most of the cocaine excreted in the fetal urine is in the form of its water-soluble metabolite, benzoylecgonine. To study this observation, we analyzed, by GC/MS, cocaine and benzoylecgonine in meconium, gastric aspirate, and urine of 10 infants (Garcia, Romero, Garcia, & Ostrea, 1994). We observed that benzoylecgonine was the principal form of cocaine in both urine and gastric aspirate with little or no cocaine (Table 10.18). Yet in meconium, cocaine was present in 50% of the specimens analyzed. The explanation for this difference is yet unknown, although the observation is clinically significant because cocaine is lipid soluble and can therefore be absorbed back into fetal circulation. Thus, this implies endogenous and repeated exposure of the fetus to cocaine.

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TABLE 10.18
Concentration (ng/mL) of Cocaine (COC) and Benzoylecgonine (BE)
in Meconium, Gastric Aspirate, and Urine of 10 Infants

	Meconium		*Gastric Aspirate*		*Urine*	
	Coc	*BE*	*Coc*	*BE*	*Coc*	*BE*
1	56.74	63.83	0	199.37	0	86.85
2	0	0	0	0	0	0
3	0	10.75	144.08	18.57	0	0
4	0	7.47	0	24.74	0	0
5	27.31	25.85	0	11.08	0	34.90
6	0	0	0	0	0	10.80
7	22.62	22.23	0	0	43.70	0
8	0	67.32	0	0	0	25.88
9	16.71	14.04	0	7.47	0	7.04
10	0	16.10	0	24.46	0	131.64

The prevalence of illicit drug exposure in infants in the intensive care unit was studied (Ostrea, Lizardo, & Transfranca, 1992). The meconium drug test was used to prospectively screen for drugs (opiates, cocaine, and cannabinoids) every infant admitted to a neonatal intensive care unit (NICU) at a high-risk perinatal center from June 10 to August 15, 1991. The morbidity, mortality, and cost of care for the infants were determined. A total of 122 infants were enrolled but 40 were not tested because of insufficient or no meconium samples collected. Of the 82 infants tested, 41 (50%) were positive for drugs, 36 (44%) positive for cocaine, 9 (11%) positive for opiates, and none for cannabinoid. The material profile or complications in the drug-positive group was: 83% Medicaid, 90% African American, 90% unmarried, 75% no prenatal care, 54% Cesarean section, 8% meconium-stained fluid, and 30% prolonged rupture of membranes. The neonatal profile was premature (78%), small gestational age (6%), weight less than 1,500g (48%), length less than 35 cm (3%), head circumference less than 28 cm (30%), and Apgar less than 6 at 1 min (29%). The total length of hospital stay of the drug-positive infants was 979 days, or an average of 26 days per infant. At an average NICU cost (excluding physician's fee) per day of $1,250, this amounted to a total cost of $1,223,750. Thus, the prevalence of drug exposure in infants admitted to the NICU is very high. Fifty percent of the morbidity, the mortality, and the high cost of care of infants in the NICU is associated with illicit drugs.

We also studied whether the recent upsurge in the incidence of congenital syphilis was a drug-related event (Sison, Ostrea, & Saleri, 1992). Although reports have shown that the resurgence of congenital syphilis is related to illicit drug use, these reports were based on a restricted population of pregnant women who have openly admitted to the use of drugs. Thus, the true

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relationship of congenital syphilis to drug use is not known because a large number of pregnant women deny their use of drugs. Infants in a high-risk nursery were screened for drugs by the meconium drug screen test, if (a) the mother admitted to the use of illicit drug during the current or past (denies current) pregnancy, (b) the mother admitted only to the use of marijuana or alcohol, (c) the mother was a "walk-in" mother with some prenatal care elsewhere, (d) the mother was a "walk-in" mother without prenatal care, (e) the infant manifested "withdrawal" and the mother denied drug use, and (f) social service request (e.g., infant for adoption/home placement, etc.). Similarly, all parturients in the obstetric service were routinely screened on admission for syphilis by the RPR and FTA-ABS (if RPR is positive) tests. Thus, in this large and more encompassing population of drug- and syphilis-screened maternal infant dyads (N = 1,012), the relationship between congenital syphilis and drug abuse in pregnancy was studied.

Meconium drug screen (MDS) was positive for one or more drugs in 449 infants (44.4%). Seventy-two mothers (7%) had positive RPR/FTA-ABS tests. Forty-six of their infants (4.5%) had congenital syphilis based on current definitions. Of 449 infants with positive MDS, 47 mothers (10.5%) were RPR positive and 32 (7%) infants had congenital syphilis, whereas of 563 infants with negative MDS, 25 (4.4%) mothers were RPR reactive and 14 (2.5%) infants had congenital syphilis (x = 13.7, $p < .001$). The incidence of positive RPR and congenital syphilis in the MDS positive group was not significantly different ($p > .10$) whether mother admitted to illicit drug use (11% and 8%, respectively) or not (8.8% and 4.4%, respectively). We conclude that maternal drug abuse is truly a significant factor that is related to the resurgence of congenital syphilis.

A study was also conducted to determine if meconium analysis can detect acute, intrapartum drug exposure. We therefore studied 12 infants, 2 control and 10 whose mothers were given meperidine during labor (Morales, Knapp, Utarnachitt, Utarnachitt, & Ostrea, 1994). The infants' meconium was collected for 2 days and individually tested for meperidine and normeperidine by GC/MS. Two infants whose mothers were given codeine just before labor were included. Meconium from the control group was negative for meperidine or normeperidine. In the treated group, meperidine was the predominant drug found in meconium and was identified in samples collected on Days 1 and 2 (Table 10.19). In one third of the samples, normeperidine was also isolated at concentrations approximately half those of meperidine. In two infants whose mothers received codeine before labor, the MDS by radioimmunoassay was positive for "opiates" and by GC/MS was positive for codeine.

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TABLE 10.19
Detection of Meperidine in Meconium

Meperidine (ng/ml)	*Day 1 Meconium*	*Day 2 Meconium*
Mean	282.9 ± 821.4	145.2± 178.2
Range	0-3,450	0-3,590

We conclude that MDS can detect both *acute* and *chronic* fetal exposure to drugs. This demonstrates that a wide range of gestational drug exposure, from early midgestation to labor, can be detected by the meconium drug test. Analgesics, which contain codeine, are easily available and are used before or during labor. This may result in a positive, nonspecific meconium drug screen for opiates and underscores the need for specific drug identification, if exposure to illicit opiates is sought.

Lastly, although the fetal effects of alcohol are known, no reliable marker of fetal exposure to alcohol has yet been identified. Fatty acid ethyl esters (FAEE) are enzymatic, nonoxidative products of in vivo ethanol metabolism, with a long half-life, and they are markers of ethanol consumption in the adult. We reported on the identification of FAEE in meconium of alcohol-exposed infants at concentrations proportional to the amount of maternal alcohol use (Mac, Pacis, Garcia, & Ostrea, 1994). Preliminary studies to determine the optimum method for extraction and isolation of FAEE in meconium were done. FAEE standards (ethyl palmitate and stearate) were spiked into meconium and extracted by acetone or hexane: water and isolated by thin layer chromatography or bonded phase column. Detection was by GC/MS. Optimum FAEE extraction and chromatogram were achieved using the hexane: water/bonded phase column combination. With this method, meconium was analyzed for FAEE in 10 control and 15 alcohol-exposed infants. In the latter, FAEE concentrations in meconium were multifold higher than control, with a wide range proportional to the amount of maternal alcohol use (Table 10.20).

TABLE 10.20
Fatty Acid Ethyl Esters in Meconium of Control and Alcohol-Exposed Infants

Mean FAEE (ng/ml)	*Ethyl Laurate*	*Ethyl Palmitate*	*Ethyl Stearate*
Control	32.0	67.2	32.3
(range)	(0-29.6)	(2-367)	(0.4-54)
Alcohol exposed	4,799	1,082	338.2
(range)	(76-36,106)	(57-8,290)	(28-1,908)

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We concluded that FAEE in meconium may serve as an important biologic marker of fetal exposure to ethanol and provide an important, objective tool for the precise study of alcohol exposure and its fetal effects.

STUDIES BY OTHERS

Meconium drug analysis has also been studied by other workers and their data confirm the sensitivity, specificity and usefulness of the test.

Drug-free meconium was spiked with benzoylecgonine and cocaine for extraction efficiencies (Clark *et al.*, 1990). The determination was sensitive for spiked samples to 0.3 each of cocaine and benzolecgonine per gram of meconium. The assay was linear for cocaine to 10/g and to 3.5 gm for benzoylecgonine. Prechromatographic extraction efficiencies were 100% for cocaine and 30% for benzoylecgonine. Cocaine and benzoylecgonine were analyzed by GC/MS in meconium of three infants of cocaine-dependent mothers: Cocaine was found in one infant whose mother used cocaine heavily in the first two trimesters; cocaine and benzoylecgonine were found in another infant, and none in the third infant who had limited in utero exposure to cocaine.

Meconium from 28 neonates born to women suspected of drug abuse were tested for cocaine, morphine, codeine, and marijuana (Maynard, Amoroso, & Oh, 1991). In each case, testing of urine from the mother, the infant, or both were done because of suspected maternal drug abuse. Seventeen of 28 (61%) meconium samples tested positive; 28 of 47 (60%) urine samples were positive. Meconium test results were concordant with the results of maternal or newborn urine testing in 24 of the 28 (86%) cases. In three cases, meconium was positive for cocaine when newborn urine was negative; in one case, meconium was negative when maternal urine was positive for cocaine. Compared with the combination of maternal and newborn urine testing, meconium testing had an 82% positive predictive value (14 of 17) and a 91% negative predictive value. The authors further added that the collection of meconium was simpler and more reliable than collection of urine and that the testing of meconium was easily incorporated into routine procedures at a busy commercial laboratory.

The sensitivity of newborn hair, meconium, and urine analyses for drugs in detecting gestational exposure to cocaine was studied (Callahan *et al*., 1992). Infants were born to 59 women who were interviewed to determine their use of cocaine during pregnancy and whose hair was analyzed for the presence of cocaine. Regression analysis was used to evaluate the relationship between cocaine in newborn hair and in maternal hair. Radioimmunoassay of newborn hair and gas

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chromatography of meconium were more sensitive than immunoassay of urine ($p < .02$). Urine immunoassay failed to identify 60% of cocaine-exposed infants.

A comparative methodologic study was done to detect in utero cocaine exposure (Bandstra, *et al.*, 1992). Maternal history was compared with various assays in meconium, maternal urine, and infant's urine, using GC/MS, EMIT, ADX, and DPC radioimmunoassay. The authors found meconium to be superior to either maternal or infant urine in detecting in utero cocaine exposure, although the need for concomitant maternal histories in some cases was emphasized.

SUMMARY

Drug abuse in pregnancy is an important health-care problem in the United States and globally, as well. In 1990, it was estimated that 4.8 million women in the United States used illicit drugs and 0.4% to 32% used illicit drugs during pregnancy. The impact of illicit drug use during pregnancy on the mother and infant are far reaching. Thus, for the appropriate management and care of the mother and infant, identification of the drug-exposed infant is vital. The meconium drug test has become an important test to detect these infants. For several reasons, meconium drug analysis is ideal for this purpose: (a) the test is sensitive and specific, (b) the test can be performed using common laboratory techniques for purposes of mass screening and with capabilities for GC/MS confirmation, (c) collection of meconium is easy and noninvasive, (d) meconium can be analyzed for a number of illicit and licit drugs, the latter including nicotine, (e) analysis of serial meconium can reflect the type, chronology, and amount of in utero drug exposure of the infant, and, (f) drugs in meconium are present up to the third day after birth; thus late testing of the infant for drugs is possible, if necessary. Meconium drug testing has therefore become an important diagnostic tool for clinical and research purposes.

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REFERENCES

Bailey, D. N. (1989). Drug screening in an unconventional matrix: Hair analysis. *Journal of the American Medical Association, 262,* 3331.

Bandstra, E., Steel, B., & Chitwood, D. (1992). Detection of in utero cocaine exposure: A comparative methodologic study. *Pediatric Research 31,* 58A.

Callahan, C. M., Grant, T. M., Phipps, B. S., Clark, G., Novack, A. H., Streissguth, A. P., & Raisys, V. A. (1992). Measurement of gestational cocaine exposure: Sensitivity of newborn hair, meconium and urine. *Journal of Pediatrics, 120,* 763-768.

Chasnoff, I. J. (1989). Drug use and women. Establishing a standard of care. *Annals of New York Academy of Science, 562,* 208-210.

Chasnoff, I. J., Burns, W. J., Schnoll, S. H., & Burns, K. A. (1985). Cocaine use in pregnancy. *New England Journal of Medicine, 313,* 666-669.

Chasnoff, I. J., Bussy, M. E., Savich, R., & Stack, C. M. (1986). Perinatal cerebral infarction and maternal cocaine use. *Journal of Pediatrics, 108,* 456-459.

Chasnoff, I. J., Hatcher, R., & Burns, W. J. (1982). Polydrug and methadone addicted newborns: A continuum of impairment. *Pediatrics, 70,* 210-213.

Chasnoff, I. J., Hunt, C. E., Kletter, R., & Kaplan, D. (1989). Prenatal cocaine exposure is associated with respirating pattern abnormalities. *American Journal of Diseases of Children, 143,* 583-5887.

Chavez, C. J., Ostrea, E. M., Jr., Stryker, J. C., & Smialek, T. (1979). Sudden infant death syndrome among infants of drug dependent mothers. *Journal of Pediatrics, 95,* 407-409.

Chavez, C. J., Ostrea, E. M., Jr., Stryker, J. C., & Strauss, M. E. (1979). Ocular abnormalities in infants as sequalae of prenatal drug addition. *Pediatric Research, 12,* 367A.

Clark, G. D., Rosenweig, B., & Raisys, V. A. (1990). Analysis of cocaine and benzoylecgonine in meconium of infants born to cocaine dependent mothers. *Clinical Chemistry, 36,* 1022A.

Fried, P. A., & Watkinson, B. (1990). 36 and 48 month neurobehavioral follow up of children prenatally exposed to marijuana, cigarettes, alcohol. *Journal of Development and Behaviour, 11,* 49-58.

Fulroth, R., Phillips, B., & Durand, D. (1989). Perinatal outcome of infants exposed to cocaine and/or heroin in utero. *American Journal of Diseases of Children, 43,* 905-910.

Garcia, D., Romero, A., Garcia, G., & Ostrea, E. M., Jr. (1994). Gastric juice analysis for drugs. *Pediatric Research, 35,* 225A.

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Gervasio, C., & Ostrea, E. M., Jr. (1991). Bedside meconium drug testing using latex agglutination inhibition test. *Pediatric Research, 29*, 215A.

Graham, K., Koren, G., Klein, J., Schneiderman, & J. Greenwald, M. (1989). Determination of gestational cocaine exposure by hair analysis. *Journal of the American Medical Association, 262,* 3328-3330.

Halstead, A. C., Godolphin, W., Lockitch, G., & Segal, S. (1988). Timing of specimens is crucial in urine screening of drug dependent mothers and infants. *Clinical Biochemistry 21,* 59-61.

Kandall, S. R., & Gartner, L. M. (1974). Late presentation of drug withdrawal symptoms in newborns. *American Journal of Diseases of Children, 12,* 58-61.

Khalsa, J. H., & Gfroerer, J. (1991). Epidemiology and health consequences of drug abuse among pregnant women. *Seminars in Perinatology, 15,* 265-270.

Lucena, J., Silvestre, M. A., Raymundo, A. L., Roxas, R., Jr., & Ostrea, E. M., Jr. (1991). The effect of timing, dosage duration of cocaine intake during pregnancy on the amount of cocaine in meconium in a rat model. *Pediatric Research, 29,* 62A.

Mac, E., Pacis, M., Garcia, G., & Ostrea, E. M., Jr. (1994). A marker of fetal exposures to alcohol by meconium analysis. *Pediatric Research, 35* 238A.

MacGregor, S. N., Keith, L. G., Chasnoff, I. J., Rosner, M. A., Chisum, G. M., Shaw, P., & Minogue, J. P. (1987). Cocaine use during pregnancy. Adverse perinatal outcome. *American Journal of Obstetrics and Gynecology, 157,* 686-690.

Maynard, E. C., Amuroso, L. P., & Oh, W. (1991). Meconium for drug testing. *American Journal of Diseases of Children, 145,* 650-652.

Montes, N., Roberto, A., Ostrea, E. M., Jr. & Ostrea, A. R. (1993). Improved method of GC/MS analysis of meconium for opiate, cocaine and cannabinoid. *Pediatric Research, 33,* 66A.

Morales, V., Knapp, D. K., Utamachitt, R., Utamachitt, D., & Ostrea, E. M., Jr. (1994). Meconium analysis will detect intrapartum drug use: Clinical implications. *Pediatric Research, 35,* 87A.

Oleske, J., Minnefor, A., Cooper, R., Thomas, K., Cruz, A. D., Ahdieh, H., Guerrero, I., Joshi, V., & Deposito, F. (1983). Immune deficiency syndrome in children. *Journal of the American Medical Association, 249,* 2345-2349.

Oro, A. S., & Dixon, S. D. (1987). Perinatal cocaine and methamphetamine exposure: Maternal and neonatal correlates. *Journal of Pediatrics, 111,* 571-578.

Ostrea, E. M., Jr., Brady, M., Gause, S., Raymundo, A. L. & Stevens, M. (1992). Drug screening of newborns by meconium analysis. A large scale, prospective, epidemiologic study. *Pediatrics, 89,* 107-113.

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Ostrea, E. M., Jr., Brady, M. J., Parks, P. M., Asensio, D. C., & Naluz, A. (1989). Drug screening of meconium in infants of drug dependent mothers. An alternative to urine screening. *Journal of Pediatrics, 115,* 474-477.

Ostrea, E. M., Jr., & Chavez, C. J. (1979). Perinatal problems (excluding neonatal withdrawal) in maternal drug addiction. A study of 830 cases. *Journal of Pediatrics, 94,* 292-295.

Ostrea, E. M., Jr., Chavez, C. J., & Strauss, M. E. (1976). A study of factors that influence the severity of neonatal narcotic withdrawal. *Journal of Pediatrics*, 88, 642-645.

Ostrea, E. M., Jr., Knapp, D. K., Romero, A., Montes, M., & Ostrea, A. R. (1994). Meconium analysis to assess fetal exposure to active and passive maternal smoking. *Journal of Pediatrics, 124,* 471-476.

Ostrea, E. M., Jr., Knapp, D. K., Romero, A., & Ostrea, A. R. (1994). Postmortem analysis of meconium in early gestation human fetuses exposed to cocaine: Clinical implications. *Journal of Pediatrics, 124,* 477-479.

Ostrea, E. M., Jr., Knapp, D. K., Ostrea, A. R., Tannenbaum, L., & Saleri, V. (1994). A prospective study comparing systematic interview and analysis of maternal hair and meconium to determine illicit drug use during pregnancy. *Pediatric Research, 35,* 245A.

Ostrea, E. M., Jr., Kresbach, P., Knapp, D. K., & Simkowski, K. (1987). Abnormal heart rate tracings and serum creatine phosphokinase in addicted neonates. *Neurotoxicology and Teratology, 9,* 305-309.

Ostrea, E. M., Jr., Lizardo, E., & Tanafranca, M. (1992). The prevalence of illicit drug exposure in infants in the NICU as determined by meconium drug screen. *Pediatric Research, 31,* 215A.

Ostrea, E. M., Jr., Lynn, S. N., Wayne, R. H., & Stryker, J. C. (1980). Tissue distribution of morphine in the newborns of addicted monkeys and humans. *Developmental Pharmacology and Therapy, 1,* 163-170.

Ostrea, E. M., Jr., Parks, P., & Brady, M. (1988). Rapid isolation and detection of drugs in meconium of infants of drug dependent mothers. *Clinical Chemistry, 34,* 2372-2373.

Ostrea, E. M., Jr., & Romero, A. (1992). Selection criteria for routine drug screening of infants by meconium analysis. *Pediatric Research 31,* 215A.

Ostrea, E. M., Jr., Romero, A., & Yee, H. (1993). Adaptation of the meconium drug test for mass screening. *Journal of Pediatrics, 122,* 152-154.

Ostrea, E. M., Jr., Yee, H., & Thrasher, S. (1992). GC/MS analysis of meconium for cocaine: Clinical implications. *Pediatric Research, 29,* 63A.

Romero, A., Mac, E., Knapp, D. K., & Ostrea, E. M., Jr. (1993). Evaluation of a rapid, meconium drug testing system for clinical use. *Pediatric Research, 33,* 68A.

Ryan, L., Ehrlich, S., & Finnegan, L. (1987). Cocaine abuse in pregnancy: Effects on the fetus and newborn. *Neurotoxicology and Teratology, 9,* 295-299.

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Silvestre, M. A., Lucena, J., & Ostrea, E. M., Jr. (1991). The effect of timing, dosage and duration of morphine intake during pregnancy on the amount of morphine in meconium in a rat model. *Pediatric Research, 29,* 66A.

Sison, C., Ostrea, E. M., Jr. & Saleri, V. (1992). Resurgence of congenital syphilis is a drug related problem. *Pediatric Research, 31,* 261A.

Wilson, G. S. (1989). Clinical studies of infants and children exposed prenatally to heroin. *Annals of the New York Academy of Science, 562,* 183-194.

Wilson, G. S., McCreary, R., Kean, J., & Baxter, J. (1979). The development of preschool children of heroin addicted mothers: A controlled study. *Pediatrics,* 63, 135-141.

Zelson, C., Rubio, E., & Wasserman, E. (1971). Neonatal narcotic addiction: 10-year observation. *Journal of Pediatrics,* 48, 178-182.

Zuckerman, B., Frank, D. A., Hingson, R., Amaro, H., Levenson, S. M., Kayne, H., Parker, S., Vinci, R., Aboagye, K., Fried, L. E., Cabral, H., Timperi, R., & Bauchner, H. (1989). Effects of maternal marijuana and cocaine use on fetal growth. *New England Journal of Medicine, 320,* 762-768.

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TABLE OF CONTENTS