6. A. E. Frankel, R. L. Neuebauer, P. J. Fischinger, *J. Virol.* **18**, 481 (1976).
7. G. F. Vande Woude, M. Oskarsson, L. W. Enquist, S. Nomura, M. Sullivan, P. J. Fischinger, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4464 (1979).
8. G. F. Vande Woude *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* **44**, 735 (1979).
9. M. Oskarsson, W. L. McClements, D. G. Blair, J. V. Maizel, G. F. Vande Woude, *Science* **207**, 1222 (1980).
10. D. G. Blair, W. McClements, M. Oskarsson, P. J. Fischinger, G. F. Vande Woude, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 3504 (1980).
11. W. L. McClements, R. Dhar, D. G. Blair, L. Enquist, M. Oskarsson, G. F. Vande Woude, *Cold Spring Harbor Symp. Quant. Biol.*, in press.
12. R. Dhar, W. L. McClements, L. W. Enquist, G. F. Vande Woude, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 3937 (1980).
13. W. L. McClements, L. W. Enquist, M. Oskarsson, M. Sullivan, G. F. Vande Woude, *J. Virol.* **35**, 488 (1980).
14. C. Shoemaker, S. Goff, E. Gilboa, M. Paskind, S. Mitra, D. Baltimore, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 3932 (1980).
15. G. Ju and M. A. Skalka, *Cell* **22**, 379 (1980).
16. W. L. McClements, D. G. Blair, M. Oskarsson, G. F. Vande Woude, in *Animal Virus Genetics*, B. N. Fields, R. Jaenisch, C. F. Fox, Eds. (Academic Press, New York, 1980), pp. 455–460.
17. T. G. Wood, in preparation.
18. C. Shih, B. Z. Shilo, M. P. Goldfarb, A. Dannenberg, R. A. Weinberg, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5714 (1979).
19. G. M. Cooper, S. A. Okenquist, L. Silverman, *Nature (London)* **284**, 418 (1980).
20. V. Krup-Konvalinkova, and K. J. van den Berg, *ibid.* **287**, 353 (1980).
21. B. Neel, W. Hayward, H. Robinson, J. Fang, S. Astrin, *Cell* **23**, 323 (1981).
22. K. Shimotohno, S. Mizutani, H. M. Temin, *Nature (London)* **285**, 550 (1980); J. G. Sutcliffe, T. M. Schininck, I. M. Verma, R. A. Lerner, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 3302 (1980); M. P. Carlos and J. H. Miller, *Cell* **20**, 579 (1980).
23. E. M. Southern, *J. Mol. Biol.* **38**, 503 (1975).
24. J. M. Bailey and N. Davidson, *Anal. Biochem.* **70**, 75 (1976).
25. J. C. Alwine, D. J. Kemp, G. R. Stark, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5350 (1977).
26. We thank A. O. Thornton for technical assistance. All experiments with recombinant DNA were carried out in accordance with the NIH Guidelines.

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Opiate Withdrawal in utero Increases Neonatal Morbidity in the Rat

Abstract. *Long-term oral administration of the long-acting opiate* l-α-*acetylmethadol (LAAM) to female rats beginning on the day of conception interfered with the dams' ability to carry litters to term. When treatment was initiated 3 weeks prior to mating this effect was not observed. Daily administration of the opiate antagonist naloxone from day 14 of gestation through term, to precipitate withdrawal in utero, resulted in increased stillbirths, decreased pup weight and size, and weight loss 24 hours after birth. These data question the validity of animal experiments which purport to be models for methadone maintenance programs but in which treatment is started immediately prior to or soon after conception. They also suggest that withdrawal in utero may be responsible for many of the adverse effects of opiates on human and animal development.*

In the United States methadone maintenance is the major form of therapy for heroin addiction. Considerable data have been gathered concerning the effects of methadone on the pregnant addict and her offspring. It is generally accepted that children born to methadone addicted women have a higher incidence of morbidity (for example, low birth weights) and mortality than do those born to nonaddicted women (*1*, *2*). These data, however, are difficult to interpret because many of the women use other drugs concurrently, few receive adequate prenatal care, and some may even experience withdrawal during pregnancy (*2*–*4*). When these factors are controlled, perinatal morbidity and mortality are significantly reduced (*2*).

Studies in the rat (*5*) have shown that offspring exposed to methadone in utero have low birth weights. The possibility that drug withdrawal in utero, rather than the direct effect of opiates on growth, may be responsible for the low birth weights has not been investigated. This is a reasonable alternative, since methadone is commonly administered once daily, even though the half-life of methadone in rat plasma after long-term administration of the drug is less than 2 hours (*6*). This schedule of administration might therefore result in opiate withdrawal prior to the subsequent injection, as has been demonstrated in rats injected twice daily with morphine (*7*). That the fetus may itself experience withdrawal has been shown in the lamb (*8*) and chick (*9*) by injection of the antagonist naloxone into the opiate-dependent fetus. Stryker (*3*) has recently presented clinical evidence for spontaneous fetal withdrawal from methadone, as shown by heart rate aberrations, increased fetal activity, and by meconium-stained amniotic fluid at birth.

The present study was undertaken to determine if withdrawal in utero, rather than opiate exposure per se, is responsible for at least some of the adverse effects of opiates in humans and other animals. We also examined the role of tolerance in the severity of so-called direct drug effects. One question was whether extended opiate treatment would attenuate any detrimental effects of the opiate that might occur as a result of the drug not being given until near the time of conception (*5*, *10*).

We administered *l*-α-acetylmethadol (LAAM), a long-acting derivative of methadone, to pregnant rats. In clinical trials for treatment of heroin addiction, LAAM is administered three times per week, whereas methadone is administered daily (*11*). Animal studies have shown that LAAM is more potent and longer acting than methadone (*12*), and results in a more stabilized state of dependence (*13*). We have shown (*14*) that operant behavior is suppressed for at least 24 hours after a single dose of LAAM, but completely recovers within 5 hours after relatively high doses of methadone. By using the long-acting opiate and by precipitating withdrawal with naloxone we hoped to distinguish between the direct effects of the opiate and those produced by withdrawal in utero. To study tolerance, we initiated treatment for one group of rats 3 weeks before they were mated and for a second group, at the time of conception.

The rats were Sprague-Dawley derived females (Holtzman) weighing approximately 280 g. LAAM (1 or 4 mg/kg) was administered orally in water, by gavage. For rats treated 3 weeks before being mated, the first three doses were spaced 3 days apart, the next two were 2 days apart, and all remaining doses were administered daily. After 21 days the females were randomly assigned to males of proved fertility (Holtzman). They were placed with the males for ten consecutive days or until mating had occurred, as determined by the presence of sperm in a vaginal lavage taken each morning. To study the effects of withdrawal in utero, naloxone (1 mg/kg) (Nx) was administered subcutaneously to half of the rats 4 and 2 hours before their dose of water (WNx) or LAAM (1LNx and 4LNx) from day 14 of gestation through parturition. The remaining rats received saline (WS, 1LS and 4LS). To study tolerance, a second group of females, as yet untreated, were mated as described. After conception (+C) they were assigned to treatment groups and given water (W+C) or LAAM (1 or 4 mg/kg) (1L+C or 4L+C) daily but were not injected with saline or naloxone during gestation.

Successful mating occurred in about 85 percent of all females, and was not affected by treatment. With the exception of the 4L+C group, 81 percent (47 of 58) of these matings resulted in the delivery of a litter. However, only 12.5 percent (one of eight) 4L+C dams delivered litters. Examination of the uteri of

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Table 1. Effects of LAAM or withdrawal from LAAM on rat pups at birth and after 24 hours. Data are expressed as mean ± SEM and were analyzed by ANOVA using litters as the experimental unit. Duncan's New Multiple Range test was utilized for comparison of individual treatment means if a significant ($p < .05$) treatment effect was observed.

Group	Number of litters	Implantation sites	Decidua	Live pups	Percentage expected*	Percentage surviving 24 hours†
WS−W+C	12	12.4 ± 0.5	11.7 ± 0.5	11.5 ± 0.5	98.5 ± 1.0	100.0 ± 0.0
WNx	4	13.5 ± 1.7	12.3 ± 2.1	12.0 ± 1.9	98.5 ± 1.5	93.8 ± 6.3
1LS	7	14.3 ± 0.6	13.1 ± 0.8	11.7 ± 1.5	88.9 ± 9.7	64.3 ± 15.3‡
1L+C	6	13.3 ± 0.8	11.8 ± 0.9	11.2 ± 0.8	95.0 ± 3.4	50.0 ± 17.1‡
1LNx	6	12.2 ± 0.6	11.0 ± 0.7	7.5 ± 1.3	68.1 ± 10.4§	36.0 ± 13.3§
4LS	6	13.5 ± 0.6	12.7 ± 0.6	10.5 ± 1.0	83.9 ± 8.5	45.8 ± 20.8‡
4LNx	5	12.4 ± 1.3	11.4 ± 1.6	8.6 ± 2.2	74.7 ± 14.4§	25.0 ± 15.8§

*Percentage expected = (live pups/decidua) × 100; data were transformed (arc sin square root) to reduce variance prior to statistical analysis. †Data represent survival only of pups injected with saline after birth (see text). ‡$P < .05$, compared to WS−W+C. §$P < .05$, compared to WNx.

the remaining dams in this group 22 days after conception (that is, on the expected day of parturition) did not reveal the presence of implantation sites or resorptions. In comparison with the 4L+C group, 85.7 percent (six of seven) 4LS dams carried their litters to term ($P < .01$, Fisher's exact probability test) (*15*).

Administration of naloxone from day 14 of gestation through parturition precipitated a daily weight loss of approximately 25 g in dams treated with either dose of LAAM (*16*). Despite the weight loss precipitated each day, this effect was only transient, since maternal weight gain during gestation was no different across treatment groups [$F(6,39) = 1.7$, $P > .10$].

Although treatment with LAAM prior to conception did not reduce the number of implantation sites or decidua or increase the number of resorptions, prenatal withdrawal from either dose of LAAM did reduce litter size (Table 1). Stillbirths, early deaths, or infanticide are presumed responsible for this effect.

Pups prenatally exposed to LAAM did not differ from controls in body weight or nose-to-tail length at birth (Fig. 1). Pups prenatally withdrawn from either dose of LAAM, however, demonstrated significantly reduced birth weights (Fig. 1A) and nose-to-tail lengths (Fig. 1B).

In an attempt to study the effects of postnatal withdrawal, litters were culled to no more than eight pups, half of which were injected subcutaneously with naloxone (0.5 μg/g), and the remainder with saline. Of 102 pups (27 litters) prenatally exposed to LAAM with or without prenatally administered naloxone, 94 died within 24 hours when given naloxone postnatally. In contrast, only one of 60 pups (16 litters) prenatally exposed to water died after postnatal administration of naloxone ($\chi^2 = 127.5$; $P < .0001$). Many of the LAAM-exposed pups appeared cyanotic soon after receiving naloxone, suggesting that their cardiovascular-pulmonary system was not sufficiently developed to cope with the stress of withdrawal. The survival for 24 hours of pups injected with saline at birth was significantly reduced in all LAAM groups (Table 1). In addition, whereas significant weight gain ($P < .05$) was observed in WS−W+C (10.3 ± 1.4 percent, mean ± standard error) and WNx (13.8 ± 1.9 percent) pups, 1LS (−0.6 ± 3.8 percent), 1L+C (−0.8 ± 2.2 percent), and 4LS (−1.4 ± 7.8 percent) pups did not gain weight, and 1LNx (−8.6 ± 4.3 percent) and 4LNx (−8.0 ± 3.4 percent) pups actually lost weight ($P < .05$). The 1L+C pups did not differ from 1LS pups on any parameter measured in this study.

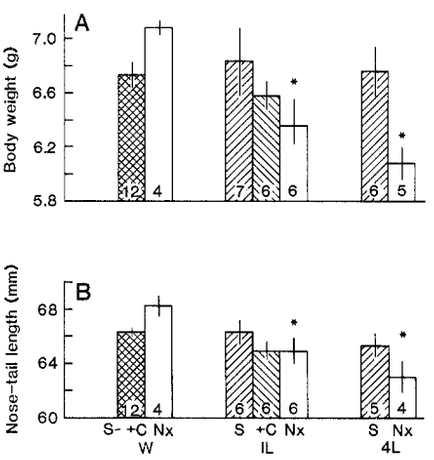

Fig. 1. Effect of prenatally administered LAAM and precipitated withdrawal from LAAM on (A) body weights and (B) nose-to-tail lengths of pups at birth. Litters were born to mothers exposed to water (*W*) or 1 or 4 mg of LAAM per kilogram per day (*1L* or *4L*) starting 3 weeks before conception and given saline (*S*) or naloxone (*Nx*) (1 mg/kg) twice daily from day 14 of gestation through parturition, or first exposed to water or LAAM (1 mg/kg per day) 1 day after conception (*W+C* or *1L+C*). Numbers at the bottom of the bars represent the number of litters per treatment group used for analysis. Data are expressed as mean ± standard error. Asterisks indicate $P < .05$ compared to WNx (*19*).

Thus, initiating long-term opiate administration at the time of conception (4L+C) interferes with normal reproductive function of females such that implantation does not occur or, if it does, hormonal balance is so altered that pregnancy cannot be maintained. Tolerance to this effect does develop since treatment for 3 to 4 weeks prior to mating (4LS) negated this effect. Although a prerequisite for admittance into methadone maintenance programs is a long-standing history of opiate addiction (*17*), in many animal studies treatment is initiated near the time of conception or sometime during gestation (*5, 10*). Such studies may lead to erroneous conclusions concerning the risk to the pregnant addict or her offspring, since the clinical use of the drug has not been approximated.

Our data clearly demonstrate the effects of withdrawal in utero on the outcome of pregnancy. Litters from dams put through withdrawal daily from day 14 of gestation through parturition had a higher incidence of stillbirths and early deaths, lower birth weights, and shorter nose-to-tail lengths. In the first 24 hours after birth these pups lost weight, whereas water-treated pups gained weight and LAAM-treated pups did not change weight. These data support the hypothesis that withdrawal in utero may be responsible for the higher incidence of stillbirths, low birth weights, and small size reported in animal and clinical studies with opiates.

The doses of LAAM used in this study were not without effect themselves. Although the LAAM-exposed pups were no different from controls at birth, differences did emerge within the next 24 hours. Survival of the LAAM-exposed pups was reduced and those which did survive did not gain weight comparable to controls. Whereas postnatal withdrawal may be responsible for the increased mortality, as was shown when withdrawal was precipitated by direct injection of the neonate with naloxone, chronic intoxication, due to the persistent activity of LAAM or its metabolites in the rat (*12, 14, 18*), might also depress feeding or other behavior necessary for normal growth and survival and thereby result in diminished growth and increased mortality.

Our data indicate that studies in which treatment of pregnant rats is initiated at the time of conception or soon afterward

may not be relevant to clinical maintenance strategies and may lead to erroneous conclusions regarding risk to the pregnant addict or her child. Furthermore, withdrawal in utero rather than continuous opiate exposure may be responsible for many of the detrimental sequelae reported for this class of drugs.

LEONARD LICHTBLAU
SHELDON B. SPARBER
Department of Pharmacology,
University of Minnesota
Minneapolis 55455

References and Notes

1. G. S. Wilson, *Addict. Dis.* **2**, 333 (1975); C. Zelson, J. C. Cook, M. Casalino, *N. Engl. J. Med.* **289**, 1216 (1973).
2. L. P. Finnegan, *N.Y. Acad. Sci.* **311**, 135 (1978); J. F. Connaughton, D. Reeser, J. Schut, L. P. Finnegan, *Am. J. Obstet. Gynecol.* **129**, 679 (1977).
3. J. C. Stryker, in *Substance Abuse: Genetic, Perinatal and Developmental Aspects*, M. C. Braude, Ed., (Raven, New York, 1981).
4. E. M. Ostrea, Jr. and C. J. Chavez, *J. Pediatr.* **94**, 292 (1979); J. L. Rementeria and L. Lotongkhom, in *Drug Abuse in Pregnancy and Neonatal Effects*, J. L. Rementeria, Ed. (Mosby, St. Louis, Mo., 1977), p. 3.
5. T. A. Slotkin, F. J. Seidler, W. L. Whitmore, *Life Sci.* **26**, 861 (1980); D. E. Hutchings, H. F. Hunt, J. P. Towey, T. S. Rosen, H. S. Gorinson, *J. Pharmacol. Exp. Ther.* **197**, 171 (1976); D. E. Hutchings, J. P. Towey, H. S. Gorinson, H. F. Hunt, *ibid.* **208**, 106 (1979); D. Buchenauer, M. Turnbow, M. A. Peters, *ibid.* **189**, 66 (1974); M. A. Peters, *ibid.* **203**, 340 (1977).
6. A. L. Misra, S. J. Mulé, R. Bloch, N. L. Vadlamari, *J. Pharmacol. Exp. Ther.* **185**, 287 (1973).
7. V. F. Gellert and S. B. Sparber, *Psychopharmacology* **60**, 165 (1979).
8. M. S. Cohen, A. M. Rudolph, K. L. Melmon, *Dev. Pharmacol. Ther.* **1**, 58 (1980).
9. M. D. Kuwahara and S. B. Sparber, *Science* **212**, 945 (1981).
10. I. S. Zagon and P. J. McLaughlin, *Experientia* **33**, 1486 (1977); *J. Neurochem.* **31**, 49 (1978).
11. J. H. Jaffe and E. C. Senay, *J. Am. Med. Assoc.* **216**, 1303 (1971); W. Ling, J. Klett, R. D. Gillis, *Arch. Gen. Psychiatry* **35**, 345 (1978).
12. J. Wax, J. R. McLean, D. K. Tessman, D. A. McCarthy, J. A. Nuite, *Res. Commun. Chem. Pathol. Pharmacol.* **12**, 613 (1975); J. E. Moreton, T. Roehrs, N. Khazan, *Psychopharmacology* **47**, 237 (1976).
13. S. Chiba, G. A. Young, J. E. Moreton, N. Khazan, *Psychopharmacology* **54**, 105 (1977).
14. S. B. Sparber and L. Lichtblau, in *Substance Abuse: Genetic, Perinatal and Developmental Aspects*, M. C. Braude, Ed. (Raven, New York, 1981).
15. Since only one litter was born to the 4L+C rats, no further statistical analyses were carried out with this group.
16. Although weight loss was no greater in the 4LNx group, additional signs of withdrawal, such as hyperirritability, wet-dog shakes, and rhinorrhea were observed, indicating that withdrawal was more severe in this group than in the 1LNx group.
17. J. H. Lowinson and R. B. Millman, in *Handbook on Drug Abuse*, R. I. Dupont, A. Goldstein, J. O'Donnel, Eds. (National Institute on Drug Abuse, Washington, D.C., 1979), p. 49.
18. L. Lichtblau and S. B. Sparber, *Neurobehav. Toxicol.* **2**, 13 (1980).
19. The data in Fig. 1 represent the average for males and females since sex distribution was approximately equal for all groups and since no sex differences in these measures were observed at birth. The data were analyzed by analysis of variance with litters, rather than individual pups, being used as the experimental unit. Duncan's new multiple range test was used to compare individual treatment means at a significance level of $P < .05$.
20. This research, supported by PHS grant DA 01880, was presented in brief at the annual meeting of the American Society of Pharmacology and Experimental Therapeutics, August 1980.

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Prenatal Withdrawal from Opiates Interferes with Hatching of Otherwise Viable Chick Fetuses

Abstract. *Fetal chicks were made opiate-dependent by injections of* N-*desmethyl*-l-α-*acetylmethadol into the chorioallantois on day 3 of embryogenesis. The injections had no effect on subsequent hatchability; however, spontaneous fetal motility was significantly depressed. Injection of naloxone caused a significant increase in the motility of the opiate-exposed fetuses but had no effect on control fetuses. That naloxone's effect was an expression of opiate withdrawal and not due to antagonism of depressed motility is also supported by the observation that naloxone significantly reduced the hatchability of opiate-exposed chicks and not of control chicks. Thus the withdrawal of a developing organism from a narcotic may be more deleterious to its survival than continued exposure.*

Both the effects of maternal narcotic addiction on the newborn infant and the neonatal narcotic withdrawal syndrome have been subjects of extensive discussion (*1–4*). Although the diagnosis of withdrawal is often difficult to make, approximately 85 percent of infants born to addicted women show some signs of withdrawal (*3, 4*). Less information is available concerning fetal withdrawal, which may present as great a threat to the fetus and neonate as does postnatal withdrawal to the neonate. Fetal withdrawal, as evidenced by an increase in fetal motility and a high incidence of placental staining by meconium (*3, 5*), may or may not be coincident with maternal withdrawal. However, maternal withdrawal may itself exacerbate the effects of fetal withdrawal (*3, 5*). The possibility of fetal distress due to withdrawal and of abortion or premature labor have prompted some clinicians to recommend maintaining pregnant addicts on low doses of methadone. Others, in view of the apparent toxic effects of opiate exposure (*6*), encourage a drug-free state during pregnancy or try to gradually reduce the doses to the lowest possible level before delivery.

We sought to determine whether the effects of an opiate are less detrimental to normal fetal development than withdrawal precipitated by naloxone. Several reports have described adverse consequences of fetal exposure to opiates (*7*); some of these effects may be attributed to a direct action on the developing fetus. However, many of these sequelae may be secondary consequences of in utero withdrawal [resulting from inappropriate dosing schedules (*8*) in species that rapidly metabolize or excrete opiates]. By using chick fetuses, we were able to examine the direct effects of opiates on a developing organism in the absence of the maternal-fetal interaction present in viviparous species (*9*). The opiate used was *N*-desmethyl-*l*-α-acetylmethadol (NLAAM) (*10*), a long-acting substance. By using NLAAM in lieu of the parent compound, we did not have to rely on the immature metabolizing capabilities of the chick embryo to ensure its exposure to the most potent metabolite, which is probably responsible for most of the pharmacological effects in animals and man (*10*).

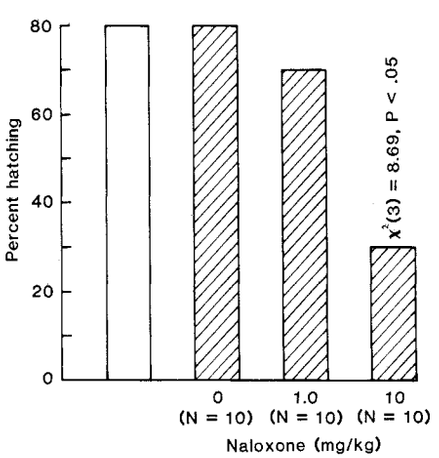

Fig. 1. Effect of naloxone on hatchability of chick fetuses exposed to propylene glycol or NLAAM. The open bar represents the hatchability of vehicle-treated fetuses given saline or naloxone on the 19th day of incubation ($N = 30$). The shaded bars represent the hatchability of fetuses treated with NLAAM (2.5 mg/kg) and given saline or naloxone on day 19.

Fertile eggs of a White Leghorn strain were obtained from a local hatchery, randomly assigned to vehicle or drug treatment groups, and placed in a rotating forced-air incubator maintained at 38°C and 58 percent relative humidity. On the third day of incubation, 30 μl of vehicle (50 percent propylene glycol) or NLAAM (2.5 or 3.4 mg per kilogram of egg) was injected into the chorioallantois (*11*).

In the first experiment we determined whether naloxone affects the hatchability of fetuses treated with NLAAM. On the 19th day of incubation, the chorioallantois of eggs treated with vehicle or NLAAM (2.5 mg/kg) was injected with 0.9 percent saline or naloxone (1.0 or 10

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