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Biphasic Effect of Methadone on Hepatic Drug Metabolism (42443)

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Abstract. When methadone HCl (30 mg/kg, po) was given acutely to mice, it was found to inhibit drug metabolism as evidenced by a prolongation of hexobarbital sleeping time and zoxazolamine paralysis time. Pharmacokinetic studies revealed that this acute dose of the narcotic analgesic could also prolong the plasma half-life of aminopyrine without any change in its volume of distribution. When added to the incubation mixture containing 10,000 g mouse liver supernatant fraction and a complete system for measuring aminopyrine *N*-demethylase or aniline hydroxylase, metadone showed a dose-dependent inhibition of the enzymes; the former enzyme was inhibited to a greater extent than the latter one. However, subacute treatment of mice with methadone HCl (30 mg/kg, po, twice daily for 3 days) resulted in increases in liver weight, microsomal protein, and cytochrome *P*-450 content in consonant with the increased activities of four hepatic drug-metabolizing enzymes: aminopyrine *N*-demethylase, aniline hydroxylase, *p*-nitroanisole,*O*-demethylase, and benzphetamine *N*-demethylase. Moreover, both hexobarbital sleeping time and zoxazolamine paralysis time were shortened. The plasma half-life of aminopyrine was decreased. These changes were prevented by simultaneous administration of puromycin diHCl (80 mg/kg, ip). Methadone thus seems to act in a manner very similar to that of propoxyphene or SKF-525A, acting as a potent inhibitor of hepatic drug metabolism when given acutely and as an inducer when given subacutely. © 1987 Society for Experimental Biology and Medicine.

Methadone is a narcotic analgesic widely used throughout the world. The indications for methadone in the management of pain are the same as those for morphine. Because of its oral effectiveness, its analgesic potency, and the lack of marked sedative effect, methadone has become a desirable drug to use for the management of intractable cancer pain or to permit substitution and withdrawal of short-acting opiates such as morphine. Clinical studies by Halpern and Bonica (1) have shown that in order to obtain optimal analgesic effects, methadone must be administered in approximately the same dose as morphine, 2.5 to 10 mg by parenteral route and 5 to 15 mg by oral administration, depending on the severity of pain.

The structural similarity among SKF-525A, propoxyphene, and methadone, as pointed out by Peterson *et al.* (2), has led several groups of investigators to speculate that these drugs might share some common pharmacologic actions. Indeed, Peterson *et al.* (2) have reported that propoxyphene has a biphasic effect on hepatic drug metabolism similar to SKF-525A, i.e., an inhibition of mixed function oxidases (MFO) when the drug is given acutely, followed by induction of several enzymes on repeated administration. SKF-525A, a model inhibitor of MFO, is well known to have this effect (3–5). In 1975, Masten *et al.* (6) showed that treatment of mice with methadone HCl (100 mg/kg, po) for 6 days brought about a 100% increase in the activity of methadone *N*-demethylase. However, little is known about the acute effect of this narcotic analgesic. In this report evidence is presented to indicate that methadone also has a biphasic effect on hepatic drug metabolism similar to SKF-525A and propoxyphene.

Materials and Methods. *Experimental animals.* Adult male Swiss mice of about 60 days of age and weighing 30–35 g were used in the study. They were supplied by the Animal Center of the Faculty of Science, Mahidol University. All animals were allowed free access to standard rat chow (Purina Laboratory Chow, Zuellig Pte., Ltd., Singapore) and tap water *ad libitum* until 16–20 hr before sacrifice, during which time they were allowed access to water only. To limit the effects of nonspecific stress, all animals used in the *in vivo* experiments were accustomed to daily handling for at least 3 days before experimentation. Further, animals were housed in the experimental environment for 24 hr prior to treatment.

Methadone HCl was given to the animals orally by means of intubation, either as a single dose or as a twice daily dose at 8:00–9:00 AM

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and 4:00–5:00 PM. They were weighed prior to each intubation and fasted for 16–20 hr after the last dose. In a study using an inhibitor of protein biosynthesis, puromycin diHCl (80 mg/kg, ip) was given once daily at 30 min prior to the administration of the second dose of methadone (4:00 PM).

Drugs and chemicals. All drugs and chemicals used were obtained commercially in the highest grade available without further purification: *dl*-methadone HCl from Siegfried Aktiengesellschaft (CH-4800 Zofinger, Switzerland), aniline hydrochloride from BDH Chemicals Ltd. (Poole, England), aminopyrine and zoxazolamine from Aldrich Chemical Co., Inc. (Milwaukee, WI), *p*-nitroanisole from Eastman Kodak Co. (Rochester, NY), and hexobarbital sodium from Sterling-Winthrop (Rensselaer, NY). Benzphetamine HCl was a gift from Upjohn Co. (Kalamazoo, MI). All other reagents and drugs were purchased from Sigma Chemical Co. (St. Louis, MO).

All drugs were freshly formulated just before use: methadone HCl as an aqueous solution (3 mg/ml) in distilled water, hexobarbital sodium (10 mg/ml) in distilled water, puromycin diHCl (20 mg/ml) in normal saline, and zoxazolamine solution (20 mg/ml) was prepared by dissolving 300 mg of the drug in 3.6 ml of 1 *N* HCl and the solution was then adjusted to 15 ml with 0.9% NaCl.

The awakening brain hexobarbital levels were determined from the animals killed by decapitation by the method of Cooper and Brodie (7) as modified by Vesell (8).

Enzyme preparations and assays. Mice were sacrificed by cervical dislocation. The abdomen was opened by scissors and the liver was quickly removed and washed with ice-cold buffered KCl (1.5% KCl in 0.1 *M* Na^+/K^+ phosphate buffer, pH 7.4) to remove the blood. The liver was blotted dry with filter paper and weighed. All subsequent procedures were performed at the temperature below 4°C. After weighing, the liver was minced with scissors and then homogenized with 7 vol of buffered KCl in a Potter–Elvehjem homogenizer using a Teflon pestle. The homogenate was centrifuged at 10,000*g* in a refrigerated centrifuge for 15 min using Beckman Centrifuge Model J-21B with JA-21 rotor. After centrifugation, the supernatant was carefully aspirated off with a Pasteur pipette. Three milliliters of this post mitochondrial fraction was further centrifuged at 105,000*g* for 60 min in a Beckman Model L5-65 refrigerated ultracentrifuge, whereas the remaining portion (about 9.0 ml) was used as the enzyme source in the *in vitro* hepatic drug-metabolizing enzyme assays. After ultracentrifugation, the soluble supernatant fraction was carefully removed with a Pasteur pipette and the microsomal pellet was rinsed and resuspended in ice-cold 0.1 *M* Na^+/K^+ phosphate buffer, pH 7.4, to 3.0 ml. This microsomal suspension was used for the determintion of microsomal protein content.

The activities of aminopyrine *N*-demethylase, aniline hydroxylase, *p*-nitroanisole,*O*-demethylase, and benzphetamine *N*-demethylase were measured in the presence of various cofactors according to the methods described in detail in previous publications (9, 10), using the 10,000*g* mouse liver supernatant fraction as the enzyme source.

To study the inhibition of aminopyrine *N*-demethylase or aniline hydroxylase by methadone HCl, the enzyme activity was measured by incubating 1.0 ml of the 10,000*g* liver supernatant fraction with 20 μmole of aminopyrine or 10 μmole of aniline and a complete supporting system plus the inhibitor at various concentrations in a final volume of 6.0 ml for aminopyrine *N*-demethylase and 4.0 ml for aniline hydroxylase, respectively. Control incubation mixture contained equivalent components except for the omission of methadone HCl.

Microsomal protein was determined by the method of Lowry *et al.* (11) with bovine serum albumin as the standard. Cytochrome *P*-450 in the microsomal pellet was estimated by the method of Omura and Sato (12).

Plasma concentration-time profile of aminopyrine. Mice were intubated with 30 mg/kg methadone HCl, either as a single dose (acute treatment) or as a twice daily dose for 3 days (subacute treatment). At 1 hr after the single dose or 16 hr after the last dose of the 3-day pretreatment, the animals were weighed and then given with aminopyrine (500 mg/kg, po). Blood was collected at various time intervals (15 min to 8 hr) by heart puncture under light ether anesthesia into heparinized tubes containing 200 units of heparin sodium/ml of blood. The plasma levels of aminopyrine as measured by the method of Brodie *et al.* (13) were then plotted against time to yield the plasma concentration-time profile and vari-

TABLE I. EFFECTS OF ACUTE AND SUBACUTE TREATMENT OF METHADONE ON THE METABOLISM OF HEXOBARBITAL AND ZOXAZOLAMINE

Treatment	Hexobarbital: Sleeping time (min)	Hexobarbital: Awakening brain level (μg/g)	Zoxazolamine paralysis time (min)
Control	48.1 ± 2.5	38.71 ± 1.82	69.0 ± 3.1
Methadone HCl			
Acute	68.7 ± 3.2*	39.20 ± 2.41	90.0 ± 5.0*
Subacute	28.7 ± 2.4	40.10 ± 1.75	19.7 ± 1.2*

Note. Mice were treated orally with methadone HCl (30 mg/kg) as a single dose (acute) or twice daily for 3 days (subacute). Control animals received an equal volume of saline. At 1 hr after the single dose or 16–20 hr after the last dose of the 3-day pretreatment, hexobarbital sodium (120 mg/kg, ip) or zoxazolamine (140 mg/kg, ip) was given. Each value represents mean ± SE from six animals.

* $P < 0.05$ (from control).

ous pharmacokinetic parameters computed according to the one-compartment open model (14).

Statistical analysis. Results are given as means ± SE. Analysis of the difference between two means was performed using Student's *t* test with the level of significance of $P < 0.05$ (15). Significant analysis of more than two experimental groups was performed by using the Newman–Keuls test (16). Regression analysis was used in drawing a line to obtain pharmacokinetic data. All statistical manipulations were conducted by using a Wang Electronic Calculator, Model 462.

Results. As shown in Table I, acute treatment of the animals with methadone HCl (30 mg/kg, po) was found to prolong zoxazolamine paralysis time and increase hexobarbital sleeping time without affecting the awakening brain barbiturate level. However, subacute treatment with this narcotic analgesic (30 mg/kg, po, twice daily for 3 days) produced the opposite results; the mice were found to display statistically shorter zoxazolamine paralysis time and hexobarbital sleeping time than those in the control group.

This biphasic effect of methadone on hepatic drug metabolism is also supported by results from the *in vivo* studies of aminopyrine disposition. As shown in Fig. 1, the plasma concentration-time profile reveals that aminopyrine absorption was delayed and declined at a slower rate in mice pretreated for 1 hr with an acute dose of methadone HCl (30 mg/

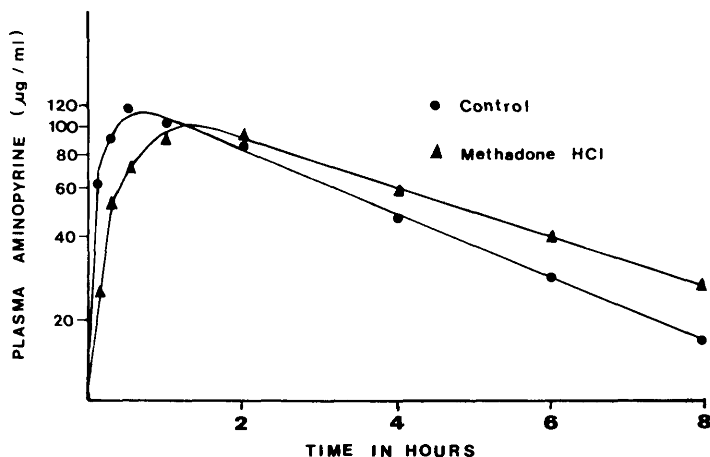

FIG. 1. Plasma concentration time profiles of aminopyrine in control and methadone-pretreated mice. Methadone HCl (30 mg/kg, ip) was given 1 hr before the intubation of aminopyrine (500 mg/kg). For earlier points up to 4 hr, each point is the mean ± SE from four mice. The SE of these points ranges from 2 to 10 μg/ml. As for the points at 6 and 8 hr, each point is the mean ± SE from three mice, and the SE is between 4 and 12 μg/ml except for the 6-hr point of the control curve which shows the maximum SE (20 μg/ml). Pharmacokinetic parameters obtained from each curve were computed according to the one-compartment open model.

kg, po) when compared with the control; a statistically longer elimination half-life of aminopyrine was found while its volume of distribution remained unchanged (Table II). In contrast, when the animals were pretreated with methadone HCl (30 mg/kg, po, twice daily for 3 days) the plasma half-life of aminopyrine was significantly shortened, again without any effect on the volume of distribution (Table II). These findings are therefore compatible with the view that methadone can produce a biphasic effect on hepatic drug metabolism *in vivo*.

As shown in Fig. 2 and Table III, methadone was found to be a more potent inhibitor of aminopyrine *N*-demethylase than aniline hydroxylase. It should be noted that the enzyme inhibition caused by methadone was dose-related and associated with two phases (Fig. 2); the inhibition was less at lower concentrations of the inhibitor (0.1 to 1.0 m*M*) but increased disproportionately at higher concentration range (1.0 to 10 m*M*). Like propoxyphene (2), methadone was also capable of inhibiting the hydroxylation of a type II compound, aniline, though the degree of inhibition was less than that for the *N*-demethylase of a model type I substrate, aminopyrine (Table III).

The effect of methadone given subacutely on various parameters of hepatic drug-metabolizing enzyme system was also investigated in detail and was found to be dose dependent (Table IV); at a lower dose (10 mg/kg, po) the drug increased only the liver weight, cytochrome *P*-450 content, and aminopyrine *N*-demethylase activity. At higher doses (20, 30, and 40 mg/kg, po) the drug produced significant increases in all of the parameters including an increase in the activities of the four drug-metabolizing enzymes, aminopyrine *N*-demethylase, aniline hydroxylase, *p*-nitroanisole *O*-demethylase, and benzphetamine *N*-demethylase. Thus, these results suggested that methadone was an inducer of hepatic drug metabolism when given subacutely, and the inductive effect was maximum at 30 mg/kg, po, twice daily for 3 days (Table IV, Fig. 3).

As shown in Table V, it was found that prior administration of puromycin diHCl could completely abolish the methadone-stimulated increase in aminopyrine *N*-demethylase activity. This evidence thus supported the possibility that the effect of methadone given subacutely on aminopyrine metabolism was most likely due to an enzyme induction.

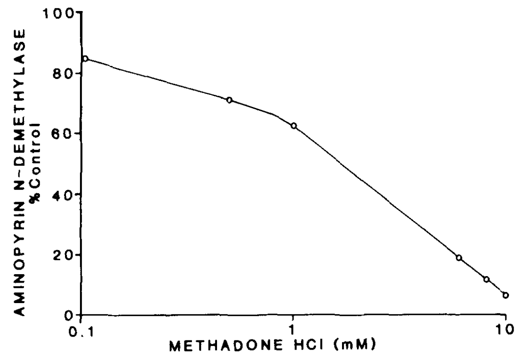

FIG. 2. Typical dose-related inhibition profile of aminopyrine *N*-demethylase by methadone HCl *in vitro*.

TABLE II. PHARMACOKINETIC PARAMETERS OF AMINOPYRINE IN ANIMALS PRETREATED ACUTELY AND SUBACUTELY WITH METHADONE HYDROCHLORIDE

Pretreatment	Half-life ($t_{\frac{1}{2}\beta}$, hr)	V_d (liters/kg)
Control	2.86 ± 0.20	4.21 ± 0.35
Methadone HCl		
Acute	3.75 ± 0.14*	3.70 ± 0.39
Subacute	2.01 ± 0.18*	4.27 ± 0.22

Note. Aminopyrine (500 mg/kg, po) was givn to mice 1 hr after the single dose (acute) or 16 hr after the last dose of the 3-day pretreatment with methadone HCl as described under Materials and Methods. The pharmacokinetic parameters were computed as described for the one-compartment open model. Each value represents mean ± SE from three separate determinations.

* $P < 0.05$ (from control).

TABLE III. INHIBITION OF AMINOPYRINE *N*-DEMETHYLASE AND ANILINE HYDROXYLASE *IN VITRO* BY METHADONE HCl

Inhibitor concentration (*M*)	% Inhibition	
	Aminopyrine *N*-demethylase	Aniline hydroxylase
10^{-2}	92 ± 4	40 ± 8
10^{-3}	37 ± 6	20 ± 2
10^{-4}	15 ± 6	3 ± 2

Note. The 10,000*g* supernatant from mouse liver was used as the enzyme source. Each value is mean ± SE from four separate determinations, each from four to six mice.

TABLE IV. EFFECT OF METHADONE HYDROCHLORIDE TREATMENT ON VARIOUS PARAMETERS OF DRUG-METABOLIZING ENZYME SYSTEMS *IN VITRO* IN THE MOUSE

Methodone HCl (po, twice daily for 3 days) (mg/kg)	Liver weight (% body wt)	Microsomal protein (mg/g liver)	Aminopyrine *N*-demethylase activity[a]	Aniline hydroxylase activity[b]	*p*-Nitroanisole *O*-demethylase activity[c]	Benzphetamine *N*-demethylase activity[d]	Cytochrome *P*-450 (nmole/mg protein)
Control	4.36 ± 0.11	26.88 ± 0.79	119.98 ± 8.67	19.49 ± 1.11	7.44 ± 0.91	82.73 ± 6.32	1.05 ± 0.16
10	4.69 ± 0.09*	26.51 ± 0.24	202.46 ± 10.18*	25.38 ± 2.27	8.18 ± 1.10	—	1.44 ± 0.10*
20	4.71 ± 0.09*	30.82 ± 1.02*	204.52 ± 9.16*	29.76 ± 0.92*	17.31 ± 1.86*	—	1.54 ± 0.10*
30	4.91 ± 0.06*	30.79 ± 0.42*	231.90 ± 15.71*	32.56 ± 3.63*	19.28 ± 4.08*	245.16 ± 9.32*	1.80 ± 0.05*
40	5.12 ± 0.09*	31.07 ± 1.63*	243.41 ± 11.88*	30.41 ± 1.56*	16.30 ± 2.73*	—	

Note. Values are expressed as mean ± SE of four to five determinations from different sources.
[a] nmole formaldehyde formed/mg protein/30 min.
[b] nmole *p*-aminophenol formed/mg protein/20 min.
[c] nmole *p*-nitrophenol formed/mg protein/10 min.
[d] nmole formaldehyde formed/mg protein/15 min.
* $P < 0.05$ (from Control, Newman–Keuls test).

Discussion. In the experiments described here, the results from *in vitro* inhibition of aminopyrine *N*-demethylase and aniline hydroxylase caused by methadone HCl clearly showed that this narcotic analgesic was a more potent inhibitor of the former enzyme (Table III). This finding was similar to that reported for propoxyphene by Peterson *et al.* (2), who found that propoxyphene inhibited the *in vitro* metabolism of a representative type I substrate, aminopyrine, much more than that of a type II substrate, aniline. The potentiation by methadone of the pharmacologic actions of hexobarbital and zoxazolamine was strikingly consistent with and indeed reflected the *in vitro* data. There was greater enhancement of the action of the type I drug, hexobarbital, than that of the type II compound, zoxazolamine (Table I).

It is not yet known whether the inhibitory effect of methadone on hepatic drug metabolism is due to the parent compound or its active metabolites or both. Studies by Sullivan *et al.* (17) have shown that α-1-methadol and α-1-*N*-demethylmethadol are active metabolites of methadone both in rats and in man. Thus, it is possible that these two metabolites might also contribute to the *in vivo* inhibition of hepatic drug metabolism caused by methadone. As pointed out by Peterson *et al.* (2), the primary structural requirement for inhibitory activity of the MFO is the presence of a diphenylmethane or a closely related grouping, which is also present in these two active metabolites of methadone.

It seems unlikely that clinically important pharmacokinetic drug interactions would result from the simultaneous administration of methadone with other MFO drug substrates because of the use of relatively low doses of methadone. However, the clinical implications of administering a chronic inhibitor of drug metabolism as a detoxification drug to heroin addicts are considerable, especially since methadone may enhance the toxicity of many drugs these individuals abuse. Furthermore, impairment of drug metabolism is a possibility in these addicts in whom a high incidence of hepatic disease is common.

Like SKF-525A and propoxyphene, methadone was also found to be a potent inducer of MFO on subacute or chronic administration (Table III). There are at least two lines of

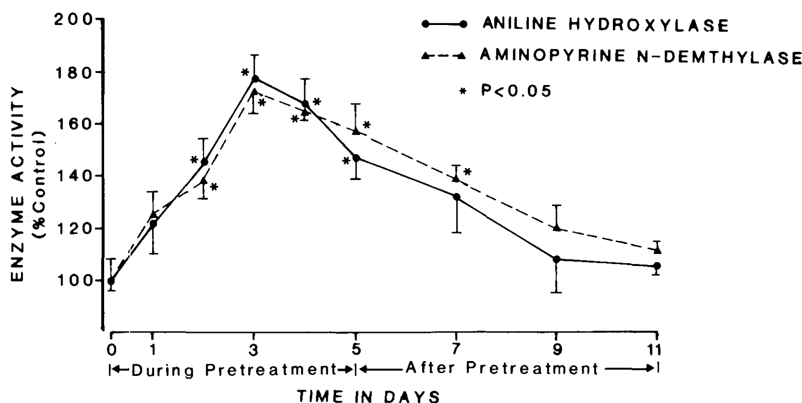

FIG. 3. Time course of induction of hepatic aminopyrine *N*-demethylase and aniline hydroxylase by methadone HCl (30 mg/kg, po, twice daily). Each point is mean ±SE from three to five mice.

evidence suggesting that the inductive nature of methadone on hepatic drug-metabolizing enzymes is similar to that of phenobarbital. First, the type of induction is broad, including benzphetamine *N*-demethylase, which is specifically induced by phenobarbital. Second, the time required for maximal induction by the barbiturate is 2–3 days and the effect lasts 4–5 days (18). In the case of methadone, the peak induction was reached in 3 days and the effect lasted for about 4 days after cessation of the drug (Fig. 3).

Methadone induction may have some clinical relevance, since this narcotic analgesic has already been shown to induce its own metabolism on chronic administration (19). For maximum efficacy, therefore, dosage regimens of high-risk drugs such as phenytoin and theophylline may have to be modified in patients undergoing treatment of opiate dependence or treated for chronic pain with methadone. Although phenytoin–methadone interaction has not been done either *in vitro* or *in vivo*, a study on the interaction of *d*-propoxyphene and phenytoin in rat microsomal preparation has been reported (20).

TABLE V. THE EFFECT OF PUROMYCIN DIHYDROCHLORIDE ON THE INDUCTION OF AMINOPYRINE *N*-DEMETHYLASE BY METHADONE HYDROCHLORIDE IN THE MOUSE

Treatment	Aminopyrine *N*-demethylase (nmole HCHO/mg protein/30 min)
Control	150.51 ± 9.84
Puromycin diHCl	121.53 ± 3.60
Methadone HCl	235.67 ± 12.27*
Methadone HCl + Puromycin diHCl	156.95 ± 5.25

Note. Puromycin diHCl (80 mg/kg, ip) was given once daily 30 min prior to the administration of methadone HCl (30 mg/kg, po, twice daily for 3 days). Each points represents mean ± SE for three separate determinations.
* $P < 0.05$ (from control).

1. Halpern LM, Bonica JJ. Analgesics. In: Modell W, Ed. Drugs of Choice. St. Louis, Mosby, p199, 1980.
2. Peterson GR, Hostetler RM, Lehman T, Covault HT. Acute inhibition of oxidative drug metabolism by propoxyphene. Biochem Pharmacol **28**:1783–1789, 1979.
3. Axelrod J, Reichenthal J, Brodie BB. Mechanism of the potentiating action of diethyl-2,2-diphenylvalerate. J Pharmacol Exp Ther **112**:49–54, 1954.
4. Fouts JR, Brodie BB. The enzymatic reduction of chloramphenicol, *p*-nitrobenzoic acid and other aromatic nitro compound in mammals. J Pharmacol Exp Ther **119**:197–207, 1957.
5. Ruemke CL, Bout J. The modification of hexobarbital narcosis by previously administered drugs. Naunyn-Schmiedebarg's Arch Exp Pathol Pharmacol **231**:333–348, 1960.
6. Masten LW, Peterson GR, Burkhalter A, Way EL. Microsomal enzyme induction by methadone and its implications on tolerance to methadone lethality. Nature (London) **253**:200–202, 1975.
7. Cooper JR, Brodie BB. The enzymatic metabolism of hexobarbital (Evipal). J Pharmacol Exp Ther **114**: 409–412, 1955.
8. Vesell ES. Induction of drug-metabolizing enzyme of liver microsomes of mice and rats by softwood bedding. Science **157**:1057–1058, 1967.

9. Fouts JR. Liver smooth endoplasmic reticulum microsomal drug metabolizing enzyme system. In: Schwartz A, Ed. Method in Pharmacology. New York, Meredith, p287, 1971.
10. Mazel P. Experimental illustrating drug metabolism in vitro. In: LaDu BN, Mondel HG, Way EL, Eds. Fundamentals of Drug Metabolism and Disposition. Baltimore, Williams & Wilkins, p546, 1971.
11. Lowry OH, Rosebrough NJ, Farr AL, Randall RI. Protein measurement with folin phenol reagent. J Biol Chem **193**:265–275, 1951.
12. Omura T, Sato R. The carbon monoxide binding pigment of liver microsomes. J Biol Chem **239**:2379–2385, 1964.
13. Brodie BB, Axelrod J. The fate of aminopyrine (pyramidon) in man and methods for the estimation of aminopyrine and its metabolites in biological meterials. J Pharmacol Exp Ther **99**:171–185, 1950.
14. Shargel L, Yu ABC. Applied Biopharmaceutics and Pharmacokinetics. New York, Appleton–Century–Crofts, p28, 1980.
15. Steele RG, Torrie JH. Principles and Procedures of Statistics. New York, McGraw–Hill, p67, 1960.
16. Grim H. Analysis of variance. In: Deriaunois AL, Ed. International Encyclopedia of Pharmacology and Therapeutics. New York, Pergamon, Vol 2:p700, 1973.
17. Sullivan HR, Due SL, McMahon RE. The identification of three new metabolites of methadone in man and in the rat. J Amer Chem Soc **94**:4050–4051, 1972.
18. Gram TE, Gillette JR. Biotransformation of drugs. In: Bacq ZM, Ed. Fundamentals of Biochemical Pharmacology. New York, Pergamon, p571, 1971.
19. Verebely K, Volavka J, Mulé S, Resnick R. Methadone in man: Pharmacokinetic and excretion studies in acute and chronic treatment. Clin Pharmacol Ther **18**:180–190, 1978.
20. Verebe K. Interaction of *d*-propoxyphene and diphenylhydantoin in rat liver microsomal preparation. Res Commun Clin Pathol Pharmacol **20**:21–29, 1978.

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