Nonspecific Stimulation of Drug Metabolism in Rats by Methoxyflurane

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Pretreatment of rats with a subanesthetic concentration (0.13 per cent) of methoxyflurane vapor, seven hours per day, resulted in the following responses relative to a control group: 1) hexobarbital sleeping time was reduced by 79 per cent when rats were exposed to the subanesthetic concentration for 15 days; 2) aminopyrine demethylase activity was markedly increased in the 9,000 × g supernate fraction of liver homogenates prepared from rats after they had been exposed for ten days; 3) a significant number of rats pretreated for ten days subsequently survived a lethal concentration of methoxyflurane, based on determination of the LD_{50}. These effects of methoxyflurane were reversed by an inhibitor of microsomal enzymic activity. These results suggest that methoxyflurane is a nonspecific stimulator of drug-metabolizing enzymes. In the 9,000 × g supernate, which contained only the microsomes and soluble cellular material, there was a marked increase in protein content, accompanied by a significant rise in drug-metabolizing activity. The increased protein may reflect an increase in microsomal enzymes and, therefore, enzyme induction. (Key words: Methoxyflurane; Hexobarbital sleeping time; Aminopyrine demethylase; Liver microsomes; Metabolism; Enzyme induction.)

MANY DRUGS that induce their own metabolic breakdown by hepatic microsome also stimulate the metabolic destruction of other pharmacologic agents.[1,2] Methoxyflurane induces its own metabolism,[3] but its ability to enhance the metabolism of other drugs by hepatic microsomes has not been demonstrated. This investigation was designed to determine if methoxyflurane is a nonspecific stimulator of hepatic microsomal enzymes. We found evidence that chronic exposure of rats to a subanesthetic concentration of methoxyflurane vapor shortens the hypnotic effect of hexobarbital, decreases the mortality of rats exposed to a lethal dose of the anesthetic, and stimulates oxidative demethylation by a 9,000 × g supernate fraction of liver homogenates.

Methods

Male Sprague-Dawley rats which initially weighed 40 to 50 g were used throughout this study. The animals were maintained on a stock diet and housed in metal cages in an air-conditioned room maintained at constant temperature. Care was taken to prevent exposure of the animals to materials known to affect drug-metabolizing enzymes, such as chlorinated insecticides[4] and cedar wood shavings.[5]

Exposure Chamber. The chamber used for administering methoxyflurane vapor to rats was a 55-l rectangular glass-sided aquarium with an inlet and an outlet at diagonally opposite ends. The aquarium could accommodate as many as 30 rats at once. Methoxyflurane in 10 l/min of filtered air was delivered through the inlet from a Pentec vaporizer. To obtain a subanesthetic concentration of methoxyflurane vapor that did not interfere with the growth of the rats, the dial of the Pentec was turned to a point just in advance of the "off" position. At this setting, the concentration of methoxyflurane in representative gas samples obtained from the aquarium was 0.13 per cent (±0.04 per cent) as determined by chromatographic analysis. The concentration was constant at 1, 3.5 and 6.5 hours during seven hours of exposure.

The control rats were placed in an identical, but separate, aquarium. They breathed only filtered air at 10 l/min.

Concentrations of carbon dioxide in the chambers, determined by gas chromatography, were 0.3 per cent after the rats had been exposed to 0.13 per cent concentration of the anesthetic for seven hours, and 0.35 to 0.4 per cent after the control rats had been exposed to air for a similar period.

Three experiments on the influence of pretreatment with methoxyflurane were performed, to determine: 1) duration of hexobarbital sleeping time; 2) tolerance to a lethal concentration of methoxyflurane; and 3) activity of aminopyrine demethylase in the 9,000 × g supernate from rat liver homogenates. In addition, the effect of butylated hydroxytoluene (BHT) on hexobarbital sleeping time was determined. BHT is the preservative in commercial methoxyflurane.

HEXOBARBITAL SLEEPING TIME

This was used as a screening test to determine if commercial methoxyflurane affected drug-metabolizing enzymes. One group of rats was exposed to 0.13 per cent methoxyflurane vapor for seven hours per day and a control group was exposed to air for seven hours per day. At the end of the seven hours the rats were returned to their cages and permitted to eat and drink *ad libitum*. Five or six rats from each group were used to measure the duration of action of hexobarbital 24 hours after the above treatment. This test was repeated after 1, 2, 3, 4, 5, 10 and 15 consecutive days of exposure to methoxyflurane. Each rat was given 125 mg/kg of sodium hexobarbital in physiologic saline solution intraperitoneally (ip) and the time interval until return of the righting reflex was measured. A final group of rats was exposed to methoxyflurane as described for ten consecutive days. Twenty-four hours after their last exposure and 40 minutes prior to determining the hexobarbital sleeping time each rat was given an ip injection of 50 mg/kg SKF 525-A [*] (beta-diethylaminodiphenylpropylacetate HCl) dissolved in physiologic saline solution. Rats employed in estimating the duration of action of hexobarbital were used only once and then destroyed.

TOLERANCE TO A LETHAL CONCENTRATION OF METHOXYFLURANE

Rats, in groups of six, were pretreated with 0.13 per cent methoxyflurane seven hours per day for ten consecutive days. Ninety-six hours later, these rats were exposed to a predetermined lethal dose of methoxyflurane (0.5 per

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TABLE 1. Results of Exposure to Various Concentrations of Methoxyflurane for Five Hours

Methoxyflurane Concentration (Per Cent)	Per Cent Mortality[*] without SKF 525-A	Per Cent Mortality[*] with SKF 525-A
0.13	0	0
0.21	16	20
0.27	33	27
0.38	86	80
0.50	100	100
LD₅₀ (Per Cent)	0.31	0.29
	(Probit analysis)	
95 (Per Cent) confidence limits	0.40–0.29	0.35–0.24

LD_{50} (Per Cent) : 0.31 / 0.29 (Probit analysis)

* 30 rats were used at each concentration.

cent). Another group of rats was pretreated with 0.13 per cent methoxyflurane as described. Forty minutes prior to exposure to the lethal concentration of the anesthetic, each rat was given an ip injection of SKF 525-A, 50 mg/kg.

The lethal dose of methoxyflurane was based on an LD_{50} determined by exposing rats in groups of six to methoxyflurane in air at 10 l/min. An unexposed group of rats was used for each of several concentrations of the anesthetic: 0.13, 0.21, 0.27, 0.38 and 0.50 per cent. The number of dead animals at the end of five hours was counted. This experiment was repeated five times for each concentration (table 1) at comparable times on different days, and the sequence of exposure was randomized. Probit analysis [6] was used to calculate the LD_{50}. This method of determining the LD_{50} of methoxyflurane vapor was repeated using rats pretreated with an ip injection of SKF 525-A, 50 mg/kg, 40 minutes prior to exposure to a given concentration of methoxyflurane.

ACTIVITY OF AMINOPYRINE DEMETHYLASE IN THE 9,000 × g SUPERNATE

Rats were divided into three groups of 12 each. One group was exposed to 0.13 per cent methoxyflurane seven hours per day for ten consecutive days; 24 hours after the last exposure these rats were killed by decapitation. The second group was treated similarly except that 40 minutes prior to decapitation each

rat was given 50 mg/kg of SKF 525-A ip. The
third, or control, group was exposed to air for
seven hours per day for ten consecutive days.

After thorough bleeding, the livers of all rats
were excised, blotted on filter paper previously
refrigerated (0 C) and moistened with ice-
cold 0.25-M sucrose. The organs were then
quickly cut into small pieces and placed in
refrigerated previously-teared beakers contain-
ing ice-cold 0.25-M sucrose. The beakers were
then weighed. The liver pieces with the su-
crose solution were transferred to ice-cold glass
homogenizer cups and additional ice-cold 0.25-
M sucrose was added sufficient to prepare 20
per cent homogenates. This was done in a
cold room (0 to 4 C) using a motor-driven
teflon-coated pestle. Homogenates were cen-
trifuged for 20 minutes at 9,000 × g at 0 C.

The ability of the 9,000 × g supernate to
demethylate aminopyrine was determined by
the amount of formaldehyde formed using the
Nash reagent by the technique of Cochin and
Axelrod.[7] The reaction mixture was prepared
as described by Argyris.[8] The incubation time
was 90 minutes. All assays were done in trip-
licate. Formaldehyde standards were used
with every determination. Protein was as-
sayed in triplicate on the 9,000 × g supernate
fraction, using the biuret reaction.[9]

Butylated hydroxytoluene (BHT) increased
the activity of hepatic microsomal enzymes
which metabolize hexobarbital when BHT was
given orally in large doses for several
days.[10] To determine if BHT administered ip
affected the hexobarbital sleeping time, 36

rats were divided into six groups; one group
served as a control, and each rat in the other
five groups received an ip injection of BHT
dissolved in 0.25 ml of corn oil twice a day
for five days. On the sixth day hexobarbital
sleeping time was determined. The dosage
schedule for BHT was: Group 1, 0.001 mg;
group 2, 0.01 mg; group 3, 0.1 mg; group 4,
1 mg; group 5, 10 mg; group 6, 0.25 ml corn
oil only.

Results

Rats exposed to 0.13 per cent methoxy-
flurane vapor seven hours per day for as long
as 15 consecutive days gained as much weight
as control rats.

HEXOBARBITAL SLEEPING TIME

Pretreatment with methoxyflurane markedly
shortened the hypnotic action of hexobarbital
(table 2). This effect appeared after two days
of treatment and persisted over the entire pe-
riod studied. The hexobarbital sleeping time
decreased 79 per cent between the first and
the fifteenth day of methoxyflurane treatment.
SKF 525-A prolonged the duration of action
of hexobarbital beyond that in the controls
(table 3).

TOLERANCE TO A LETHAL CONCENTRATION
OF METHOXYFLURANE

The difference between the LD_{50} of me-
thoxyflurane in normal rats and that in normal
rats pretreated with SKF 525-A was not sig-
nificant (table 1). Exposure of both groups
of rats to methoxyflurane vapor, 0.5 per cent,

TABLE 2. Mean Hexobarbital Sleeping Times of Rats 24 Hours Following
Last Exposure to Methoxyflurane Vapor

Number of Consecutive Days of Exposure (Seven Hours per Day)	Number of Rats	Control Sleeping Times ± SD (minutes)	Number of Rats	Sleeping Times after Methoxyflurane, 0.13 Per Cent, ± SD (minutes)
1	5	157 ± 46.8	5	96 ± 23.3
2	17	255 ± 54.5	17	68 ± 11.3*
3	33	122 ± 19.1	33	43 ± 14.9*
4	12	161 ± 45.1	23	43 ± 10.7*
5	6	102 ± 17.4	6	34 ± 7.6*
10	6	105 ± 14.5	6	22 ± 3.0*
15	6	112 ± 20.4	6	21 ± 3.2*

* $p < 0.001$ compared with corresponding control value.

TABLE 3. Mean Hexobarbital Sleeping Times of Rats Following Treatment with
Methoxyflurane Vapor and SKF 525-A

Number of Consecutive Days of Exposure (Seven Hours per Day)	Number of Rats	Control Sleeping Time ± SD (minutes)	Sleeping Time after Methoxyflurane 0.13 per cent, ± SD	Number of Rats	Sleeping Time after Methoxyflurane, 0.13 per cent; SKF 525-A, 50 mg/kg, ± SD
10	6	105 ± 14.5	22 ± 3.0	6	144 ± 11.5

resulted in 100 per cent mortality. However, mortality at this concentration was reduced 43 per cent if the rats were first exposed to a subanesthetic concentration of the anesthetic. The decrease in mortality of pretreated rats after 0.5 per cent methoxyflurane was abolished by SKF 525-A.

ACTIVITY OF AMINOPYRINE DEMETHYLASE IN THE 9,000 × g SUPERNATE

There was a marked increase in N-demethylase activity in the 9,000 × g supernate of liver homogenates obtained from rats which had been pretreated with a subanesthetic concentration of methoxyflurane, compared with the activity in the same fraction obtained from control rats (table 5). SKF 525-A significantly inhibited N-demethylase activity in the 9,000 × g supernate prepared from livers of rats pretreated with methoxyflurane. Enzymic activity in the 9,000 × g supernate from rats given the inhibitor was significantly lower than control values.

The amount of protein in the hepatic microsomes from rats pretreated with methoxyflurane was markedly increased (table 6). However, livers of the drug-treated rats were not significantly heavier than livers from controls. The mean wet weights of the control and drug-treated rat livers were 5.5 ± S.D. 0.56 g and 5.3 ± S.D. 0.31 g, respectively. SKF 525-A as used in these experiments did not affect the amount of 9,000 × g supernatant protein (mean weight 767.5 ± S.D. 68.24 mg), nor did it affect liver growth (mean wet weight 5.0 ± S.D. 0.48 g).

BHT administered to rats in a dose of 10 mg twice a day for five days resulted in a marked reduction in the hexobarbital sleeping time, 28.8 ± S.D. 8.1 min, compared with the control value, 89.8 ± S.D. 23.8 min. The other doses employed failed to affect hexobarbital sleeping time.

Discussion

Van Dyke[3] demonstrated that chronic exposure of rats to a subanesthetic concentration of methoxyflurane vapor enhanced the ability of hepatic microsomes to dechlorinate methoxyflurane. The work described in this paper indicates that methoxyflurane is also a nonspecific stimulator of drug metabolism. The observation that the hexobarbital sleeping time was significantly decreased in rats pretreated with methoxyflurane is indirect evidence for this conclusion. The hepatic microsomal enzyme inhibitor SKF 525-A reversed this effect. The use of the hexobarbital sleeping time as a screening test to determine if methoxyflurane affected microsomal enzyme systems other than the dechlorinating system was based on the report[2,11] that the duration of hexobarbital hypnosis is largely regulated by the level of hepatic microsomes that inactivate this drug. Many drugs known to stimulate drug-metabolizing enzymes by hepatic microsomes also stimulate metabolism of hexobarbital.[2,12] SKF 525-A prolongs the duration of action of hexobarbital by inhibiting hepatic microsomal enzymic activity,[2,12] without producing sedative effects of its own[13,14] or eliciting any other physiologic response[13-15] in the dose used.

Direct evidence for the nonspecific stimulating effect of methoxyflurane on drug metabolism was the marked increase in aminopyrine demethylase activity in the 9,000 × g supernate prepared from livers of rats pretreated with a subanesthetic concentration of the anesthetic. Aminopyrine demethylase activity has been demonstrated in rat hepatic microsomes (9,000 × g fraction).[2] Pretreatment of rats with many pharmacologic agents enhances the activity of this enzyme. Recent evidence[16] suggests that aminopyrine demethylase activity may be inhibited by excess aminopyrine in the incubation medium. La

TABLE 4. Effect of Pretreatment on Mortality after
Five Hours of 0.5 Per Cent Methoxyflurane

Number of Rats	Pretreatment	Mortality (Per Cent)
30	None	100
21	0.13 per cent methoxyflurane, 7 hours/day × 10 days	43
12	0.13 per cent methoxyflurane, 7 hours/day × 10 days; SKF 525-A, 50 mg/kg	100

Du et al.[17] found that demethylation of amino-
pyrine by hepatic microsomes occurs in two
steps: 1) demethylation of aminopyrine to
monomethyl antipyrine (MMAP) and formal-
dehyde; 2) demethylation of MMAP to 4-
aminoantipyrine (4-AAP) and formaldehyde.
Step 2 was found to be inhibited by 2×10^{-3}
M aminopyrine, but maximum formaldehyde
formation required more than this concentra-
tion of substrate.[16] Gram et al.[16] noted that
the amount of 4-AAP formed, compared with
the amount of formaldehyde formed, was 3
per cent after 30, and 12.5 per cent after 90,
minutes of incubation of 2×10^{-3} M amino-
pyrine with rat hepatic microsomes ($9,000 \times g$
fraction). Since 5×10^{-3} M aminopyrine was
used in our study, the amount of 4-AAP
formed was probably much less than 12.5 per
cent, because 5×10^{-3} M aminopyrine inhibits
the formation of 4-AAP.[16] Therefore, formal-
dehyde formation presumably resulted from
reaction 1, with an insignificant amount of
4-AAP formed during the incubation period.
Since we found a significant increase in form-
aldehyde formation in the $9,000 \times g$ supernate
of livers prepared from rats pretreated with
methoxyflurane, compared with untreated con-
trols, quantitative estimation of formaldehyde
as an index of aminopyrine demethylase ac-
tivity under these conditions seems justified.

Administration of SKF 525-A to rats inhibits
aminopyrine demethylase activity.[2, 12, 15] Ami-
nopyrine demethylase activity was inhibited in
rats pretreated with methoxyflurane and given
SKF 525-A. Only pretreated rats were given
SKF 525-A, as in the experiment to determine
the hexobarbital sleeping time. This was done
because it had been reported[18] that SKF
525-A sometimes fails to inhibit enzymic ac-

tivity in microsomes prepared from control
rats, but is an excellent inhibitor of enzymic
activity in microsomes prepared from drug-
treated rats. In this study SKF 525-A was
used to confirm that the effects of pretreat-
ment with methoxyflurane were related to mi-
crosomal enzymic activity.

The $9,000 \times g$ supernatant fraction of a he-
patic homogenate contains only microsomes
and soluble cellular material.[19-21] In this frac-
tion prepared from livers of rats pretreated
with methoxyflurane there was a marked in-
crease in protein content, accompanied by a
significant rise in drug-metabolizing activity.
The increased protein may reflect an increase
in microsomal enzyme and, therefore, induc-
tion. Evidence that drug-induced increases in
microsomal enzymic activity represent in-
creases in enzymic protein has been reported.[2]
However, from our data it is not possible to
conclude unequivocally that methoxyflurane
induced microsomal enzymes.

There was no significant increase in hepatic
weight in rats pretreated with methoxyflurane.
This finding is inconsistent with reports[2, 8, 22]
that many inducers of drug metabolism by he-
patic microsomes also cause increases in he-
patic weight. In one study[22] chronic ad-
ministration of an anesthetic concentration
of methoxyflurane to mice increased hepatic
weight but failed to stimulate drug-metabo-

TABLE 5. N-Demethylase Activity in Livers
from Rats Exposed to Methoxyflurane*

Treatment†	Dose	μg of For-maldehyde Formed per g Wet Weight of Liver (Mean ± SD)
Control	—	1.16 ± 0.86
Methoxyflurane	0.13 per cent 7 hours/day × 10 days	3.65 ± 1.20
Methoxyflurane; SKF 525-A	0.13 per cent 7 hours/day × 10 days	0.78 ± 0.50

* Control and methoxyflurane, $P < .001$.
Methoxyflurane and methoxyflurane–SKF
525A, $P < .001$.
Control and methoxyflurane–SKF 525A,
$P < .025$.
† Six assays per treatment. Each assay represents
the livers from three rats.

lizing enzymes in the liver, as determined by activity of a TPNH oxidase system. However, the discrepancies between the results of that study and those of our study may be explained by differences in the species used, concentrations of methoxyflurane employed, frequency and duration of exposure to anesthetic, and/or methods employed to detect drug-metabolizing activity.

The similarity between the values of LD_{50} of methoxyflurane in normal rats with and without pretreatment with SKF 525-A indicated that this agent exerted no additive or synergistic effects on the dose–response curve of methoxyflurane. This finding is consistent with reports [13-15] demonstrating that SKF 525-A possesses no (or minimal) pharmacologic activity except that of an inhibitor of microsomal enzymic activity. Therefore, the observation that pretreatment of rats with a subanesthetic concentration of methoxyflurane vapor significantly reduced their mortality when the rats were exposed to a concentration of anesthetic lethal to control rats, and the blocking of this response by SKF 525-A, strongly suggest that the reduced mortality may be related (in part) to enhanced drug metabolism. The data in this study do not exclude the possibility that factors other than drug metabolism contributed to the increased survival after a lethal dose of methoxyflurane.

A minimum of 24 hours was necessary between the time the rats had been given their last pretreatment with a subanesthetic concentration of methoxyflurane and the determination of hexobarbital sleeping time or aminopyrine demethylase activity. When this interval was shortened, we could not demonstrate a significant decrease in hexobarbital sleeping time or an increase in aminopyrine demethylase activity. We could not demonstrate increased tolerance to a toxic dose of methoxyflurane following pretreatment unless the interval between pretreatment and exposure to the lethal concentration was at least 96 hours. These observations suggest that methoxyflurane may exert a biphasic effect on drug metabolism, that is, it first inhibits and then stimulates drug metabolism after 12 hours.[2] The apparent delay before stimulation of drug metabolism by methoxyflurane may represent the time re-

TABLE 6. Increased Protein in $9,000 \times g$ Supernate from Liver Homogenates of Methoxyflurane-treated Rats

Pretreatment*	$9,000 \times g$ Protein mg/g Wet Weight of Liver (Mean ± SD)
Control	636.1 ± 49.52
Methoxyflurane, 0.13 per cent, 7 hours/day × 10 days	799.1 ± 60.62†

* Six assays per treatment. Each assay represents the livers from three rats.
† $P < 0.001$.

quired to synthesize enzymic protein, and/or time required to eliminate methoxyflurane.

The behavior of rats exposed to a subanesthetic concentration of methoxyflurane as used in this study could not be differentiated from that of control rats. The possibility that hypoxia and/or hypercarbia occurred during pretreatment with the subanesthetic concentration of the methoxyflurane used in this study, while unlikely, cannot be ruled out. The effects on drug metabolism of chronic exposure to hypoxia and hypercarbia are under investigation.

The screening test to determine if ip administration of BHT affected microsomal enzymic activity indicated that fairly large doses of BHT were necessary for demonstration of a decrease in hexobarbital sleeping time. The weights of the rats at the beginning of the experiment were between 40 to 50 g; weights (control and treated) at the end of the experiment averaged 90 g. The dose of BHT which markedly shortened the hexobarbital sleeping time was 10 mg twice a day per rat. Converting this dose to a kilogram body weight basis, it would be 200 to 250 mg administered twice a day at the beginning of the experiment and 11.5 mg twice a day at the end. It is extremely doubtful that BHT would volatilize under the conditions used to vaporize methoxyflurane in this study (personal communication, E. R. Larson, Dow Chemical Company), so it can be presumed that rats exposed to methoxyflurane did not inhale an amount of BHT sufficient to affect drug metabolism.

References

1. Conney, A. H.: Drug metabolism and thera- peutics, New Eng. J. Med. 280: 653, 1969.

2. Conney, A. H.: Pharmacological implications of microsomal enzyme induction, Pharmacol. Rev. 19: 317, 1967.

3. Van Dyke, R. A.: Metabolism of volatile anes- thetics. III. Induction of microsomal de- chlorinating and ether-cleaving enzymes, J. Pharmacol. Exp. Ther. 154: 364, 1965.

4. Hart, L. G., and Foutes, J. R.: Effects of acute and chronic DDT administration on hepatic microsomal drug metabolism in rat, Proc. Soc. Exp. Biol. Med. 113: 388, 1963.

5. Vesell, E. S.: Induction of drug metabolizing enzymes in liver microsomes of mice and rats by softwood bedding, Science 157: 1058, 1967.

6. Finney, D. J.: Statistical Methods in Biologi- cal Assay. Second ed. New York, Harner Publishing Co., 1964.

7. Cohn, J., and Axelrod, J.: Biochemical and pharmacological changes in the rat following chronic administration of morphine, nalor- phine, and normorphine, J. Pharmacol. Exp. Ther. 125: 105, 1959.

8. Argyris, T. S.: Liver growth associated with the induction of aminopyrine demethylase activity after phenobarbital in adult male rats, J. Pharmacol. Exp. Ther. 164: 405, 1968.

9. Layne, E.: Spectrophotometric and turbid- metric methods for measuring proteins. In Colowick, S. P. and Kaplan, N. O. (eds.): Methods in Enzymology. Volume 3. New York, Academic Press, 1957, pp. 447–454.

10. Creaven, P. J., Davies, W. H., and Williams, R. T.: The effect of butylated hydroxy- toluene, butylated hydroxyanisole and octyl gallate upon liver weight and biphenyl-4- hydroxyase activity in the rat, J. Pharm. Pharmacol. 18: 485, 1966.

11. Conney, A. H., Davison, C., Gastel, R., and Burns, J. J.: Adaptive increases in drug- metabolizing enzymes induced by pheno- barbital and other drugs, J. Pharmacol. Exp. Ther. 180: 1, 1960.

12. Axelrod, J., Reichenthal, J., and Brodie, B. B.: Mechanism of the potential action of beta-

13. Cook, W., Toner, J. J., and Fellows, E. J.: The effect of beta-diethylaminoethyl di- phenyl propylacetate hydrochloride (SKF 525-A) on hexobarbital, J. Pharmacol. Exp. Ther. 111: 131, 1954.

14. Cook, W., Macko, E., and Fellows, E. J.: The effect of beta-diethylaminoethyldiphenylpro- pylacetate hydrochloride on the action of a series of barbiturates and CNS depressants, J. Pharmacol. Exp. Ther. 112: 382, 1954.

15. Mannering, G. J.: Significance of stimulation and inhibition of drug metabolism in phar- macologic testing. In Tedeschi, D. H., and Tedeschi, R. E. (eds.): Importance of Fun- damental Principles in Drug Evaluation. New York, Raven Press, 1968, pp. 106–127.

16. Gram, T. E., Wilson, J. T., and Fouts, J. R.: Some characteristics of hepatic microsomal systems which metabolize aminopyrine in the rat and rabbit, J. Pharmacol. Exp. Ther. 159: 172, 1968.

17. La Du, B. N., Gaudette, W., Trousof, N., and Brodie, B. B.: Enzymatic dealkylation of aminopyrine (Pyramidon) and other alkyl- amines, J. Biol. Chem. 214: 741, 1953.

18. Mannering, G. J.: In Gillette, L. R., Cosmides, G. J., Estabrook, R. W., Fouts, J. R., and Mannering, G. (eds.): Microsomes and Drug Oxidations. New York, Academic Press, 1969, p. 367.

19. Axelrod, J.: Enzymatic determination of am- phetamine, J. Biol. Chem. 214: 753, 1955.

20. Fouts, J. R., and Brodie, B. B.: Enzymatic reduction of chloramphenicol, p-nitrobenzoic acid and other aromatic compounds in mam- mals, J. Pharmacol. Exp. Ther. 119: 197, 1957.

21. Fouts, J. R., and Brodie, B. B.: Inhibition of drug metabolic pathways by potentiating agent 2,4-dichloro-6-phenyl-phenoxyethyldi- ethylamine, J. Pharmacol. Exp. Ther. 115: 68, 1955.

22. Kunz, W., Schaude, G., Schmiassek, H., Schmid, W., and Siess, J.: Stimulation of liver growth by drugs. II. Biochemical analysis. In Alcock, S. J., deC. Baker, S. B., Scott, A. T., and Tucker, M. J. (eds.): Pro- ceedings of the European Society of the Study of Drug Toxicity. New York, Ex- cerpta Medica Foundation, 7: 138, 1966.

diethylaminoethyldiphenylpropylacetate, J. Pharmacol. Exp. Ther. 112: 49, 1954.