Acknowledgements—This work was supported by United States Public Health Service Grant AM13070. Adrenal glands were generously provided by Mr. W. E. Romero of Wilson & Company, Denver, Colo.

Department of Pharmacology,
University of Colorado Medical Center,
Denver, Colo. 80220, *U.S.A.*

LORIN A. WAGNER
ROBERT L. KOERKER
FREDERICK. H SCHNEIDER*

REFERENCES

1. W. W. DOUGLAS and R. P. RUBIN, *Nature, Lond.* **192,** 1087 (1961).
2. W. W. DOUGLAS and R. P. RUBIN, *J. Physiol., Lond.* **159,** 40 (1961).
3. W. W. DOUGLAS and R. P. RUBIN, *J. Physiol., Lond.* **167,** 288 (1963).
4. W. W. DOUGLAS and R. P. RUBIN, *J. Physiol., Lond.* **174,** 231 (1964).
5. A. PHILIPPU and H. J. SCHÜMANN, *Experientia* **18,** 138 (1962).
6. A. M. POISNER and W. W. DOUGLAS, *Proc. Soc. exp. Biol. Med.* **123,** 62 (1966).
7. R. P. RUBIN and S. D. JAANUS, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **254,** 125 (1966).
8. N. A. HILLARP, *Acta physiol. Scand.* **43,** 292 (1958).
9. A. M. POISNER and J. M. TRIFARÓ, *Molec. Pharmac.* **3,** 561 (1967).
10. F. LISHAJKO, *Acta physiol. Scand.* Suppl. **362** (1971).
11. F. LISHAJKO, *Acta physiol. Scand.* **75,** 255 (1969).
12. M. OKA, T. OHUCHI, H. YOSHIDA and R. IMAIZUMI, *Jap. J. Pharmac.* **17,** 199 (1967).
13. U. S. VON EULER and U. HAMBERG, *Acta physiol. Scand.* **19,** 74 (1949).
14. J. GOA, *Scand. J. Lab. clin. Invest.* **5,** 218 (1953).
15. F. H. SCHNEIDER, *J. Pharmac. exp. Ther.* **183,** 80 (1972).

* Established Investigator of the American Heart Association.

Biochemical Pharmacology, Vol. 22, pp. 432–436. Pergamon Press, 1973. Printed in Great Britain.

Effect of dietary lipid ingestion on the induction of drug-metabolizing enzymes by phenobarbital

(*Received* 27 *June* 1972; *accepted* 8 *September* 1972)

PREVIOUS reports from this laboratory[1–5] and by others[6,7] have implicated a role for dietary fatty acids in the metabolism of various substrates by liver enzymes of the rat. As little as 3 weeks' feeding of a fat-free diet results in depression of hexobarbital, heptachlor, aniline and ethylmorphine metabolism, decreased levels of cytochrome P-450, decreased binding of aniline and hexobarbital to washed microsomes, and a decrease in the ratio of absorbance peaks when ethyl isocyanide is used as a ligand for cytochrome P-450. Induction of microsomal drug-hydroxylating enzymes by phenobarbital appears to be enhanced in rats whose diets are supplemented with polyunsaturated fatty acids.[6,7] The present research describes the effects of dietary fatty acid consumption and phenobarbital pretreatment on the kinetics of drug hydroxylation by washed microsomes and on substrate binding to microsomal cytochrome P-450. These data plus those provided by the ethyl isocyanide difference spectra suggest a requirement of dietary polyunsaturated fatty acids for the full expresion of microsomal changes produced by the administration of phenobarbital.

Male Sprague–Dawley rats* (50–55 g) were fed a synthetic diet[2] containing 0 or 3 per cent corn oil or 3 per cent coconut oil (substituted for part of the sucrose) for 3 weeks. On four successive days prior to sacrifice each animal received phenobarbital (80 mg/kg i.p.) or saline (1·0 ml/kg i.p.). The animals were decapitated 24 hr after the last injection, and washed microsomes were prepared.[5] Protein content was assayed,[8] and cytochrome P-450 content, rates of metabolism of aniline and

* The Holtzman Company, Madison, Wis.

TABLE 1. EFFECT OF DIETARY LIPID CONSUMPTION AND PHENOBARBITAL PRETREATMENT ON SOME HEPATIC MICROSOMAL PARAMETERS OF MALE RATS*

Parameter	Fat-free diet†		3% Corn oil diet†	
	Saline	Phenobarbital	Saline	Phenobarbital
Liver weight (g/100 g body wt)	5·10 ± 0·13‡	5·93 ± 0·09§	5·02 ± 0·13‡	6·72 ± 0·09‖
Microsomal protein (mg/g liver)	19·3 ± 1·6‡	25·4 ± 2·3§,‖	21·2 ± 1·8‡,§	28·6 ± 1·1‖
Cytochrome P-450 content**	0·985 ± 0·124‡	1·618 ± 0·090§	1·241 ± 0·126‡	2·660 ± 0·153‖
Ethyl isocyanide ratio††	0·858 ± 0·087‡	1·213 ± 0·043§	0·897 ± 0·032‡	1·467 ± 0·056‖

* Values represent means ±S. E. of 7–18 animals per group.

† Animals were maintained on dietary regimen 21 days. On 4 successive days prior to sacrifice, each animal received saline (1·0 ml/kg i.p.) or phenobarbital (80 mg/kg i.p.).

‡,§,‖ Values for each parameter without common superscripts differ significantly ($P < 0·05$).

** Nanomoles of cytochrome P-450/milligram of microsomal protein.

†† Ratio of absorbance peak at 455 nm to peak at 430 nm.

TABLE 2. EFFECT OF DIETARY LIPID CONSUMPTION AND PHENOBARBITAL PRETREATMENT ON KINETICS OF SUBSTRATE METABOLISM AND SPECTRAL PROPERTIES OF LIVER MICROSOMES FROM MALE RATS*

Parameter	Fat-free diet		3% Corn oil diet	
	Saline	Phenobarbital	Saline	Phenobarbital
Hexobarbital K_m (mM)	0·270 ± 0·079†	0·222 ± 0·035†	0·211 ± 0·118†	0·248 ± 0·072†
Hexobarbital V_{max}‡	331·2 ± 43·1†	621·2 ± 34·9§	386·2 ± 73·0†	839·4 ± 90·2§
Aniline K_m (mM)	0·014 ± 0·008†	0·049 ± 0·020†,§	0·051 ± 0·018†,§	0·085 ± 0·019§
Aniline V_{max}‖	40·0 ± 5·1†	75·5 ± 11·2§	85·6 ± 11·5§	160·8 ± 14·3**
Hexobarbital K_s (mM)	0·217 ± 0·006†	0·151 ± 0·006§	0·143 ± 0·013§,**	0·131 ± 0·004**
Hexobarbital A_{max}††	20·8 ± 0·2†	38·0 ± 0·4§	31·8 ± 0·7**	60·4 ± 0·4‡‡
Aniline K_s (mM)	0·269 ± 0·019†,§	0·321 ± 0·037†	0·222 ± 0·020§	0·306 ± 0·016†
Aniline A_{max}††	9·3 ± 0·3†	22·4 ± 1·1§	16·9 ± 0·5**	31·1 ± 0·7‡‡

* K_m and V_{max} values are means ±S. E. determined from four points on Lineweaver–Burk plots using pooled livers of four rats per group. K_s and A_{max} values are means ±S. E. determined from seven points on Lineweaver–Burk plots using pooled livers of four rats per group.

†,§,**,‡‡ Values for each parameter without common superscripts differ significantly ($P < 0·05$).

‡ Nanomoles of hexobarbital metabolized/milligram of microsomal protein/hr.

‖ Nanomoles of *p*-aminophenol formed/milligram of microsomal protein/hr.

†† Δ A per milligram of microsomal protein × 10^3.

hexobarbital, spectral binding of aniline and hexobarbital, and ethyl isocyanide difference spectra were determined as previously described.[5] The Student's *t*-test was used to determine significant differences between treatment groups. The K_m and V_{max} values and the K_s and A_{max} values were determined according to Wilkinson,[9] where the standard errors of these values are obtained from residual errors of the weighted least square estimates.

Phenobarbital increased the liver weight/body weight ratio to a greater extent in animals receiving the diet supplemented with 3 per cent corn oil (Table 1). Microsomal protein content, however, was increased to the same extent in phenobarbital-pretreated animals receiving the two diets. The content of microsomal cytochrome P-450 and the ratio of ethyl isocyanide absorbance peaks were increased by phenobarbital to a greater extent in rats fed the corn oil diet. The results indicate that polyunsaturated fatty acids of corn oil allow greater induction of specific enzyme by phenobarbital. It is of interest that phenobarbital altered the ratio of ethyl isocyanide peak heights in these experiments, since this does not occur in phenobarbital-pretreated animals provided a diet of laboratory chow.[10] The ratio of ethyl isocyanide peak heights is believed to indicate relative content of different cytochrome P-450 species contained in microsomes.[10] It is conceivable that feeding the synthetic diet produces a particular pattern of cytochrome P-450 species and that phenobarbital administration alters this pattern. The finding that phenobarbital increases the ethyl isocyanide absorbance ratio more in rats fed the corn oil diet than in rats fed a fat-free diet indicates a role of essential fatty acid in determining relative amounts of cytochrome P-450 species.

The apparent V_{max} for microsomal metabolism of the type I substrate, hexobarbital, and the type II substrate, aniline, was elevated more by phenobarbital pretreatment of rats fed the corn oil-supplemented diet than by similar pretreatment of rats fed the fat-free diet (Table 2). The apparent Michaelis constants (K_m) were not significantly altered by phenobarbital pretreatment. Phenobarbital-induced increases in the binding of substrates to microsomes were also enhanced by corn oil feeding, as indicated by elevated maximal spectral shifts (A_{max}). The spectral binding constant (K_s) for hexobarbital was depressed by phenobarbital administration, while the aniline K_s tended to be increased.

Feeding a diet containing coconut oil (largely saturated fatty acids) failed to allow the level of phenobarbital induction of hexobarbital oxidase and aniline hydroxylase afforded by the diet containing corn oil (Table 3). Maximum binding (A_{max}) of hexobarbital was unchanged in liver microsomes from rats fed the diet supplemented with coconut oil; however, the A_{max} of aniline with microsomes from animals on this diet was intermediate between the A_{max} values obtained with microsomes from rats fed fat-free or corn oil-containing diets. This finding implies that the role of dietary lipid in enhancing induction of enzyme by phenobarbital administration is due to polyunsatured fatty acids rather than to saturated fatty acids.

The type of dietary lipid has a profound effect on the make-up of phospholipids of microsomal membranes.[5] Phospholipid is important in maintaining an active form of cytochrome P-450 in drug,

TABLE 3. EFFECT OF DIETARY LIPID CONSUMPTION ON KINETIC AND SPECTRAL PARAMETERS OF HEPATIC MICROSOMES FROM PHENOBARBITAL-PRETREATED MALE RATS*

Parameter	Diet		
	Fat-free	3% Coconut oil	3% Corn oil
Hexobarbital K_m (mM)	0·101 ± 0·063†	0·122 ± 0·047†	0·193 ± 0·020†
Hexobarbital V_{max}‡	563·2 ± 73·9†	629·1 ± 57·2†	857·0 ± 29·9§
Aniline K_m (mM)	0·089 ± 0·023†	0·100 ± 0·023†	0·172 ± 0·030†
Aniline V_{max}‖	117·1 ± 13·1†	154·1 ± 16·0†,§	205·2 ± 17·7§
Hexobarbital K_s (mM)	0·405 ± 0·046†	0·306 ± 0·008†	0·237 ± 0·006§
Hexobarbital A_{max}**	44·8 ± 2·2†	48·3 ± 0·5†	62·8 ± 0·5§
Aniline K_s (mM)	0·310 ± 0·044†	0·317 ± 0·041†	0·308 ± 0·060†
Aniline A_{max} **	12·5 ± 0·7†	15·2 ± 0·8§	18·5 ± 1·6§

* K_m and V_{max} values are means ±S. E. determined from four points on Lineweaver–Burk plots using pooled livers of four rats per group. K_s and A_{max} values are means ±S. E. determined from seven points on Lineweaver–Burk plots using pooled livers of four rats per group.

†,§ Values for each parameter without common superscripts differ significantly ($P < 0·05$).

‡ Nanomoles of hexobarbital metabolized/milligram of microsomal protein/hr.

‖ Nanomoles of *p*-aminophenol formed/milligram of microsomal protein/hr.

** Δ A per milligram of microsomal protein $\times 10^3$.

steroid and fatty acid metabolism,[11,12] and in maintaining integrity of the type I binding site.[13] The results of this study suggest that changes in fatty acid composition of the microsomal phospholipids produce changes in enzyme activity and binding properties of the microsomal drug-metabolizing system. That such changes in fatty acid constitution alter the induction processes is further evidence for this concept.

Marshall and McLean[6] have reported that dietary unsaturated fatty acids have a "permissive effect" in allowing the induction of cytochrome P-450 and hydroxylating enzymes produced by phenobarbital. These workers proposed a mechanism whereby fatty acids enhance the ability of phenobarbital to block hydroxylation, and thus inactivation, of an endogenous factor by cytochrome P-450. The endogenous factor is believed to be the "mediator" between inducing agents and increased content of cytochrome P-450, and interruption by phenobarbital of the normal inactivation of the factor results in initiation of the inductive processes. In view of the results of the present report, this theory appears to be an oversimplification in that the possible role of dietary fat in conformationally altering microsomal membranes, and therefore properties of cytochrome P-450, is not considered. Furthermore, the theory does not explain the fact that rats fed a fat-free diet usually have significantly less cytochrome P-450 than rats fed diets containing corn oil.[5] However, the theory may explain the lower degree of induction observed when phenobarbital was administered to rats fed a fat-free or coconut oil supplemented diet when compared to rats fed diets supplemented with corn oil.

Acknowledgements—The authors wish to thank Mrs. Betty C. Wu for technical assistance and Dr. J. J. Peifer for helpful discussion and comments.

WILLIAM P. NORRED*
ADELBERT E. WADE

Department of Pharmacology,
School of Pharmacy,
University of Georgia,
Athens, Ga. 30601, *U.S.A.*

REFERENCES

1. A. E. WADE, W. O. CASTER, F. E. GREENE and J. S. MEADOWS, *Archs int. Pharmacodyn. Thér.* **181,** 466 (1969).
2. W. O. CASTER, A. E. WADE, F. E. GREENE and J. S. MEADOWS, *Life Sci.* **9,** 181 (1970).
3. W. O. CASTER, A. E. WADE, W. P. NORRED and R. E. BARGMANN, *Pharmacology* **3,** 177 (1970).
4. W. P. NORRED, B. C. WU and A. E. WADE, *Fedn Proc.* **30,** 281 (1971).
5. W. P. NORRED and A. E. WADE, *Biochem. Pharmac.*, **21,** 2887 (1972).
6. W. J. MARSHALL and A. E. M. MCLEAN, *Biochem. J.* **122,** 569 (1971).
7. R. KASCHNITZ, *Hoppe-Seyler's Z. physiol. Chem.* **351,** 771 (1970).
8. A. G. GORNALL, C. J. BARDAWILL and M. M. DAVID, *J. biol. Chem.* **177,** 751 (1949).
9. G. N. WILKINSON, *Biochem. J.* **80,** 324 (1961).
10. N. E. SLADEK and G. J. MANNERING, *Biochem. biophys. Res. Commun.* **24,** 668 (1966).
11. H. W. STROBEL, A. Y. H. LU, J. HEIDEMA and M. J. COON, *J. biol. Chem.* **245,** 4851 (1970).
12. H. W. STROBEL, A. P. AUTOR, J. HEIDEMA and M. J. COON, *Fedn Proc.* **30,** 1091 (1971).
13. M. D. CHAPLIN and G. J. MANNERING, *Molec. Pharmac.* **6,** 631 (1970).

* NDEA Title IV Fellow. Present address: Pharmacology Laboratory, Richard B. Russell Agricultural Reesarch Center, USDA, Athens, Ga. 30601.