Biochemical Pharmacology, Vol. 34, No. 20, pp. 3747–3754, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

DIETARY FAT—A REQUIREMENT FOR INDUCTION OF MIXED-FUNCTION OXIDASE ACTIVITIES IN STARVED–REFED RATS*

ADELBERT E. WADE,† ROBERT A. WHITE, LINDA C. WALTON and JENELLA T. BELLOWS
Department of Pharmacology and Toxicology, College of Pharmacy, The University of Georgia, Athens, GA 30602, U.S.A.

(*Received* 31 *July* 1984; *accepted* 28 *February* 1985)

Abstract—Male rats were starved 0–48 hr, and then refed diets containing 0% (F.F.) to 20% corn oil (C.O.) lab chow or 20% coconut oil (C.C.O.) for 1–4 days. Some received phenobarbital sodium (80 mg/kg, i.p. daily) for 1–3 days prior to decapitation. Five cytochrome P-450-dependent indicators were assayed as measures of altered hepatic microsomal function: ethylmorphine *N*-demethylase (EMDM), *N*-nitrosodimethylamine (DMN)-*N* demethylase, aniline hydroxylase (AH), benzo[*a*]pyrene hydroxylase (AHH) and CO-difference spectra (P-450). Increasing dietary corn oil (0, 0.5, 10, 20%) in control rats resulted in a progressive increase in the activities of these five enzymes. Dietary fat influenced phenobarbital (Pb) inducibility of all mixed-function oxidase (MFO) enzymes measured except AHH. Pb induced the remaining enzymes only 11–22% in animals fed fat-free diet as compared to 119–246% in animals fed coconut oil and corn oil. Rats fed fat-free diet for 21 days without prior food deprivation and administered Pb had 79% more EMDM, 34% more AH and 120% more P-450 than non-induced controls, whereas rats fed 20% corn oil diet had 227% more EMDM, 143% more AH and 128% more P-450. A requirement of dietary fat for induction of MFO by Pb was demonstrated by these starvation–refeeding experiments. Coupled with data recovered from the 21-day studies, these experiments suggest that a compensatory mechanism may be operative during chronic feeding of the fat-free diet to partially return inducibility to the drug-metabolizing system.

Previous studies relating *in vitro* hepatic drug metabolism activities to dietary fat ingestion have utilized feeding schedules of 10 [1] to 21 or more days [2–4]. In each of these studies, the activities of several enzymes have been reduced in animals fed diets low in or devoid of essential fatty acids. More marked, however, has been the consistent reduction in the response of rats and mice to the administration of phenobarbital or 3-methylcholanthrene [3, 5–7]. Thus, the chronic administration of diets low in unsaturated fatty acids has been found to increase the sleeping time resulting from hexobarbital administration [8, 9] and to decrease the rate of clearance of drugs from plasma [10], the acute toxicity of drugs and chemicals which require metabolic activation (unpublished observations) and the *in vitro* activation of the mutagenic compounds *N*-nitrosodimethylamine [11] and 2-acetylamino fluorene [12] and the metabolic activation of benzo[*a*] pyrene [13]. These latter influences may be responsible, in part, for the increased susceptibility of laboratory animals fed high fat diets to chemically-induced cancer.

Starvation for 48–72 hr reportedly depresses, in male rats, the sex-dependent hepatic microsomal drug-metabolizing enzymes that are responsible for metabolizing hexobarbital (and increasing hexobarbital sleeping time), aminopyrine, pentobarbital and morphine but enhances levels of cytochrome P-450 and the metabolism of the sex-independent zoxazolamine hydroxylase, ethylmorphine *N*-demethylase and aniline hydroxylase [14–16]. Food restriction for 28 days resulted in reduced hexobarbital sleep time and enhanced cytochrome P-450 content. The drug-metabolizing enzymes aniline hydroxylase, *p*-chloro-*N*-methylaniline demethylase and *p*-nitrobenzoate reductase as well as those enzymes involved in generating NADPH were also increased [17].

The cause of changes in the mixed-function oxidases has been postulated to involve the appearance or disappearance of enzyme activators or inhibitors, allosteric enzymic effects produced by substances elaborated during starvation, conformational changes in the microsomal membrane, or enzymes which might influence enzyme substrate binding [16].

With the current practice of dietary weight-loss regimens which require varying food-deprivation schedules, it seemed prudent to investigate the influence of food deprivation preceding the feeding of diets with varied fat content on the activities and inducibilities of hepatic drug-metabolizing enzymes of the rat.

EXPERIMENTAL

Animals and treatment. Male Sprague–Dawley rats (100–124 g, Harlan, Madison, WI) were admin-

* This research was supported by NIH BRGS RR07025-17 and a grant from the American Institute for Cancer Research. Preliminary results from this research were presented at the annual FASEB meeting in St. Louis, MO, April 1984, and at IUPHAR, London, August 1984.

† Author to whom all correspondence should be addressed.

Table 1. Constituents of diet*

Ingredient	Fat free	20% Fat diet
Caseine (vitamin free)	18%	18%
Sucrose	25%	5%
Wheat starch	44.4%	44.4%
AIN 76 salt mix	4.0%	4.0%
Vitamin mix (26)	4.6%	4.6%
Alphacel	4.0%	4.0%
Fat	0	20%

* In diets containing other levels of fat, an equal weight of sucrose was substituted for the decrease in fat. All ingredients except sucrose were purchased from the United States Biochemical Corp., Cleveland, OH.

istered Purina Rodent Laboratory Chow 5001 for 7 days prior to food deprivation for 0–48 hr. Following this period, rats were refed *ad lib.* the Purina Rodent Chow or synthetic diets (compounded in our laboratory) containing 0–20% fat in the form of Mazola Corn Oil (Best Foods, Inc.) or hydrogenated coconut oil (Table 1). Other rats (same source) weighing 50–65 g were fed the same 0 or 20% fat diets for 21 days with no prior starvation. Some rats were administered saline or phenobarbital sodium (80 mg/kg, i.p., at 9:00 a.m. daily) beginning 1 day after refeeding and continuing until the day before decapitation (maximum of three injections). Rats on the 21-day feeding experiment were administered the same doses of phenobarbital sodium daily for 3 days prior to decapitation.

Rats were killed between 9:00 and 10:00 a.m., and livers were removed, chilled, and homogenized in 0.1 M phosphate buffer, pH 7.4, containing 1 mM dithiothreitol (DTT) and 1 mM EDTA. Microsomes were prepared from the 9000 *g* supernatant fraction [18] and stored at −80° suspended in 0.1 M phosphate buffer containing 20% glycerol, 1 mM DTT, 1 mM EDTA, pH 7.4, at a protein concentration of approximately 20 mg/ml.

Metabolic incubation studies. Microsomes were thawed and then diluted with 0.1 M phosphate buffer (pH 7.4) containing 1 mM DTT and EDTA; protein concentration was determined by the method of Lowry *et al.* [19]. Cytochrome P-450 concentration was measured by the CO difference method of Omura and Sato [20] using the Aminco DW-2 spectrophotometer in the dual-wavelength mode. N-Demethylation of ethylmorphine and *N*-nitrosodimethylamine (DMN) was determined by measuring formaldehyde formation [21]. Aniline hydroxylase was estimated by measuring the amount of *p*-aminophenol formed [22] and benzo[*a*]pyrene hydroxylase was measured by the fluorimetric method of Dehnen *et al.* [23]. Apparent kinetics of these reactions were measured using six substrate concentrations spanning the expected K_m.

Data analysis. Apparent V_{max} and K_m for these reactions were calculated using Wilkinson's computerized adaptation of the Lineweaver–Burk method [24]. Statistical differences between control and phenobarbital-treated animals were estimated by Student's *t*-test using N-2 degrees of freedom.

RESULTS

Effect of refeeding dietary corn oil following 48 hr of food deprivation. Increasing the corn oil content of the diets from 0 to 20% increased the apparent V_{max} of ethylmorphine *N*-demethylase, aniline hydroxylase, *N*-nitrosodimethylamine *N*-demethylase I, benzo[*a*]pyrene hydroxylase and cytochrome P-450 in a concentration-related manner (Table 2). The apparent K_m values for these reactions were not altered in a diet-related manner. The influence of the starvation–refeeding regimen affected cytochrome b_5 concentration only slightly.

Phenobarbital administered during the last 3 days of the refeeding period produced a differential effect on induction of drug-metabolizing enzymes, an effect dependent on the concentration and type of fat in the diet (Table 3). Not only were the control and induced activities higher, but the percentage induction in all enzymes tested except benzo[*a*]pyrene hydroxylase was increased by feeding a source of corn oil in the diet. Microsomes from rats refed the saturated fat (C.C.O.) exhibited higher enzyme activities than those from rats refed the fat-free diet. Induction of these enzymes by phenobarbital in rats refed the hydrogenated coconut oil was intermediate between the animals refed fat-free diet and those refed corn oil.

Effect of dietary fat on the temporal development of phenobarbital-induced drug-metabolizing enzymes following 48 hr of food deprivation. Figure 1 depicts the temporal effects of refeeding diets containing 0 or 20% corn oil or hydrogenated coconut oil on the apparent V_{max} of ethylmorphine *N*-demethylase (EMDM). One and two days of food deprivation slightly elevated the apparent V_{max} of EMDM. However, refeeding the fat-free diet appeared to depress the activity to below pre-fasting levels. The refeeding

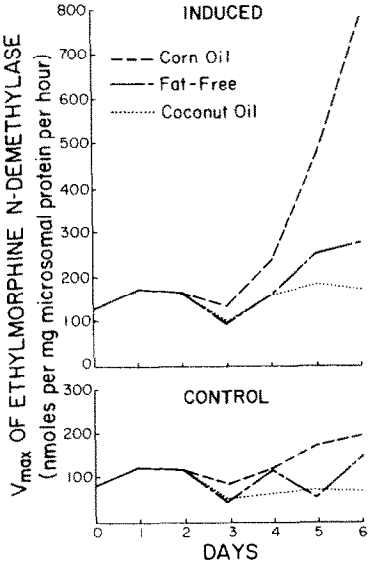

Fig. 1. Temporal effects of refeeding diets containing 0 or 20% corn oil or hydrogenated coconut oil on the apparent V_{max} of ethylmorphine *N*-demethylase. After the 2 days of food deprivation, rats were refed the specified diet 1–4 days before decapitation.

Table 2. Influence of diets containing various levels of corn oil on hepatic drug-metabolizing enzymes in starved–refed rats*

Diet	Ethylmorphine *N*-demethylase		Aniline hydroxylase		DMN *N*-demethylase		BaP hydroxylase		Cytochrome P-450	b_5
	V_{max}†	K_m (mM)	V_{max}†	K_m (mM)	V_{max}†	K_m (mM)	V_{max}‡	K_m (μM)	nmoles/mg microsomal protein)	
F.F.	96.4 ± 2.5	0.233 ± 0.019	11.2 ± 0.3	0.023 ± 0.004	23.4 ± 3.7	0.237 ± 0.166	14.83 ± 0.90	4.24 ± 0.67	0.365	0.427
0.5% C.O.	128.0 ± 4.2	0.188 ± 0.021	12.3 ± 0.1	0.034 ± 0.002	29.7 ± 0.9	0.181 ± 0.024	22.07 ± 2.20	5.34 ± 1.02	0.465	0.544
10% C.O.	160.9 ± 2.3	0.163 ± 0.008	15.1 ± 0.4	0.026 ± 0.004	40.3 ± 1.5	0.146 ± 0.025	28.23 ± 1.47	5.44 ± 0.70	0.624	0.557
20% C.O.	175.3 ± 4.2	0.197 ± 0.016	16.8 ± 0.3	0.012 ± 0.002	56.7 ± 1.6	0.156 ± 0.020	29.50 ± 2.93	6.12 ± 1.28	0.821	0.570

* Rats were deprived of food for 48 hr prior to refeeding for 4 days. The kinetic parameters were calculated from six points on the Lineweaver–Burk plots and are the means of two separate experiments using pooled microsomes from two to three rats.

† Expressed in nmoles product formed per mg microsomal protein per hr ± S.E.M.

‡ Expressed in pmoles 3-hydroxybenzo[*a*]pyrene formed per mg microsomal protein per 10 min ± S.E.M.

Table 3. Effect of phenobarbital administration in rats subjected to starvation followed by refeeding 0 or 20% fat in their diets*

Treatment	Ethylmorphine *N*-demethylase		Aniline hydroxylase		Benzo[*a*]pyrene hydroxylase		Cytochrome P-450§ (nmoles/mg microsomal protein)
	V_{max}†	K_m (mM)	V_{max}†	K_m (mM)	V_{max}‡	K_m (μM)	
F.F. (control)	116.69 ± 2.86 (4)	0.243 ± 0.019	12.56 ± 0.14 (3)	0.025 ± 0.002	13.63 ± 0.70 (1)	2.85 ± 0.40	0.403
F.F. (Pb)	142.33 ± 5.06‖ (4)	0.341 ± 0.035¶	14.75 ± 0.50‖ (3)	0.034 ± 0.007	18.21 ± 1.15¶ (1)	6.28 ± 0.93¶	0.449
20% C.O. (control)	194.89 ± 3.08 (3)	0.197 ± 0.011	18.83 ± 1.12 (2)	0.035 ± 0.011	28.05 ± 1.44 (1)	2.85 ± 0.40	0.725
20% C.O. (Pb)	673.48 ± 9.71‖ (3)	0.351 ± 0.015‖	53.61 ± 1.67‖ (2)	0.053 ± 0.008	35.51 ± 2.12¶ (1)	4.11 ± 0.64	1.844
20% C.C.O. (control)	173.40 ± 3.00 (2)	0.373 ± 0.018	16.61 ± 0.73 (1)	0.020 ± 0.007	19.65 ± 1.12 (1)	1.66 ± 0.30	0.547
20% C.C.O. (Pb)	379.42 ± 14.37 (2)	0.475 ± 0.046	40.85 ± 2.04‖ (1)	0.051 ± 0.013	24.44 ± 2.75 (1)	2.83 ± 0.83	1.697

* Numbers in parentheses represent the number of times the starvation–refeeding experiment was conducted. Rats were starved for 48 hr and then refed for 4 days.

† Expressed in nmoles product formed per mg microsomal protein per hr ± S.E.M.

‡ Expressed in pmoles 3-hydroxybenzo[*a*]pyrene formed per mg microsomal protein per 10 min ± S.E.M.

§ Values are means of two experiments using pooled microsomes from two to four rats.

‖,¶ Statistically different from uninduced control fed same diet: ‖$P < 0.01$ and ¶$P < 0.05$.

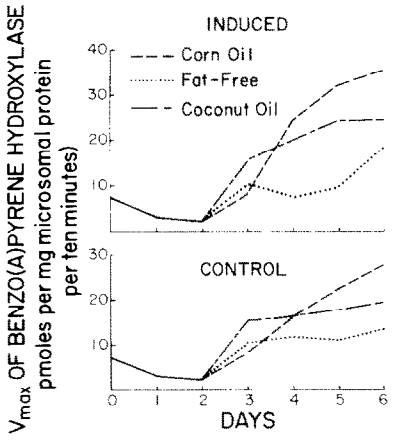

Fig. 2. Temporal effects of refeeding diets containing 0 or 20% corn oil or hydrogenated coconut oil on the apparent V_{max} of benzo[*a*]pyrene hydroxylase. Rats were starved for 2 days and then refed the specified diet for 1–4 days before decapitation.

of 20% corn oil had a marked stimulatory effect beginning at day 2 of refeeding whereas the hydrogenated coconut oil was only moderately effective in elevating the activity of EMDM. The effect of phenobarbital was negligible in rats fed the fat-free diet but most marked in enhancing ethylmorphine *N*-demethylase in rats refed the diet containing 20% corn oil.

Figure 2 depicts the temporal effects of refeeding diets containing 0 or 20% corn oil or hydrogenated coconut oil on the apparent kinetics of benzo[*a*] pyrene hydroxylase activity. Unlike ethylmorphine *N*-demethylase, benzo[*a*]pyrene hydroxylase activity was slightly depressed during starvation but increased following the refeeding period. Like ethylmorphine *N*-demethylase, the increase in activity was dependent upon the level and type of fat in the diet, with corn oil providing more enhancement than coconut oil. The effect of phenobarbital was only slightly more pronounced in rats fed the corn and coconut oil diets than in those fed the fat-free diet and much less marked than the same treatment on EMDM activity.

Effect of duration of food deprivation on induction of the microsomal drug-metabolizing enzymes. Figures 3–5 depict the effects that duration of food deprivation followed by 4 days of refeeding had on ethylmorphine *N*-demethylase, aniline hydroxylase and cytochrome P-450 respectively. Animals refed the fat-free diet had lower microsomal enzyme levels and responded least to phenobarbital administration if starved for 48 hr. Lesser periods of food deprivation resulted in higher basal levels of these enzymes and appeared to allow for some induction. Induction was uniformly less in animals fed the fat-free diet than in those fed the diet containing corn oil.

Table 4 contains data relating food deprivation to DMN *N*-demethylase activity in rats refed fat-free or 20% corn oil containing diets. Although microsomes from rats refed the corn oil diet demonstrated higher DMN *N*-demethylase activity regardless of the period of food deprivation, only rats fed the fat-free diet responded to phenobarbital induction. This unexpected phenomenon was also demonstrated in rats fed fat-free diet for 21 days [11].

DISCUSSION

Gram *et al.* [16] reported that refeeding lab chow for 72 hr following 72-hr starvation generally results in depressed concentrations of hepatic microsomal cytochrome P-450, ethylmorphine *N*-demethylase and aniline hydroxylase in a manner not unlike the present results in rats refed the fat-free diet. Refeeding lab chow 4 days following 48-hr starvation in the present study also resulted in depressed ethylmorphine *N*-demethylase activity (Fig. 6).

The most profound changes in enzyme activities and cytochrome P-450 content yet reported were

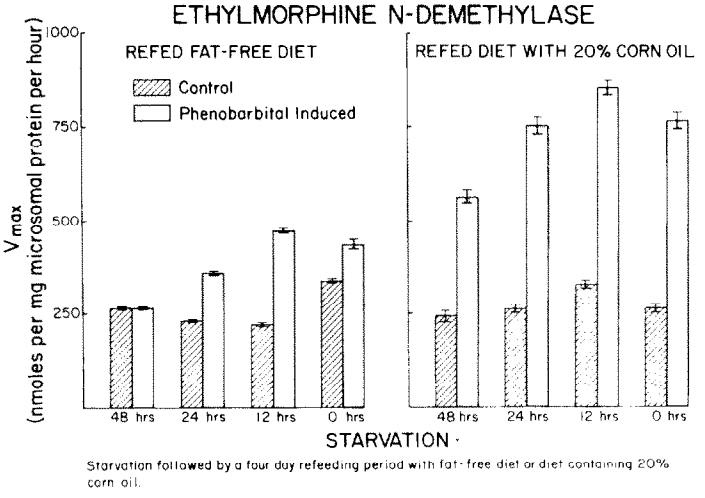

Fig. 3. Effect of duration of food deprivation followed by 4 days of refeeding on ethylmorphine *N*-demethylase.

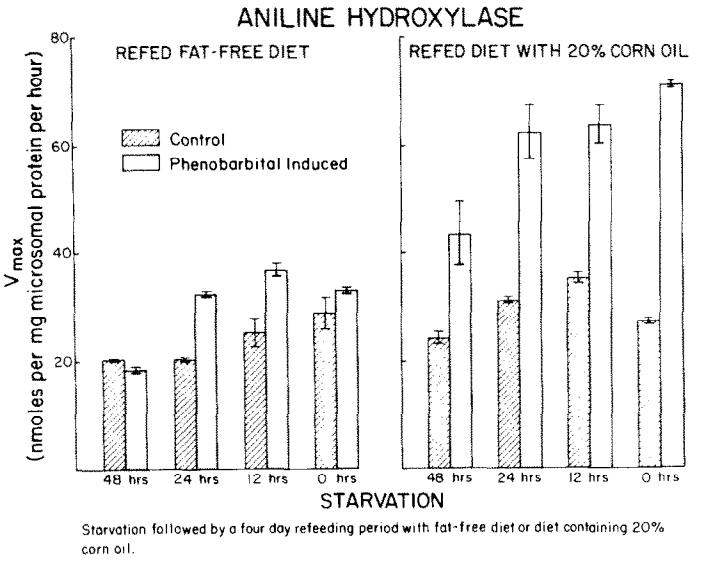

Fig. 4. Effect of duration of food deprivation followed by 4 days of refeeding on aniline hydroxylase.

seen in the present studies in which animals were fed various levels of fat incorporated into a synthetic diet. The refeeding of increasing amounts of corn oil (up to 20% by weight) enhanced the activities of these enzymes in a progressive manner. Since essential fatty acid deficiency evokes changes in fatty acid composition of the endoplasmic reticulum within 4 days (e.g. decrease in linoleic and arachidonic and increase in palmitic) [25], it seems reasonable to postulate a probable causal relationship based on these changes. The diet containing 0.5% corn oil most likely supplied levels of linoleate adequate to prevent essential fatty acid deficiency symptoms during the 4 days of these refeeding experiments. However, this level of corn oil did not support the maximum inductive response to phenobarbital. Thus, two phenomena appear to operate simultaneously and in concert. (a) Diet-related changes in fatty acid composition of phospholipids of the endoplasmic reticulum which alter the unsaturated:saturated fatty acid ratio [25] may be responsible, in part, for the decreased mixed-function oxidase activity observed in microsomes from rats on the low fat diets. However, when dietary fat is increased from 0.5 to 10% or 20%, there is additional dietary fat-related increases in drug-metabolizing enzyme

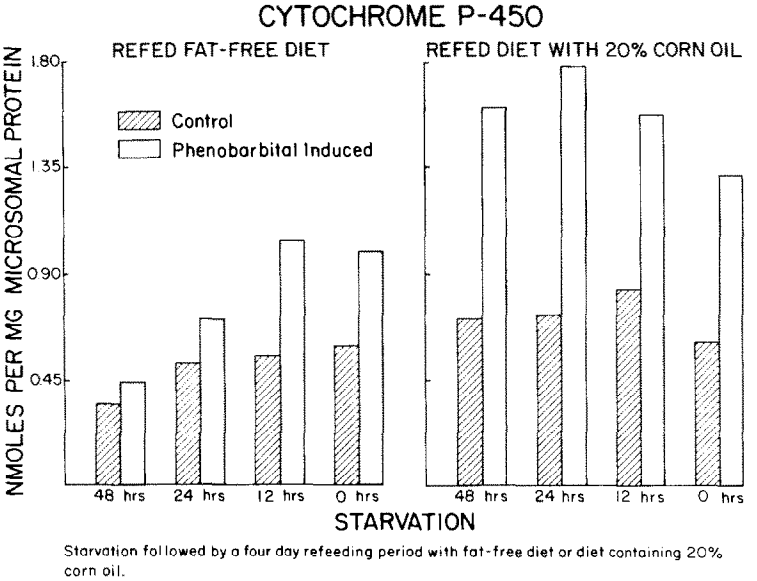

Fig. 5. Effect of duration of food deprivation followed by 4 days of refeeding on concentration of cytochrome P-450.

Table 4. Effects of starvation period and phenobarbital administration on the kinetics of *N*-nitrosodimethylamine *N*-demethylase of hepatic microsomes

Duration of food deprivation (hr)	Diet and treatment	V_{max}*	K_m (mM)
48	F.F. (control)	26.5 ± 3.4	1.22 ± 0.32
	F.F. (Pb)	42.1 ± 5.2†	0.49 ± 0.16
	20% C.O. (control)	38.9 ± 3.7	0.04 ± 0.03
	20% C.O. (Pb)	36.9 ± 3.5	0.17 ± 0.07
24	F.F. (control)	28.3 ± 2.5	0.29 ± 0.08
	F.F. (Pb)	35.7 ± 1.8†	0.16 ± 0.03
	20% C.O. (control)	53.6 ± 2.7	0.09 ± 0.03
	20% C.O. (Pb)	58.2 ± 7.1	0.20 ± 0.09
12	F.F. (control)	27.7 ± 3.4	0.12 ± 0.06
	F.F. (Pb)	51.0 ± 2.0‡	0.25 ± 0.04
	20% C.O. (control)	56.6 ± 2.7	0.21 ± 0.04
	20% C.O. (Pb)	65.9 ± 5.8	0.24 ± 0.08

* V_{max} = nmoles formaldehyde generated per mg microsomal protein/hr.
†,‡ Statistically different from uninduced controls: †$P < 0.05$, and ‡$P < 0.01$.

activity. (b) Thus, the second contributing factor appears to be the level of dietary corn oil and may suggest an effect on membrane fluidity or other alterations of unknown secondary reactions. The finding that 20% coconut oil, which has little or no polyunsaturated fatty acids, supported enzyme induction (although less than the corn oil) also suggests a relative contribution of total fat ingestion. On the other hand, those differences in enzyme activity observed in microsomes from rats consuming fat-free versus 0.5% corn oil diets, or the difference resulting from feeding 20% coconut oil versus 20% corn oil diets may reflect the contribution of essential fatty acids to the maintenance and inducibility of the mixed-function oxidases.

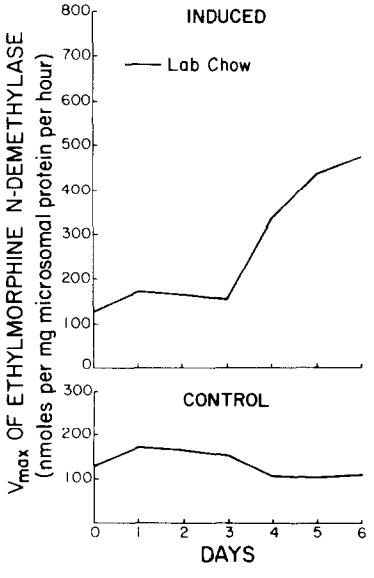

Fig. 6. Temporal effects of refeeding lab chow on the apparent V_{max} of ethylmorphine *N*-demethylase. Rats were starved for 2 days and then refed lab chow for 1–4 days before decapitation.

The fact that these acute changes in mixed-function oxidase activities are more pronounced than those reported for 21-day feeding experiments (Table 5) suggests that a compensatory response develops with prolonged feeding of fat-free diet. That this compensatory response may involve an altered phospholipid fatty acid profile in the endoplasmic reticulum is suggested by the findings of Brenner *et al.* [25]. These investigators reported that shortly after 4 days on a fat-free diet a gradual increase of the monoenoic acids (oleic and palmitoleic) occurs until day 23. Also, by 21–23 days an elevation in eicosa-5,8,11-trienoic acid occurs [2, 25]. Thus, the ratio of unsaturated to saturated fatty acids falls rapidly by day 4 and continues to decline slowly to about day 11 when it reverses this trend and is largely back to normal at day 23. Although this ratio has returned to normal and the resulting membrane fluidity has essentially returned to normal at day 23, the levels of polyunsaturated fatty acids (e.g. arachidonic, linoleic) remain significantly lowered [2, 25].

Although the changes in unsaturated:saturated fatty acid ratios which result in changes of membrane fluidity may be involved in the basal levels of MFO in microsomes and may be related to the abilities of substrates to reach the active sites on these enzymes, or the rapid transfer of electrons between NADPH, the reductases and cytochrome P-450, this does not adequately explain the relative inability of the system to respond to the enzyme inducer phenobarbital.

Since fatty acid deficiency results in rapid induction of Δ9 and Δ6 desaturases in liver microsomes, it is possible that competition for amino acid incorporation into these newly synthesized enzyme proteins might reduce the response to phenobarbital. However, this would only explain that portion of the difference in induction resulting from EFA defici-

Table 5. Effect of phenobarbital administration to rats fed 0 or 20% fat for 21 days on hepatic microsomal drug-metabolizing enzymes

Diet and treatment	Ethylmorphine *N*-demethylase		Aniline hydroxylase		Cytochrome P-450† (nmoles/mg microsomal protein)
	V_{max}*	K_m (mM)	V_{max}*	K_m (mM)	
F.F. (control)	337 ± 4.5	0.130 ± 0.007	43.6 ± 0.40	0.017 ± 0.002	0.421
F.F. (Pb)	604 ± 10.0‡	0.296 ± 0.015‡	58.4 ± 0.70‡	0.052 ± 0.003‡	0.926
20% C.O. (control)	271 ± 10.0	0.244 ± 0.028	30.4 ± 0.50	0.010 ± 0.002	0.500
20% C.O. (Pb)	885 ± 7.5‡	0.313 ± 0.008§	73.8 ± 5.8‡	0.082 ± 0.028§	1.409
20% C.C.O. (control)	289 ± 8.0	0.261 ± 0.023	29.7 ± 0.70	0.034 ± 0.005	0.429
20% C.C.O. (Pb)	715 ± 8.0‡	0.353 ± 0.011‡	53.3 ± 0.09‡	0.061 ± 0.005‡	0.848

* V_{max} = nmoles product formed per mg microsomal protein per hr ± S.E.M.
† Values are means of two experiments using pooled microsomes from two to four rats.
‡,§ Statistically different from non-induced controls: ‡$P < 0.01$, and §$P < 0.05$.

ency and would not explain the apparent total dietary fat effect. The diets used in these experiments had different caloric densities, and rats are known to eat calories rather than food quantity. Thus, rats fed the lower fat diets would be expected to ingest more diet and thus more carbohydrate. Carbohydrate has been reported to decrease the basal level of several MFOs, but a 10–20% increase of carbohydrate in the diet of rats has not prevented induction of MFO activity by Pb.

Attempts to quantitate differences in cytochrome P-450 hemoprotein content of microsomes from rats fed 0 or 10% corn oil for 21 days by sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed that there was no apparent decrease in hemoprotein concentration in microsomes from rats fed the fat-free diet. This led us [26] to postulate that membrane phospholipid composition affected the expression of mixed-function oxidase activity. That this may not be due to a specific polyunsaturated fatty acid (ex. arachidonic) is suggested by the work of Wills [1]. Although a diet containing herring oil (rich in $\omega 3$ fatty acids) reduced the arachidonic acid content of phospholipids of endoplasmic reticulum 50% within 10 days, it supported mixed-function oxidase levels in a manner similar to that afforded by a linoleate containing diet. That the level of fat, although important in determining basal activity, failed to alter benzo[*a*]pyrene hydroxylase inducibility by phenobarbital may be in part related to the multiple pathways of benzo[*a*]pyrene degradation or the limitations of the 3-OH-B[*a*]P assay used in our present studies. We found [27], for example, that feeding rats a diet containing 10% corn oil for 21 days increased benzo[*a*]pyrene metabolism to the dihydrodiols more than to the phenols. The AHH activity reported in the current experiments measured the formation of phenols only.

This phenomenon of a dietary-fat related decrease and/or short-term suppression of MFO inducibility after 48-hr food deprivation may have important medical implications. Increases in specific cytochrome P-450-requiring detoxification/activation activities appear to require the ingestion of both unsaturated fats as well as general dietary fats and may account for some of the increased susceptibility of laboratory animals fed high-fat diets to chemically-induced cancer. On the other hand, starvation followed by refeeding of a low fat diet may render individuals more sensitive or susceptible to chemicals or drugs which are normally detoxified by the hepatic mixed-function oxidase system.

REFERENCES

1. E. D. Wills, in *Microsomes, Drug Oxidation and Chemical Carcinogenesis* (Eds. M. J. Coon, A. H. Conney, R. W. Estabrook, H. V. Gelboin, J. Gillette and P. J. O'Brien), Vol. I. Academic Press, New York (1980).
2. W. P. Norred and A. E. Wade, *Biochem. Pharmac.* **21**, 2887 (1972).
3. A. E. Wade and W. P. Norred, *Fedn Proc.* **35**, 2475 (1976).
4. W. O. Caster, A. E. Wade, F. E. Greene and J. S. Meadows, *Life Sci.* **9**, 181 (1970).
5. W. P. Norred and A. E. Wade, *Biochem. Pharmac.* **22**, 432 (1973).
6. W. J. Marshall and A. E. M. McLean, *Biochem. J.* **122**, 569 (1971).
7. B. Century, *J. Pharmac. exp. Ther.* **185**, 185 (1973).
8. A. E. Wade, W. P. Norred and J. S. Evans, in *Nutrition and Drug Interrelations* (Eds. J. N. Hathcock and J. Coon), p. 475. Academic Press, New York (1978).
9. C. Hetu and J. G. Joly, *Biochem. Pharmac.* **28**, 841 (1979).
10. R. W. Chadwick, W. S. Simmons, C. C. Bryden, L. T. Chuang, L. M. Key and C. J. Chadwick, *Toxic. appl. Pharmac.* **39**, 391 (1977).
11. A. E. Wade, W. Harley and O. R. Bunce, *Mutation Res.* **102**, 113 (1982).
12. C. E. Castro, I. C. Felkner and S. P. Yang, *Cancer Res.* **38**, 2836 (1978).
13. M. T. Baker, S. W. Karr and A. E. Wade, *Carcinogenesis* **4**, 9 (1983).
14. R. Kato and J. R. Gillette, *J. Pharmac. exp. Ther.* **150**, 279 (1965).
15. R. Kato, K. Onoda and M. Sasajima, *Jap. J. Pharmac.* **20**, 194 (1970).
16. T. E. Gram, A. M. Guarino, D. H. Schroeder, D. C. Davis, R. L. Reagan and J. R. Gillette, *J. Pharmac. exp. Ther.* **175**, 12 (1970).
17. D. S. Sachan, *Biochem. biophys. Res. Commun.* **104**, 984 (1982).

18. A. E. Wade, B. C. Wu, C. M. Holbrook and W. O. Caster, *Biochem. Pharmac.* **22**, 1573 (1973).
19. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
20. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
21. T. Nash, *Biochemistry* **55**, 416 (1953).
22. R. Kato and J. R. Gillette, *J. Pharmac. exp. Ther.* **150**, 285 (1965).
23. W. Dehnen, R. Tomingas and J. Roos, *Analyt. Biochem.* **53**, 373 (1973).
24. G. N. Wilkinson, *Biochem. J.* **80**, 324 (1961).
25. R. R. Brenner, H. Garda, I. N. T. de Gomez Dumm and H. Pezzano, in *Progress in Lipid Research* (Ed. R. T. Holman), Vol. 20, p. 315. Pergamon Press, New York (1982).
26. K-C. Cheng, W. L. Ragland and A. E. Wade, *J. environ. Path. Toxic.* **4**, 219 (1980).
27. T-C. L. Lam and A. E. Wade, *Drug-Nutrient Interact.* **1**, 31 (1981).