Biochemical Pharmacology, Vol. 26, pp. 1299–1302. Pergamon Press, 1977. Printed in Great Britain.

COMBINED EFFECTS OF LOW DOSES OF DDT AND PHENOBARBITAL ON CYTOCHROME P450 AND AMIDOPYRINE DEMETHYLATION

ANDRÉ E. M. MCLEAN* and H. ELIZABETH DRIVER

Department of Experimental Pathology, University College Hospital Medical School, University Street, London WC1, England

(*Received* 5 *August* 1976; *accepted* 13 *January* 1977)

Abstract—Low doses of DDT (2–5 mg/kg) and phenobarbital (~2 mg/kg/day) cause an increase of the cytochrome P450 and amidopyrine demethylation activity of rat liver. The combined stimuli have an added effect. It is suggested that other inducers probably have similar additional effects and that the known increase in drug metabolism produced by feeding natural diets is due to the summation of many inducer effects caused by different substances each at low dosage.

A large number of natural substances, including flavones, indoles and terpenes [1–3] have the property of inducing synthesis of cytochrome P450 and drug metabolizing enzymes in the liver. In addition, foods contain additives, such as antioxidants, and contaminants, such as DDT and benzo(a)pyrene, which also have inducing effects [4–7].

It has been shown that maximal doses of two inducers of the same type do not have additive effects [5], so that no further induction takes place when animals are dosed with DDT as well as a maximal stimulating dose of phenobarbital. The question arises whether much smaller doses of several inducers, such as are likely to occur in normal diets and in the environment, are additive.

If induction is sufficient to alter drug metabolism, this could be either harmful or beneficial, depending on what substances the individual meets at work or as medication [7].

If there is a threshold below which these drugs have no effect, as has been suggested [4, 14] and if these sub-threshold doses do not add together or even interfere with each other [16] then as long as dietary levels of all such compounds are kept below threshold, there need be no concern about public health just because compounds in the environment show the property of inducing drug metabolizing enzymes. For instance, it is argued that the inducing activity of compounds such as the antioxidant butylated hydroxy anisole (BHA) is of no consequence because the amounts used are below the "threshold" where induction occurs [4].

This argument depends on two unrelated and often unstated assumptions. Firstly, that there exists a threshold dose below which there is no effect, and secondly that the "sub-threshold" doses of the multitude of inducing substances that we meet, do not add up.

We suggest that there is no evidence for any threshold, though there are, of course, dose levels so low that biological and experimental variation conceal the effects especially when small populations are studied. The graphs showing supposed threshold values [4, 17] are misleading since they are log–dose graphs on which zero cannot be shown and where a response which is in fact directly proportional to the dose would give just such an apparent kink in the curve.

We describe the simple additional effect of DDT and phenobarbital at doses close to those where induction first becomes measurable. We also show that diets commonly eaten by people have a substantial inducing capacity.

MATERIALS AND METHODS

Two sets of experiments were performed. In the first set, male albino rats of initial body weight 135–210 g (A. Tuck and Son, Rayleigh, Essex) were housed in raised mesh cages and given a purified diet consisting of 20% casein, 10% corn oil, 65% maize starch and sucrose, vitamins and minerals to eat *ad lib.* [15]. DDT was dissolved in the corn oil and incorporated in the purified diet. Drinking water was either distilled water containing Na phenobarbital at the indicated concentration [8], or distilled water alone. Phenobarbital was given in the drinking water for 8 days: at a water intake of approximately 100 ml/kg body wt/day this gave the rats a phenobarbital intake of 2 mg/kg/day for the 20 μg/ml concentration.

In the second set of experiments, male albino rats of initial body weight 60–80 g were given stock pellets to eat *ad lib.* (Modified Diet 41B, Oxoid Ltd). The DDT (dissolved in corn oil) was given by two intraperitoneal injections of the dose stated, 8 and 4 days before the rats were killed. Phenobarbital was given as before.

In addition, one group of rats was fed on an "Indian diet" consisting of homogenized curry dishes and rice (from a local restaurant), and another group was fed on a "British diet" consisting of hospital food. All were given distilled water to drink.

*Present address: Laboratory of Toxicology and Pharmacokinetics, Department of Clinical Pharmacology, University College Hospital Medical School.

Table 1. Effect of some diets on amidopyrine demethylation

Diet	Amidopyrene demethylation μmoles/g/hr	Amidopyrene demethylation as % of control value
Purified casein diet	21.5 ± 4.9	—
41B Stock pellets	28.7 ± 2.0*	133.4
British hospital diet	24.8 ± 5.4	115.2
Indian restaurant diet	33.4 ± 3.1†	155.0

Results are expressed as means ± ISD for 5 or more rats at each point. Significant differences from the control casein group are indicated as follows: * $P < 0.01$; † < 0.001.

The rats were killed by exsanguination under ether anaesthesia, the livers rapidly removed, cooled and weighed, and a 2 g piece homogenized in 18 ml 150 mM KCl containing 20 mM Tris MCl buffer pH 7.4 at 5° [9].

Assays. Cytochrome P450 in the liver homogenates was measured by the method previously described [9]. Demethylation of amidopyrine to give formaldehyde was measured by minor modification of the methods of Fouts and Nash [5, 9, 10]. 18 ml of the 10% liver homogenate was centrifuged at 7000 *g* for 10 min and the post-mitochondrial supernatant decanted. The incubation mixture consisted of 1 ml of the 10% post-mitochondrial supernatant in a final volume of 5 ml containing the following: $MgCl_2$ 50 μmoles, Na isocitrate 20 μmoles, KH_2PO_4 buffer (adjusted to pH 7.5 with KOH) 600 μmoles, semicarbazide (adjusted to pH 7 with NaOH) 40 μmoles, NADP3 μmoles.

The flasks were incubated for 2 min at 37°, in air, a 1 ml blank sample taken and the reaction started by addition of amidopyrine to give a final concentration of 6 mM. Duplicate samples were taken 10 min after initiating the reaction. The 1 ml samples were deproteinized with 1 ml saturated Ba $(OH)_2$, followed by 1 ml 20% $ZnSO_4$, and centrifuged at 1000 rpm for 10 min. 1 ml of the clear supernatant was incubated at 60° for 30 min with 0.5 ml water and 1 ml double strength Nash reagent [9, 10]. After cooling on ice and extraction into 1.5 ml amyl alcohol the optical density was measured at 412 nm.

RESULTS

Table 1 shows that a substantial degree of enzyme induction is produced by ordinary dietary components. The "British diet" had the least inducing effect and the "Indian diet" the most, while stock pellets had an intermediate effect.

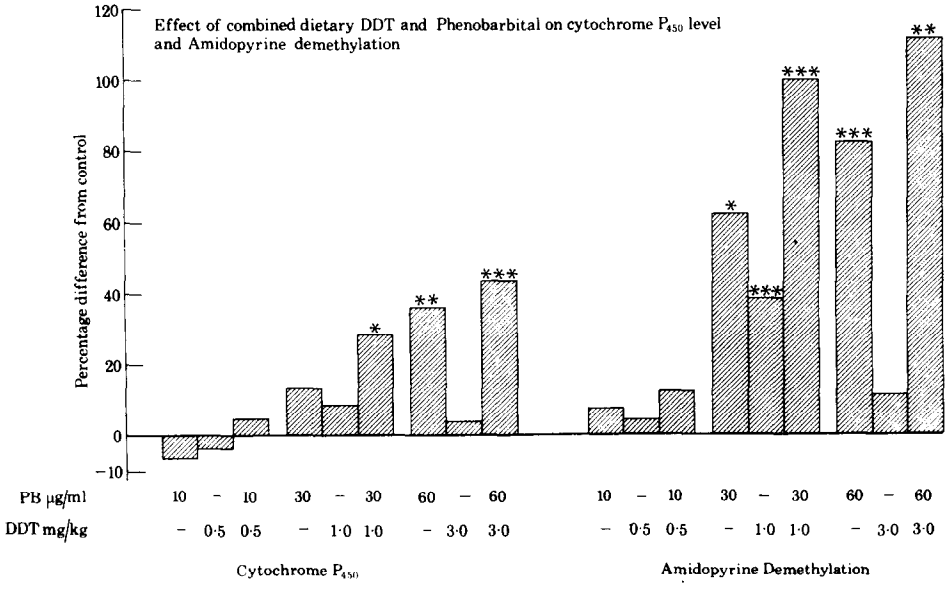

Fig. 1. Each bar represents the mean for four or more rats of body weight 170–260 g. Results are expressed as percentage difference from the control group fed on casein diet alone. Significant differences of the dosed groups from the control are indicated: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. The combination of DDT and phenobarbital was significantly different from the single doses at the following doses: Amidopyrine demethylation DDT 1.0 mg/kg $P < 0.01$; PB 30 μg/ml $P < 0.05$; DDT 3.0 mg/kg $P < 0.01$; PB 60 μg/ml $P < 0.05$; Cytochrome P450 level DDT 3.0 mg/kg $P < 0.001$. The controls, fed on purified casein diet and distilled water, had a mean cytochrome P450 level of 36.7 ± 5.4 nmols/g (mean for 15 rats) and a mean amidopyrine demethylation rate of 21.9 ± 5.2 μmoles/g/hr (mean for 14 rats).

Table 2. Effect of phenobarbital and injected DDT on cytochrome P450 level and amidopyrine demethylation

Treatment	Amidopyrene demethylation μmoles formaldehyde/g/hr	Cytochrome P450 nmol/g liver
Stock pellets (41B) controls	17.4 ± 1.2	28.9 ± 1.1
20 μg/ml PB	28.7 ± 3.6	41.3 ± 4.9
40 μg/ml PB	34.0 ± 5.6	59.8 ± 12.1
80 μg/ml PB	45.8 ± 3.9	83.9 ± 5.5
5 mg/kg DDT	28.1 ± 3.1	38.9 ± 4.3
DDT 5 + PB 20	30.3 ± 7.0	44.3 ± 1.7
10 mg/kg DDT	32.2 ± 5.0	51.7 ± 4.1
DDT 10 + PB 20	34.7 ± 3.2	59.7 ± 4.8
20 mg/kg DDT	36.4 ± 2.9	61.1 ± 2.1
DDT 20 + PB 20	41.9 ± 4.5	71.2 ± 5.6
50 mg/kg DDT	45.9 ± 4.6	69.6 ± 11.4

Results are expressed as means ± ISD for 4 or more rats at each point.
The rats weighed 120–160 g and were fed stock pellets.

Figure 1 shows that when DDT is given in the diet and phenobarbital in the drinking water, the inducing effect of the two given together is greater than that of either substance given alone, at all doses used.

At the very low doses (10 μg/ml phenobarbital and 0.5 mg/kg DDT), there is no significant difference between any of the groups and the control, yet it is shown that the effects of DDT and phenobarbital may be additive even at this level.

At a slightly higher dose (30 μg/ml phenobarbital and 1.0 mg/kg DDT) there is no significant difference between either substance given alone and the control for cytochrome P450, but when the two compounds are given together, the result is a significant alteration in the cytochrome P450 content of the liver.

Table 2 shows that when DDT or phenobarbital is given in small to medium doses there is a near linear response in cytochrome P450 content per gram of liver, and a similar increase in the amidopyrine demethylation in the post-mitochondrial supernatant fraction of the liver.

Table 2 and Fig. 2 also show that addition of 20 μg/ml of phenobarbital to the drinking water leads to an increase in cytochrome P450 level and in amidopyrine demethylation approximately equal to that produced by two injections of 5 mg/kg DDT.

The addition of phenobarbital at a constant dose of 20 μg/ml to the DDT treatment leads to an increase in cytochrome P450 and in amidopyrine demethylation, at each level of DDT treatment, which is close to a simple addition of effects, without alteration in the slope of the dose-response curve. The only exception is that the lower end of the dose–response curve (0–5 mg/kg DDT or 0–20 μg/ml phenobarbital) seems to have a steeper slope.

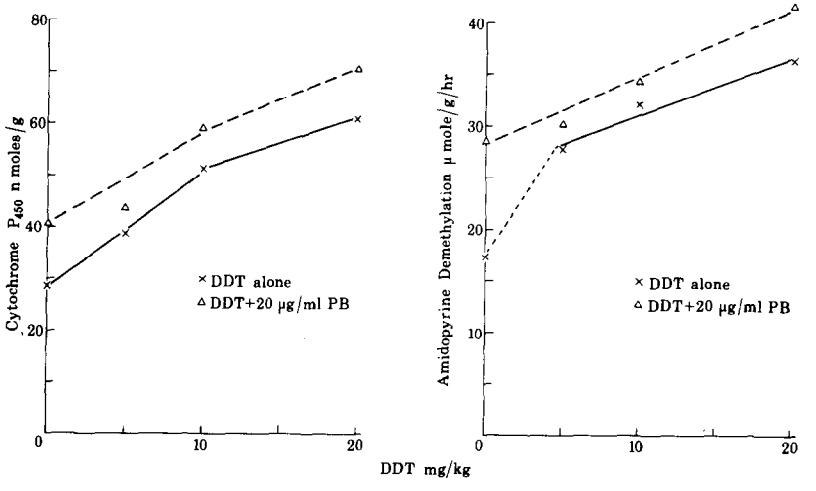

Fig. 2. Dose response relation for drug metabolizing enzymes in the liver on simultaneous treatment with DDT and phenobarbital. DDT was given as two i.p. injections and phenobarbital was given in the drinking water at a dose of 20 μg/ml (approximately 2 mg/kg body weight/day). The lines in Fig. 2 were fitted to the points by eye and the regression lines were calculated separately. The slopes of the dose–response lines did not differ significantly (demethylation = a + 0.73 DDT dose and P450 = b + 1.58 DDT dose), but the positions of the lines differed in a highly significant way: $P < 0.001$ in each case. a = 17 and 29 and b = 29 and 41 without and with phenobarbital respectively.

DISCUSSION

A large number of inducers are present in foods, at low doses, not only as contaminants and additives, but as an integral part of the diet [4, 11]. It seems likely that there is no threshold for their effects, and that their actions will add together to produce a characteristic induction effect for any diet. The lipid and protein content of the diet may also alter responses to inducers, especially in societies where low protein or low fat diets are eaten [7, 11].

Since the response to potentially toxic substances in the environment, or taken as medication, frequently depends on the level of activity of the oxidative enzymes of drug metabolism, centred on cytochrome P450 [12, 13], changes in P450 may lead to changes in public health. The present findings suggest that it is not enough to keep individual components of the diet, such as DDT or BHA, at sub-inducing levels, since these are simply the levels where the single effect is lost in the variation between animals, or people. Whole diets must be monitored to see whether changes in food technology are likely to lead to a shift in the inducing action of diets, with a consequent change in drug metabolizing activity over the whole population. The problem is made more complex by our lack of knowledge of the public health consequences of changes in drug metabolizing activity, though we can probably be assured that in spite of hepatoma formation in mice fed inducers such as phenobarbital, increased induction does not lead to increased tumour formation in man [13].

Acknowledgement—This work was supported by the Cancer Research Campaign and the Halley Stewart Foundation.

REFERENCES

1. D. L. Cinti, M. A. Lemelin and J. Christian, *Biochem. Pharmac.* **25,** 100 (1966).
2. L. W. Wattenberg, M. A. Page and J. L. Leong, *Cancer Res.* **28,** 934 (1968).
3. L. W. Wattenberg, *Cancer Res.* **35,** 3326 (1975).
4. D. Gilbert and L. Golberg, *Fd. Cosmet. Toxic.* **3,** 417 (1965).
5. L. G. Hart and J. R. Fouts, *Biochem. Pharmac.* **14,** 263 (1965).
6. A. E. M. McLean and E. K. McLean, *Biochem. J.* **100,** 564 (1966).
7. A. E. M. McLean, *Fedn Proc.* (in press) (1977).
8. W. J. Marshall and A. E. M. McLean, *Biochem. Pharmac.* **18,** 153 (1969).
9. A. E. M. McLean and P. Day, *Biochem. Pharmac.* **23,** 1173 (1974).
10. T. Nash, *Biochem. J.* **55,** 416 (1953).
11. A. E. M. McLean, *Proc. Nutr. Soc.* **33,** 197 (1974).
12. A. E. M. McLean and P. Day, *Biochem. Pharmac.* **24,** 37 (1975).
13. Editorial, *Lancet*, **II,** 629 (1974).
14. J. M. Barnes, *Br. Med. Bull.* **31,** 196 (1975).
15. A. E. M. McLean and P. A. Day, *Biochem. Pharmac.* **24,** 37 (1975).
16. J. R. Fouts, *Toxic. Appl. Pharmac.* **17,** 804 (1970).
17. D. M. Valerino, E. S. Vesell, A. O. Johnson and K. C. Aurori, *Drug Metab. Disp.* **I,** 669 (1973).