Discussion. These data suggest that nicotine administration augments restraint-cold-induced gastric ulcers. Similar results have been reported using other ulcerogenic techniques[7]. In addition, l-ascorbic acid was shown to increase restraint-cold-induced ulcers relative to control animals, confirming earlier results from this laboratory[10]. Especially interesting is the relationship between l-ascorbic acid intake and glandular ulcer severity. Perhaps l-ascorbic acid has more than a sensitizing effect on the stomach. Establishing a dose-response relation-ship between ascorbic acid administration and gastric damage is clearly indicated. Preliminary observations in this laboratory indicate that increasing doses of l-ascorbic acid are associated with increasing degrees of gastric damage in both rats and guinea-pigs. Finally, it appears that nicotine and l-ascorbic acid do not act synergistically to potentiate stress-induced gastric ulceration. Rats treated with the combination of both substances exhibited a level of gastric damage similar to that of control animals. It appears that l-ascorbic acid exerts a protective effect against nicotine-induced gastic ulcer. The combination of nicotine and l-ascorbic acid resulted in less frequent and less severe gastric damage than nicotine administered alone. Following Robert's[8] data concerning nicotine and intestinal damage, it is evident that several doses of nicotine and several doses of l-ascorbic acid must be explored in the context of their effects upon restraint-induced gastric ulcer.

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2 Reprint request to: G.B.G., Department of Psychology, University of Winnipeg, Winnipeg (Manitoba, Canada R3B 2E9).
3 I. Ehrenfeld and M. Sturdevant, Am. J. med. Sci. *201*, 81 (1941).
4 F. Steigmann, R. Dolehide and L. Kaminski, Am. J. Gastroent. *22*, 399 (1954).
5 P. Cooper and J. Knight, New Engl. J. Med. *255*, 17 (1956).
6 J. Thompson, Am. J. Dig. Dis. *15*, 209 (1970).
7 W. Paré, Biochem. Med. *14*, 51 (1975).
8 A. Robert, Proc. Soc. exp. Biol. Med. *139*, 319 (1972).
9 A. Robert, P. Stowe and J. Nezamis, Nature *233*, 497 (1971).
10 G. Glavin, W. Paré and G. Vincent, J. Nutr. *108*, 1969 (1978).
11 G.L. Lo and F. Konishi, Am. J. clin. Nutr. *31*, 1397 (1978).
12 G. Vincent, G. Glavin, J. Rutkowski and W. Paré, Gastroent. clin. Biol. *1*, 539 (1977).
13 G. Glavin, G. Vincent and W. Paré, unpublished results.

Effect of topically applied phenobarbital on O-dealkylase activity in mouse skin

A. Pannatier, B. Testa[1] and J.-C. Etter

School of Pharmacy, University of Lausanne, CH-1005 Lausanne (Switzerland), 6 August 1981

Summary. Topical application of phenobarbital to mice produces an increase in cutaneous microsomal proteins and apparently also of cytochrome P450, but results in a kinetically noncompetitive inhibition of p-nitrophenethole O-dealkylase activity in preparations of 10,000 × g supernatant from skin.

The significance of the skin as a drug-metabolizing organ is gaining increasing recognition and has been recently reviewed[2]. We have previously examined the ability of skin 10,000 × g supernatant preparations from untreated mice to O-dealkylate p-nitroanisole and p-nitrophenetole[3]. Detectable and quantifiable O-dealkylase activity was shown to exist in these preparations, the reaction being NADPH-dependent and mediated by cytochrome P450. In the present study, we examine the effect of topically applied phenobarbital, chosen as an inducer of cytochrome P450 (as opposed to cytochrome P448). It is known that classical inducers of hepatic enzymes do not necessarily have the same effects in extrahepatic tissues[4,5], and drug-drug or drug-excipient interactions are conceivable at the cutaneous monooxygenase level following topical medication.

Methods. White Swiss E mice aged 8–10 weeks were used. Batches of 10 animals (5 males and 5 females) were carefully shaved on the back and abdomen, and after 24 h an acetone solution of phenobarbital was sprayed on a dorsal and on an abdominal area, during 4 days at 24-h intervals. The animals were killed by cervical dislocation 24 h after the last treatment. The preparation of skin homogenates and of fractions from it (10,000 × g supernatant and microsomes), the incubation conditions and the GC analytical method were as previously described[3]. The microsomal protein concentration was determined according to Lowry et al.[6]. The K_m- and V_{max}-values were calculated by the method of Lineweaver and Burk[7]. The SD of these values were calculated by the method of error propagation[3,8]. For the spectral determination of cytochrome P450, microsomes isolated at 106,000 × g were resuspended in 6 ml of a 0.05 M PO_4 buffer pH 7.6 containing EDTA 10^{-3} M. A Hewlett-Packard 8450A spectrophotometer was used.

Results. Phenobarbital causes a clear and statistically significant increase in microsomal protein content (table). Under the conditions of this study however, the increase is not dose-dependent, the 2 extreme doses of PB causing practically identical increases; 33 and 32%, respectively.

The determination of cytochrome P450 in mouse cutaneous microsomes is rendered difficult by large quantitites of

The effect of toppically applied phenobarbital on protein content of mouse skin microsomes and p-nitrophenetole O-dealkylation by cutaneous 10,000 × g supernatant preparations

Animals	Proteins[c] (mg/g tissue)	Number[e]	K_m (μM)	V_{max} (nmole/mg protein/min)
Untreated	1.45 ± 0.03	6	1.21 ± 0.30[f]	0.239 ± 0.046[f]
Acetone[a]	ND[d]	8	1.20 ± 0.21	0.231 ± 0.036
Phenobarbital 0.75[b])	1.93 ± 0.001	12	1.26 ± 0.10	0.194 ± 0.013[g]
Phenobarbital 7.5[b])	ND[d]	6	1.19 ± 0.15	0.172 ± 0.020[g]
Phenobarbital 75[b]	1.91 ± 0.08	6	1.22 ± 0.13	0.173 ± 0.015[g]

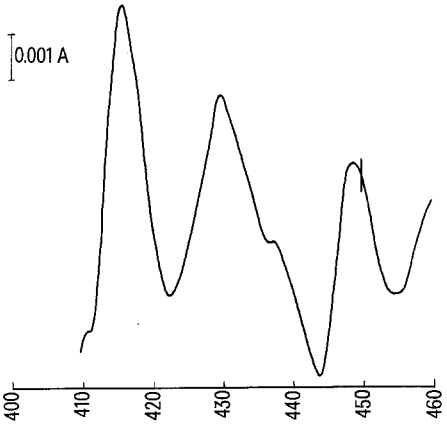
[a]Animals treated with acetone only; [b]animals receiving 0.75, 7.5, or 75 mg phenobarbital/kg/day during 4 days, respectively; [c]average values (± SD) for 3 batches (3 animals per batch); [d]not determined; [e]No. of assays (2 assays for each concentration of substrate and phenobarbital); [f]values from Pannatier et al.[3]. [g]$p < 0.01$ from control.

what appears to be cytochrome oxidase and cytochrome P420. However, spectra accumulation reveals peak at 450 nm in cutaneous microsomes from untreated mice; this peak markedly increases in the cutaneous microsomes from mice treated with 75 mg phenobarbital/kg/day. This increase is obvious in the computed difference between the 2 spectra (fig.), the maximum difference being located at 449 nm. Such a spectral effect probably corresponds to an increase in cytochrome P450 levels. However, it may not be due solely to cytochrome P450 since other effects are also apparent, particularly in the 430 nm region, and could perhaps secondarily affect the 450 nm region. The effect in the 430 nm region may indicate an influence of phenobarbital on cytochrome oxidase and other constituents and/or contaminants of the microsomal fraction. The figure thus indicates that topically applied phenobarbital apparently acts as an inducer of cytochrome P450, but a quantitative assessment of this increase cannot be made at this stage.

Due to the low proportion of substrate consumed (less than 1%), the Lineweaver-Burk method[7] is ideally suited in our case for the calculation of K_m and V_{max}-values[9,10]. The table shows that pretreatment with acetone alone does not influence O-dealkylase activity. In contrast, and this is an unexpected finding, phenobarbital acts as an apparent inhibitor of cutaneous O-dealkylase activity. The inhibition is of a noncompetitive type (identical K_m, different V_{max}), meaning that it is independent of substrate concentration. The dose dependence of this inhibition is difficult to assess; while the smallest dose causes the smallest inhibition, the 2 larger doses have practically the same effect.

Discussion. The present study shows that topically applied phenobarbital displays a dual influence on cutaneous monooxygenase activity in that it increases microsomal protein concentrations and apparently induces cytochrome P450, but inhibits p-nitrophenetole O-dealkylase activity in a noncompetitive and partly dose-dependent manner. The persistence of phenobarbital in the skin after topical applications may perhaps explain this inhibitory effect, the mechanism of which however is unknown.

It is therefore suggested that the use of inducers when investigating cutaneous monooxygenase activity may lead to situations more complex than those encountered when studying internal, well irrigated organs. Furthermore, the ability of cutaneous monooxygenases to be simultaneously induced and inhibited by the same compound may not be without consequences regarding drug interactions at the dermatological level.



Difference spectra (CO/dithionite-reduced minus CO)[11] were obtained with a HP 8450A spectrophotometer (50 scans minus 50 scans) from mouse skin microsomes. These spectra were A) untreated mice (baseline substracted), and B) mice treated with 75 mg phenobarbital/kg/day during 4 days (baseline subtracted). The figure shows the difference spectrum obtained by subtracting spectrum A from spectrum B.

1 To whom reprint requests should be addressed.
2 A. Pannatier, P. Jenner, B. Testa and J.C. Etter, Drug Metab. Rev. *8*, 319 (1978).
3 A. Pannatier, B. Testa and J.C. Etter, Xenobiotica *11*, 345 (1981).
4 G. Feuer, J.C. Sosa-Lucero, G. Lumg and G. Moddel, Toxic. appl. Pharmac. *19*, 579 (1971).
5 B.G. Lake, R. Hopkins, J. Chakraborty, J.W. Bridges and D.V. Parke, Drug Metab. Disposit. *1*, 342 (1973).
6 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
7 H. Lineweaver and D. Burk, J. Am. chem. Soc. *56*, 658 (1934).
8 G.D. Chase and J.L. Rabinowitz, Principles of radioisotope methodology, chapter 4. Burgess Publ. Co., Minneapolis 1965.
9 H.J. Lee and I.B. Wilson, Biochim. biophys. Acta *242*, 519 (1971).
10 N. Glick, A.D. Landman and B.D. Roufogalis, Trends Biochem. Sci. *4*, N82 (1979).
11 R.J. Pohl, R.M. Philpot and J.R. Fouts, Drug Metab. Disposit. *4*, 442 (1976).

The effect of activated dimethicone, other antacid constituents, and kaolin on the absorption of propranolol

J.C. McElnay, P.F. D'Arcy and J.K. Leonard[1]

Department of Pharmacy, The Queen's University of Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL (Northern Ireland), 3 September 1981

Summary. A study was made of the effect of 6 commonly used gastrointestinal preparations on the absorption of propranolol using an in vitro experimental model. The constituents examined were activated dimethicone, aluminium hydroxide gel, bismuth carbonate, kaolin, magnesium carbonate, and magnesium trisilicate. A slight decreased propranolol absorption was given by kaolin (−13.0%), the other components showed smaller effects ranging from −6.8% to +6.6%. None of the results were statistically significantly different from control absorption values.

Administration of antacids may result in a slowed or incomplete absorption of many drugs and this is probably a common but often unrecognized cause of therapeutic failure[2]. The mechanisms by which absorption interactions may occur with antacids are via chelation or complexation giving rise to nonabsorbable complexes, by adsorption, due to antacid-induced changes in gastric emptying rate and gastrointestinal motility, or via changes in gastrointestinal pH. Constituents of antacid preparations, for example, have been shown to effect the absorption of digoxin[3–6],