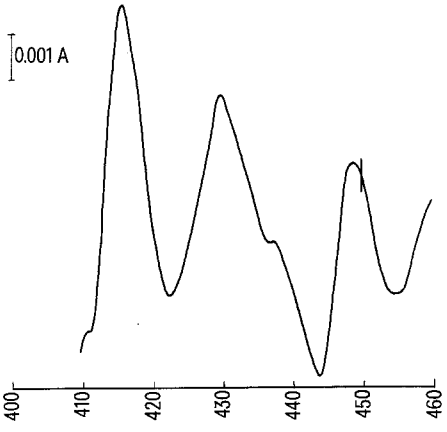
what appears to be cytochrome oxidase and cytochrome P420. However, spectra accumulation reveals peak at 450 nm in cutaneous microsomes from untreated mice; this peak markedly increases in the cutaneous microsomes from mice treated with 75 mg phenobarbital/kg/day. This increase is obvious in the computed 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.

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

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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],

phenytoin[7,8], and chloroquine[9]. It has also been reported that the administration of aluminium hydroxide gel decreased the absorption of concomitant propranolol in 4 of 5 healthy male volunteers[10]. Such an interaction is of particular relevance as patients with chronic renal failure and hypertension will often receive beta-blocker therapy and also aluminium antacid preparations (phosphate binders).

The object of the present study was to investigate the effects of activated dimethicone (an antiflatulent agent used in certain proprietary antacid preparations), other commonly used antacid agents including aluminium hydroxide gel, and the adsorbent antidiarrhoeal agent kaolin on the transfer of propranolol across intestinal membrane.

Materials and methods. An in vitro model of drug interaction in the gut was used. This model, which utilizes everted rat intestine, has already been shown to give results reflecting the clinical situation[11], for example, results obtained with the tetracycline/metal ion interaction mirrored the clinical data of Neuvonen et al.[12]. A schematic illustration of the model has been published previously[8]. The technique involved the estimation of propranolol absorbed across 2 everted intestinal segments (7.5 cm). Drug absorbed across the intestine was collected by the infusion of buffer through the segments. During each experimental run, 2 consecutive segments from the same rat were used as control and test. The segments were bathed in a phosphate buffered physiological solution at pH 7.4 (based on that of Chowhan and Amaro[13]); the control chamber contained propranolol hydrochloride (160 mg in 120 ml) while the test chamber contained the same amount of propranolol plus either antacid or antidiarrhoeal constituent (table). Infused samples (10 ml) of buffer of the same composition used for the bathing solution were collected each 10 min for 100 min; the samples were assayed for propranolol content spectrophotometrically (289 mm). Each constituent was tested in triplicate and the average cumulative absorption values for the 100-min time period of the test runs were compared with those for the controls. The sources of all materials used are given in the table.

Changes in propranolol absorption in the presence of antacid constituents and kaolin. The in vitro absorption of propranolol (160 mg of hydrochloride salt) was compared while alone and while in combination with the given quantities of constituents. The results are expressed as percentage changed absorption of propranolol in the presence of the gastrointestinal medicament with respect to control (propranolol alone) values. None of the test results were significantly different from the control experiments

Medicament tested	Amount of constituent used	Percent change in total propranolol absorption (± SE)
35% aqueous emulsion of activated dimethicone (Galen Ltd, N. Ireland)	1 ml	−5.6±0.9
Aluminium hydroxide gel (Wyeth Laboratories, England)	10 ml	+6.6±3.3
Bismuth carbonate (Evans Medical, England)	500 mg	−6.8±2.1
Kaolin (light) (B.D.H., England)	2 g	−13.0±3.4
Magnesium carbonate (light) (Evans Medical, England)	500 mg	−4.1±0.4
Magnesium trisilicate (B.D.H., England)	500 mg	−1.2±0.2

Results. A typical absorption profile is shown in the figure. Experimental results are summarized in the table. Kaolin gave rise to the largest change in propranolol transfer (a 13.0% decreased absorption was recorded). Statistical testing (unpaired t-test), however, showed that neither this result nor any of the changes found with other medicaments reached a significant level ($p > 0.05$ in all cases). Activated dimethicone, bismuth carbonate, magnesium carbonate, and magnesium trisilicate all gave slight decreases in propranolol absorption, ranging from 1.2% to 6.8% (table). There was also little change in propranolol transfer when in the presence of aluminium hydroxide gel (increased absorption by 6.6%).

Comment. The results indicate that the dimethicone, which as well as being used as an antiflatulent agent is also utilized as a 'crisping' agent in certain vegetable cooking oils, is unlikely to be involved with adsorption interactions with propranolol. Aluminium hydroxide gel, bismuth carbonate, light magnesium carbonate, and magnesium trisilicate were also free from direct interaction with propranolol, i.e., they were not involved in a complexation or adsorption interaction with the beta-blocker.

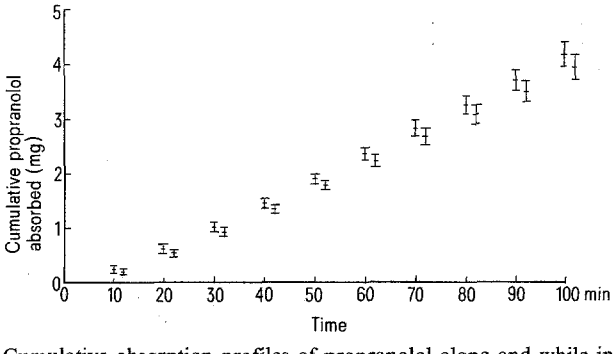
Kaolin resulted in a 13.0% decrease in propranolol transfer. Although this changed absorption did not reach statistical significance, kaolin in large doses may have the potential to interfere with propranolol bioavailability.

The present data indicate that the in vivo interaction between propranolol and aluminium hydroxide reported by Dobbs et al[10]. was not due to adsorption or complexation but was likely due to a decreased gastric emptying rate and decreased gastrointestinal motility caused by the aluminium ions of the antacid. Other antacids, which do not decrease gastrointestinal motility, would therefore not interact in this way with propranolol.

A further possibility is that an increased gastrointestinal pH, due to the antacid in the in vivo study[10], may have influenced drug release from the dosage form, drug dissolution, and drug ionization giving rise to decreased bioavailability. If this interaction mechanism played a role then other antacid agents would be expected to give rise to similar effects.

Further study is therefore required to fully resolve the interaction situation between propranolol and gastrointestinal medicaments, in particular studies with these agents in 1. in vitro drug release and dissolution studies and 2. in vivo multidose studies with propranolol at steady state (as medications may change first pass metabolism of propranolol).

In conclusion, the present data indicate clearly that propranolol is not susceptible to major adsorption or complex-



Cumulative absorption profiles of propranolol alone and while in combination with activated dimethicone across everted rat intestine. Each point represents the mean (± SE) of 3 determinations in segments from 3 individual rats. a) propranolol hydrochloride alone (control); b) propranolol hydrochloride + activated dimethicone (test). Test data displaced to right for clarity.

ation interactions with the range of antacid constituents listed. Although these data clearly elucidate the involvement of these mechasims in the reported interaction, further work is required to explore the other possible interaction mechanisms outlined.

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Effect of acetylcholine acetyl-hydrolase (E.C. 3.1.1.7) inhibition on the accumulation of pp' DDT in various brain regions of rats[1,2]

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Summary. The concentration of pp' DDT given intraperitoneally in rats was determined in different brain regions. Maximum accumulation of pp' DDT was found in the corpus striatum, followed by cerebellum and cerebral cortex in that order; following pretreatment with paraoxon the concentrations of pp' DDT were increased in all brain regions studied.

The widespread use of DDT (dichlorodiphenyl trichloroethane) has presented a potential health hazard to mammalian organisms including man. The commonly used technical DDT consists of 2 isomers, op' DDT and pp' DDT[3]. The symptoms of acute DDT poisoning - hyperexcitability tremors or convulsions - are caused by the effect of pp' DDT on the central nervous system[4], the severity of the symptoms being directly related to the concentration of the compound in the brain[5]. Tremors which appear early during the course of acute poisoning are believed to be caused by the accumulation of pp' DDT in the cerebellum[6,7], which is important in the regulation of motor function. Corpus striatum is another area of the brain which has a high concentration of certain neurochemical substances[8-10] and is also important in the regulation of motor activity. Further, prior administration of certain anticholinesterases has been reported to modify or alter the pharmacological effects or concentration of certain drugs and chemicals in the brain[11,12]. The present report is concerned with determining the concentration of pp' DDT in the corpus striatum and other brain regions of rats after treatment with pp' DDT alone or with anticholinesterases.

Methods. Adult male albino rats, 100± 10 g, were used. pp' DDT was dissolved in peanut oil. The animals were fasted for 18 h before use, since preliminary experiments indicated that more uniform results were obtained in this manner. The animals were divided into 4 groups. The animals of group 1 served as controls and received the oily vehicle. The animals of group 2 received pp' DDT (10 mg/kg, i.p.). The animals of group 3 received paraoxon (0.1 mg/kg, s.c.) while those of the group 4 were treated with paraoxon (0.1 mg/kg, s.c.) followed after 10 min by pp' DDT (10 mg/kg, i.p.).

The animals were decapitated 1 h after treatment with pp' DDT. The various brain regions were quickly separated. Corpus striatum was dissected according to the method of Glowinski and Iverson[13]. pp' DDT was extracted with hexane and assayed on GLC according to the procedure described by Maunder et al.[14]. The level of acetylcholinesterase in various brain regions was determined spectrophotometrically by the method of Ellman et al.[15]. Rat brain has been reported to contain almost exclusively the acetylcholinesterase[16,17]. It was further identified by using inhibitors and the substrates acetylthiocholine and butyrylcholine (which is not hydrolyzed by the acetylcholinesterase of rat brain[15]) as described elsewhere[16]. The acetylcholinesterase activity of rat brain was similar to that reported by others[18], and the values were in agreement with the values reported by us elsewhere[19,20].

Results. The level of acetylcholinesterase in different brain regions of paraoxon- and pp' DDT-treated animals is given in the table. Paraoxon induced a significant inhibition of acetylcholinesterase activity in all the brain regions. The concentration of pp' DDT was higher in corpus striatum than the cerebellum and cortex; pretreatment with paraoxon increased the concentration of pp' DDT in all the brain regions (table).

Discussion. The present study showed that brain concentrations of pp' DDT are increased in rats previously exposed to the organophosphorous compound paraoxon. Since paraoxon, a matabolite of parathion[21], is a potent inhibitor of acetylcholinesterase activity (table), the increase of brain concentration of pp' DDT is tentatively explained by cholinesterase inhibition, although the exact mechanism of this phenomenon is not clear. It was previously reported that pretreatment of animals with physostigmine caused greater penetration of certain chemicals and drugs[11,12] (e.g. barbital) through the blood-brain barrier resulting in their enhanced pharmacological effects or accumulation in the brain. Thus the level of acetylcholinesterase activity may influence in some way the permeability of the blood-brain barrier or the accumulation of drugs or chemicals in the brain.

Our results further indicate that the accumulation of pp' DDT in rats was significantly greater in corpus striatum than cortex or cerebellum. It was previously reported that the concentration of pp' DDT in the cerebellum was directly related to the production of tremors, which is an early manifestation of DDT toxicity[6,7]. Since the corpus striatum is an area of the brain which is important in the regulation