tives. Apparently the *m/e* 178 was not the parent ion of Met-1 and C-2, but of the fragment $(M-H_2O)^+$ and $(M-O)^+$. These interpretations remain tentative, however, until the metabolites are synthesized and the spectra are analyzed.

2-Nitrosonaphthalene [15] and niridazole [16] are reduced by NADPH in the presence of NADPH–cyt. *c* reductase to the corresponding hydroxylamines. The hydroxylamine of niridazole can be trimethylsilylated by *bis*(trimethylsilyl)acetamide (BSA) and identified by GLC–MS [16]; however, attempts to trimethylsilylate Met-1 with BSA were unsuccessful.

5-Nitrofurans are reduced by several enzyme systems. The cytosol contains xanthine oxidase, aldehyde oxidase, DT diaphorase and lipoyl dehydrogenase, which are capable of reducing 5-nitrofurans [3]. Mouse liver cytosol enzymes, which reduce MNFT or Met-1 to the corresponding amine, remain to be characterized.

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Influence of esterase inhibitors on platelet aggregation and release induced by phorbol myristate acetate*

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A tumor-promoting principle derived from croton oil, phorbol myristate acetate (PMA), is a powerful platelet-aggregating agent [1]. Minute amounts of PMA labilize platelet granules, convert the organelles to swollen vacuoles, stimulate the release of serotonin and adenine nucleotides, induce clumping of discoid platelets, and cause irreversible aggregation [2, 3]. Platelets washed and resuspended in Hank's balanced salt solution (AWP) are more sensitive to PMA and less variable in response than platelets in citrate-platelet-rich plasma (C-PRP) [1, 4] (Fig. 1). This observation suggested that some factor or factors present in plasma might interfere with the action of PMA on platelets in C-PRP. Since PMA (12-0-tetradecanyol phorbol-13-acetate) is a fatty acid ester, it seemed possible that esterases might modify or inhibit the agent. Acetyl esterases and pseudocholinesterases are known to be present in variable amounts in human plasma [5], and their activity can be blocked by esterase inhibitors [6]. The present investigation was instituted to determine if inhibitors of plasma esterase activity could modify the influence of PMA on platelets. C-PRP and AWP were prepared from the same samples of normal blood in each experiment. The methods used to obtain blood, mix the samples immediately with 3·8% trisodium citrate in a ratio of 9 parts blood to 1 part anticoagulant, separate C-PRP by centrifugation at room temperature [7], prepare AWP after sedimentation on albumin by a modification [8] of the technique of Walsh [9], and study the response of C-PRP and AWP to aggregating agents by recording nephelometry have been described in recent pub-

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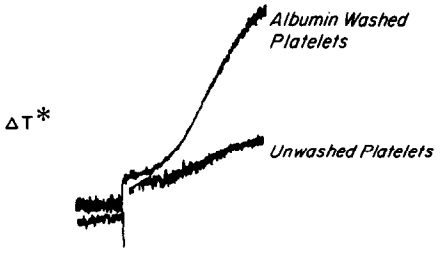
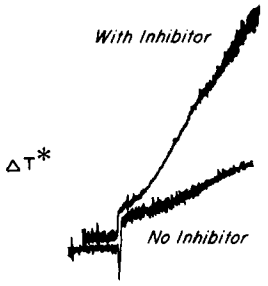
lications [10]. PMA was dissolved in dimethylsulfoxide (DMSO) at 1 mg/ml and diluted to desired concentrations in acetic acid–sodium acetate buffer, pH 6·5. The agent was evaluated by ultraviolet, infrared and mass spectral analysis and by high pressure liquid chromatography and found to be authentic and free of contaminants and degradation products [11]. The two esterase inhibitors used in this study, eserine and paraoxon, were added to C-PRP or AWP (platelet counts adjusted to 300–500 × 10^3/mm^3) on the platelet aggregometer 30 sec before the addition of PMA. In some experiments, the platelets were labeled with ^{14}C-serotonin by a modification [4] of the method introduced by Jerushalmy and Zucker[12], and the release reaction stimulated by PMA was followed by determining the percentage of isotopically labeled 5-hydroxytryptamine released into the supernatant at regular intervals.



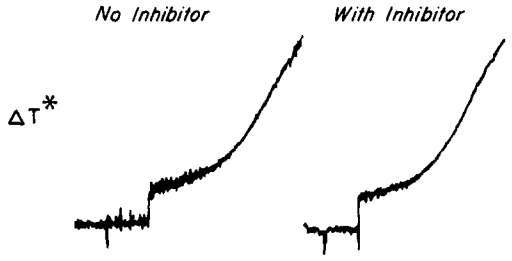
*ΔT, change in light transmission.

Fig. 1 Response of washed and unwashed platelets to phorbol myristate acetate (0·16 μM).



*ΔT, change in light transmission.

Fig. 2. Effect of paraoxon (5 × 10^{-5} M) on the response of platelets to 0·16 μM phorbol myristate acetate.



*ΔT, change in light transmission.

Fig. 3. Effect of paraoxon (5 × 10^{-5} M) on the response of albumin-washed platelets to 0·16 μM phorbol myristate acetate.

PMA-induced aggregation of C-PRP was enhanced markedly in the presence of paraoxon at a concentration of 5·0 × 10^{-5} M (Fig. 2). The esterase inhibitor did not influence aggregation in AWP (Fig. 3). Eserine added at a final concentration of 5·0 × 10^{-5} M modified PMA-induced aggregation in a manner identical to paraoxon. Effects of esterase inhibitors on aggregation in C-PRP were evident only when the concentration of PMA was 0·16 μM or less, because larger amounts of PMA produced maximal rates of platelet clumping [2, 3].

The influence of eserine and paraoxon was also evident when the release reaction stimulated by PMA was measured (Table 1). Three min after addition of 0·16 μM PMA to C-PRP, about 20 percent of the ^{14}C-serotonin was released. In the presence of paraoxon or eserine, the amount of ^{14}C-serotonin released by platelets 3 min after addition of PMA increased to 33–35 per cent. A 75–100 per cent increase in the amount of ^{14}C-serotonin released from platelets by PMA in the presence of an esterase inhibitor has been observed in 14 separate experiments. Paraoxon and eserine also increased the amount of ^{14}C-serotonin secreted by AWP 3 min after exposure to PMA, but the differences were not statistically significant. The esterase inhibitors alone did not cause aggregation or release in samples of C-PRP or AWP.

The results of this study indicate that paraoxon and eserine which block plasma esterases can enhance platelet aggregation and release induced by PMA in C-PRP but have little effect on the action of PMA in AWP. Thus, the response of C-PRP from different donors to PMA can be explained, at least in part, by the known individual variations in levels of plasma esterases [5]. PMA itself appears to be a stable compound, and spectral analysis of samples maintained in the DMSO carrier for over a year failed to demonstrate contamination or degradation [11]. As a result, the variability from donor to donor does not appear related to degradation of PMA.

The possibility that the activity of PMA can be influenced by endogenous factors appears to have been given little con-

Table 1. Effect of inhibitors on the release of ^{14}C-5HT after addition of PMA to C-PRP*

	Pellet	Supernatant	% Release
Control	14·4	0·42	2·8
PMA	13·0	3·2	19·6
Paraoxon (5 × 10^{-5} M) +PMA	10·0	5·1	33·7
Paraoxon	14·2	0·58	3·9
Eserine (5 × 10^{-5} M)	10·0	5·4	35·0
Eserine	14·6	0·52	3·4

* Effect of inhibitors on the release of ^{14}C-5HT (^{14}C-serotonin) from platelets in stirred C-PRP 3 min after the addition of 0·16 μM phorbol myristate acetate.

sideration in the literature [13]. As a result, the findings obtained in this investigation may be applicable to the study of PMA in other cell systems. According to Augustinsson [5], each esterase type exists in multiple forms and each animal species has its own typical spectrum of esterases in plasma and tissues [14]. In the presence of marked variations in location, form and activity, one might expect significant differences in the effects of drugs which stimulate or inhibit these enzymes in different species. If the pharmacological action of a drug is dependent on ester groups, then the presence of esterases in all affected cells and tissues would have to be considered in evaluating the influence of the agent. PMA has been used extensively as a co-carcinogen in animal studies [13]. It would be of interest to determine if endogenous esterases or the concomitant use of esterase inhibitors would influence the tumor-promoting action of PMA.

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Drug and fatty acid hydroxylation by solubilized human liver microsomal cytochrome P-450—Phospholipid requirement*

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The enzyme system in rabbit liver microsomes which catalyzes the hydroxylation of a variety of fatty acids, hydrocarbons, drugs and other foreign compounds was solubilized by Lu and Coon [1, 2], and resolved into three components: cytochrome P-450, NADPH–cytochrome P-450 reductase, and a heat-stable substance called Factor B [3]. The heat-stable component was identified as phosphatidylcholine and was shown to be essential for the reductase-catalyzed transfer of electrons from NADPH to cytochrome P-450 [4]. Synthetically prepared compounds such as lauroylglyceryl-3-phosphorylcholine (lauroyl-GPC) were found to be active when substituted for the microsomal phosphatidylcholine fraction. Subsequently, the corresponding enzyme systems in rat [5, 6] and mouse [7] liver microsomes as well as in a yeast, *Candida tropicalis* [8, 9], were reconstituted by similar procedures. The cytochrome P-448-containing enzyme system, which oxidizes polycyclic aromatic carcinogens, was also reconstituted and was found to require the reductase and phosphatidylcholine for activity, as shown with 3-methylcholanthrene-treated rats [10] and β-naphthoflavone-treated mice of both inducible and noninducible strains [7].

The present paper describes the solubilization of human liver microsomal cytochrome P-450 and its reconstitution into a functional hydroxylating system by the addition of the reductase and phosphatidylcholine. The presence of cytochrome P-450 in human liver microsomes was first reported by Alvares *et al.* [11], and the hydroxylation of various substrates has been shown to occur in the microsomal fraction obtained from human liver biopsy samples [12, 13], fetal liver [14], and postmortem liver from patients with abnormal medical histories [15] or apparently normal individuals who met sudden deaths [16].

A liver sample from a 21-year-old male who had met an accidental death about 4 hr previously was cut into pieces, washed with 0·25 M sucrose and frozen. All procedures were carried out at 4° unless stated otherwise. The microsomal fraction was isolated according to Nelson *et al.* [16], except that 0·5 mM dithiothreitol was added to all solutions, and was stored at −20° under nitrogen. The yield of microsomal protein was 13 mg/g of liver, wet wt. Cytochrome P-450 and P-420 were present in the amounts of 0·25 and 0·42 nmole/

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