ter, *Lahillia larseni* (Sharman and Newton), *Eutrephoceras argentinae* del Valle and Forucade, *Aturia* sp., *Eurhomalia antarctica* (Wilckens), *Nielo* n. sp., and a number of undescribed gastropods.

10. Bartonian (European marine stage) and Runangan (New Zealand marine stage). Closest regional biogeographic affinities are with the invertebrate faunas of the early Paleogene of New Zealand. The La Meseta molluscs show surprisingly weak affinities with contemporaneous faunas of southern South America (*13*).
11. The Sobral and Lopez de Bertodano formations (Campanian, ?Danian in age) were proposed and characterized by C. A. Rinaldi, A. Massabie, J. Morelli, L. Roseman, and R. Del Valle, [*Inst. Antarct. Argent. Contrib.* **217**, 1 (1978)].
12. Marsupials are known to have populated North America, western Europe, South America, and Antarctica, and are inferred to have inhabited Australia during the late Cretaceous and early Cenozoic [R. H. Tedford, *Soc. Econ. Paleontol. Mineral. Spec. Publ.* **21**, 109, (1974)]. Placental mammals radiated vigorously in North America during the late Cretaceous and early Cenozoic, [G. G. Simpson, "Evolution and geography," Condon lectures, Oregon State System of Higher Education, Eugene (1953), pp. 1–64] and a large group of nearly endemic, herbivorous, placental mammals were contemporaneous in South America [G. G. Simpson, *Proc. Am. Phil. Soc.* **122**, 318 (1978); B. Patterson and R. Pascual, *Q. Rev. Biol.* **43**, 409 (1968)]. These are generally bulky, medium-sized herbivorous animals, considered to have been selectively disadvantaged with respect to dispersal across shallow or deep water barriers, and none of these is known to have populated Australia, which had, by about 30 million years ago, and probably earlier as well, a strong diversity of endemic and largely herbivorous marsupials [W. D. L. Ride, *J. R. Soc. West Aust.* **47**, 97, 1964; J. A. Kirsch, *Nature (London)* **217**, 415 (1968); M. Archer, *J. Linn. Soc. Lond. Zool.* **59**, 217 (1976); M. Archer and A. Bartholomai, *Alcheringa (Adelaide)* **2**, 1 (1978)].
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16. Critical logistical support, without which the work could not have been accomplished, was provided by personnel of the U.S. Coast Guard Cutter *Glacier*, J. W. Coste, commanding officer, with invaluable assistance by Aviation Detachment 88, Lieutenant Commander J. Williams, leader. Scientific personnel included W. J. Zinsmeister, P. Webb, T. de Vries, C. Macellari, and B. Huber (Institute of Polar Studies, Ohio State University; invertebrate paleontology); M. O. Woodburne and W. R. Daily (Department of Earth Sciences, University of California, Riverside; fossil mammals and birds); S. Chatterjee (The Museum, Texas Tech University, Lubbock; fossil reptiles and fish); R. Askin and F. Fleming (Department of Geology, Colorado School of Mines, Golden, and Department of Geology, University of Colorado, Boulder; palynology). Supported by NSF grant DP8020096 to D.H.E. and W.J.Z.

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## Nonimmunological Production of Leukotrienes Induced by Platelet-Activating Factor

Abstract. *Platelet-activating factor caused rapid pulmonary vasoconstriction and edema in isolated lungs perfused with albumin-free salt solution devoid of formed blood elements. These effects may be due in part to the action of leukotrienes $D_4$ and $C_4$, which were identified by bioassay and high-pressure liquid chromatography in the lung effluent after stimulation by platelet-activating factor. These findings help illuminate some of the deleterious effects that platelet-activating factor elicits in anaphylactic reactions and possibly in other forms of lung injury.*

Platelet-activating factor (PAF; 1-0-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine) (*1*) was first derived from rabbit basophils that had been sensitized with a specific immunoglobulin E–antibody (*2*) and has since been identified from neutrophils, alveolar macrophages, and monocytes (*3–5*) of various species including man. The factor is known to promote calcium-dependent platelet aggregation, serotonin secretion, and thromboxane $A_2$ production (*6–7*). In the guinea pig, aspirin-resistant bronchoconstriction has been reported after intravenous PAF administration (*8*). In addition, intravenous injection of PAF in the rabbit induced bronchoconstriction and increased right ventricular pressure that appeared to be platelet independent (*9*). We have now conducted experiments showing that PAF causes pulmonary vasoconstriction in isolated lungs perfused with albumin-free salt solution (*10*).

Lungs were isolated from male Sprague-Dawley rats weighing 250 to 300 g and perfused as previously described with a warm (37°C), oxygenated Green-berg-Bohr buffer solution (*11*), which was osmotically stabilized with Ficoll (Sigma; 4 percent by volume). The lungs were pump-perfused at a constant rate of 0.03 ml per gram of body weight per minute and ventilated with a Harvard animal respirator with a gas mixture containing 21 percent $O_2$, 5 percent $CO_2$, and 74 percent $N_2$ (tidal volume 2 $cm^3$, rate 60 per minute). Thirty minutes of nonrecirculating perfusion allowed stable baseline conditions (the perfusion pressure was 7 ± 1 mmHg) and a practically cell-free lung effluent (platelets 500, leukocytes 0.01, erythrocytes 0.01 per milliliter) perfusate. Semisynthetic PAF was obtained from Avanti (Birmingham, Alabama) or Bachem (Bubendorf, Switzerland). The latter compound produced a single molecular species ($MH^+$, mass to charge ratio, 524) when analyzed by fast atom bombardment mass spectrometry. The PAF was injected rapidly as a bolus into the pulmonary artery cannula (1 to 5 μg in 0.1 ml of normal saline). The pulmonary artery pressure change after injection was measured with a Statham P23AA transducer and the lung weight change with a force displacement transducer (Statham G1-15-300). Both variables were recorded on a Gilson polygraph recorder.

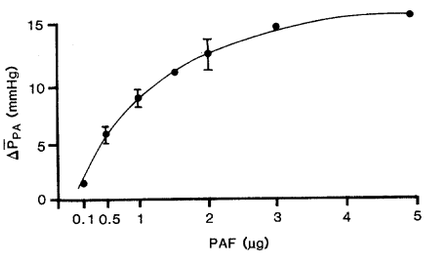


Fig. 1. Dose-response characteristic of bolus injection of PAF into the pulmonary artery. The PAF was stored in chloroform and freshly made up in 0.25 percent bovine serum albumin and Tyrode solution. Dots represent single experiments; closed circles and bars represent the mean (± standard error) of four experiments. The increase in mean pulmonary artery pressure ($\Delta\bar{P}_{PA}$) was determined three minutes after PAF injection.

The injection of PAF caused rapid dose-dependent vasoconstriction (Fig. 1) and edema (as measured by lung weight gain) within 1 minute. The lung weight gain was arbitrarily measured at 3 minutes after PAF injection and was 0.8 to 1.6 g in three lungs that received 1 μg and 2.4 to 7 g in three lungs that received 2 μg. Two lungs receiving 5 μg of the lysoanalog of PAF developed neither vasoconstriction nor edema. In three lungs, two doses of 1 μg of PAF were administered 15 minutes apart. The second injection caused less vasoconstriction, suggesting development of tachyphylaxis.

Prior treatment of the rats with indomethacin (Sigma; 5 mg/kg) and the addition of indomethacin (0.5 μg/ml) to the perfusate blocked the lung cyclooxygenase pathway. The concentration of 6-keto-prostaglandin $F_{1\alpha}$ in the lung effluent was less than 25 pg/ml, compared to greater than 1000 pg/ml in the lung effluent from untreated rats (*12*). In addition, indomethacin blunted the vasoconstriction response and delayed edema formation after PAF administration (Fig. 2). In the indomethacin-treated lungs the maximum rise in mean pulmonary artery pressure after the administration of 2 μg of PAF was 10 to 11.5 mmHg ($N$ = 3) and the weight gain in the third minute after PAF administration was 2.0 to 6 g ($N$ = 3). Thus cyclooxygenase products were probably not the major cause of this PAF effect on the lungs.

In three lungs perfused with physiological salt solution containing diethylcarbamazine (Sigma; 1 mg/ml), an inhibitor of leukotriene biosynthesis (*13–14*), vasoconstriction and edema did not occur after PAF administration. Since the


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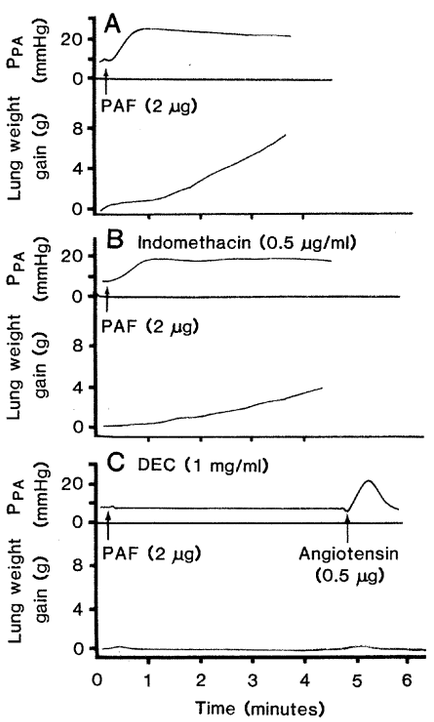
lungs responded to angiotensin II injection with vasoconstriction, diethylcarbamazine had not acted as a nonspecific inhibitor of smooth muscle contraction; furthermore, the diethylcarbamazine was used in a dose that had previously been shown in perfused guinea pig lung not to affect prostaglandin production (*13*).

These data suggested that the PAF effect could be mediated by leukotriene (SRS-A) release. We therefore analyzed the lung effluent after PAF injection for the presence of various leukotrienes. The lung effluent from eight rats was collected in ice-cold flasks for 10 minutes beginning with the injection of 1 μg of PAF (80 to 100 ml were collected in each experiment). A portion of each effluent was assayed on the stripped longitudinal muscle of the guinea pig terminal ileum for leukotriene-like activity (*15*). The effluent from PAF-stimulated lungs contracted the guinea pig ileum; and these contractions were abolished by the end-organ antagonist of SRS-A, FPL 55712 (*16*) (Fig. 3A). Effluent from lungs not treated with PAF did not cause constriction of the guinea pig ileum; PAF itself caused guinea pig ileum contractions that were not inhibited by FPL 55712.

The effluent volumes from the eight rat lungs were then pooled, and the Ficoll was removed by XAD-7 chromatography. The ethanol eluate from the XAD-7 was subjected to further purification by silicic acid chromatography, as previously described (*17*). After batch elution with ethyl acetate (10 percent methanol in ethyl acetate, which eluted no myotropic components), the final 100 percent methanol eluate was subjected to reverse phase high-pressure liquid chromatography (HPLC) (*14*). Two HPLC peaks (Fig. 3B, peaks A and B) were found to have SRS-like biological activity (see Fig. 3B, inset). Furthermore, these components had ultraviolet-absorption maxima at 280 nm characteristic of the peptidolipid leukotrienes (*17*). Component B, the minor constituent, coeluted with authentic leukotriene $C_4$ and fraction A, the major constituent, coeluted with authenic leukotriene $D_4$ upon coinjection on reverse phase HPCL (*14*).

In conclusion, PAF caused vasoconstriction and edema in nonsensitized rat



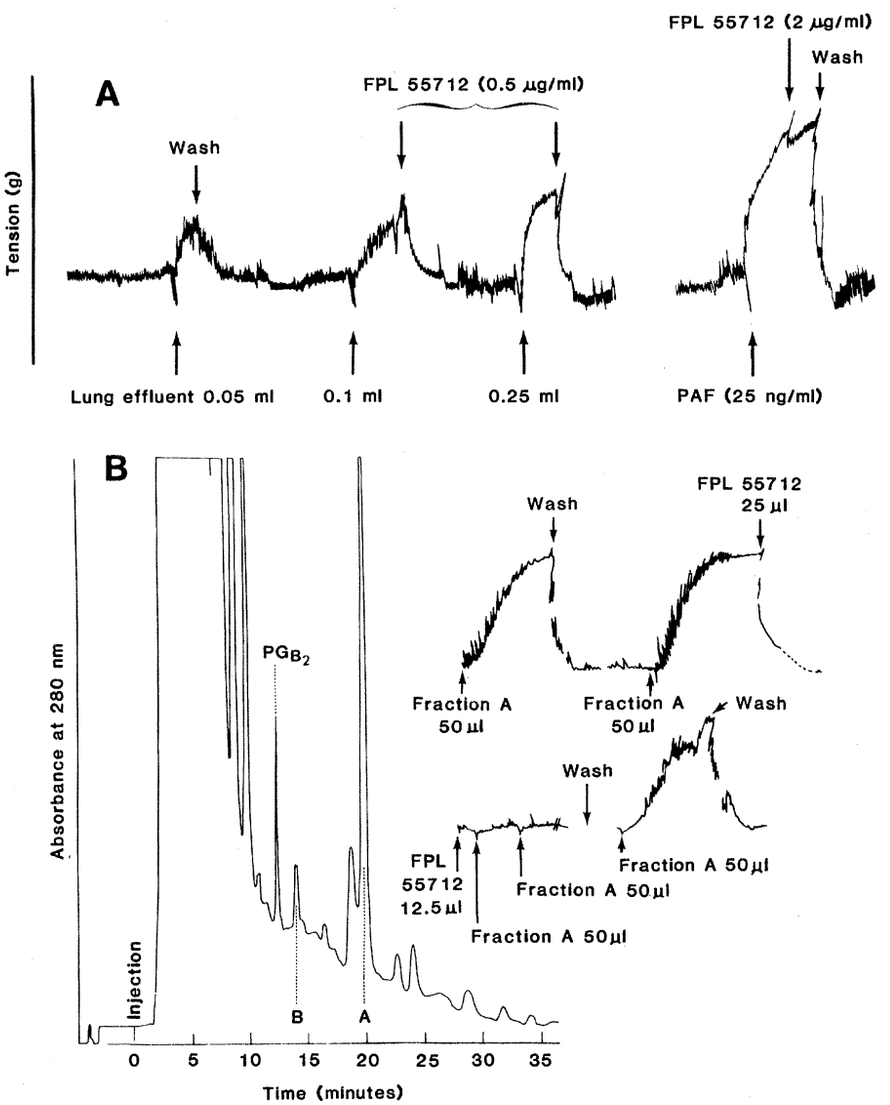




Fig. 2 (left). Representative examples of three different experiments showing pulmonary artery pressure ($P_{PA}$) tracing and lung weight gain. (A) Physiological salt solution was used as the perfusate. (B) The rats received an intraperitoneal injection of indomethacin (5 mg/kg) before they were killed and the lungs were perfused with a physiological salt solution containing indomethacin (0.5 μg/ml). (C) The perfusate contained diethylcarbamazine (*DEC*, 1 mg/ml); angiotensin II was injected into the pulmonary artery 5 minutes after PAF. Fig. 3 (right). (A) Contractions of the isolated guinea pig ileum suspended in Tyrode solution, *p*H 7.3, containing $10^{-6}M$ atropine and $10^{-6}M$ pyrilamine, and bubbled with air. Effluent from lungs that received a 1-μg bolus injection of PAF contracted the guinea pig ileum; the contractions were immediately terminated upon addition of FPL 55712 (0.5 μg/ml) to the bath fluid. The direct application of PAF to the guinea pig ileum caused a contraction that was not terminated by FPL 55712. (B) Reverse phase HPLC of partially purified extract of perfusate of rat lungs stimulated with PAF. The column was Sphereosorb C-18, 5 μm, 25 by 200 mm. The mobile phase used was methanol, water, and acetic acid (65:35:0.02 by volume), *p*H 5.1. The flow rate was 1 ml/min; the ultraviolet monitor was set at 280 nm. Prostaglandin $B_2$ was added as the elution reference. Inset: guinea pig ileum bioassay of portions of fraction A illustrating inhibition of SRS-A–like response by FPL 55712 added to the assay cuvette as indicated by arrows. FPL 55712 inhibits ongoing contraction and in a lower dose prevents contraction if it is added to the ileum before application of fraction A.

lungs, and these effects were inhibited by diethylcarbamazine but not by indomethacin. The injection of PAF into lungs perfused with physiological salt solution stimulated a lung cell to produce leukotrienes. Since the isolated rat lung responds to the injection of synthetic leukotrienes $C_4$ and $D_4$ with vasoconstriction (*18*), the vasoconstriction and edema after PAF injection may be due in part to the action of these leukotrienes.

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## Inhibitory Action of Gossypol on Enzymes and Growth of *Trypanosoma cruzi*

Abstract. *Gossypol, a phenolic compound isolated from the cotton plant, is a powerful inhibitor of nicotinamide adenine dinucleotide–linked enzymes (α-hydroxyacid dehydrogenase and malate dehydrogenase) of* Trypanosoma cruzi, *the parasite that causes Chagas' disease. Parasites at the epimastigote stage that were incubated for 5 minutes with 100 micromolar gossypol were completely immobilized. Concentrations of gossypol as low as 0.01 micromolar markedly reduced the growth rate of* T. cruzi *in culture.*

There is evidence (*1*) that gossypol (1,1′, 6,6′, 7,7′-hexahydroxy-5,5′-diisopropyl-3,3′-dimethyl-(2,2′-binaphthalene)-8,8′-dicarboxaldehyde) is an active male contraceptive agent. Oral administration of this compound produces oligospermia and azoospermia in humans and other species. Studies by Lee and Malling (*2*) suggest that gossypol acts by inhibiting selectively the lactate dehydrogenase (E.C. 1.1.1.27) isozyme X, which plays an important role in sperm metabolism. Isozyme X (LDH X or $C_4$) is associated with spermatozoa and spermatogenic cells (*3*). On the basis of its substrate specificity and subcellular distribution, LDH X has been postulated to integrate a shuttle system transferring H from cytosol to mitochondria (*4*). It therefore appears that LDH X is related to metabolic processes that provide energy for motility and survival of spermatozoa.

Studies in our laboratory (*5*) also indicate that gossypol inhibits the activity of human LDH X. We found that the compound is a noncompetitive inhibitor of LDH X with respect to pyruvate (inhibition constant, $K_i$, 11.0 $\mu M$) and is an uncompetitive inhibitor with respect to reduced nicotinamide adenine dinucleotide (NADH) ($K_i$ = 7.0 $\mu M$). These studies prompted us to investigate the action of gossypol on the α-hydroxyacid dehydrogenase and other oxidoreductases of the flagellate protozoan *Trypanosoma cruzi*. Our interest was justified by the fact that *T. cruzi* is the agent of Chagas' disease, for which there is no satisfactory drug.

Cultured epimastigotes of *T. cruzi* contain an NAD-linked oxidoreductase designated α-hydroxyacid dehydrogenase (*6*). The enzyme is present as two isozymes with different properties (*7*). The substrate specificity of these isozymes is similar to that of LDH X; in particular, isozyme II exhibits a spectrum of substrates almost identical to that demonstrated for mouse isozyme X (*7*).

The parasites were cultured and extracts were prepared as described previously (*7*). The isozyme II of α-hydroxyacid dehydrogenase and malate dehydrogenase (E.C. 1.1.1.37) were partially purified from aqueous extracts of *T. cruzi* by chromatography on a column of DEAE cellulose and by precipitation with ammonium sulfate (*7*). Succinate dehydrogenase (E.C. 1.3.99.1) was obtained by following the method of Weeger *et al.* (*8*), except that the epimastigotes were suspended in 100 m*M* phosphate buffer, *p*H 7.4, homogenized, and then centrifuged at 10,000*g* for 30 minutes. The pellet was washed three times with distilled water and finally suspended in 20 m*M* phosphate buffer, 1 m*M* EDTA, *p*H 7.6. We then continued the procedure as described by Weeger *et al.*

Table 1. Effect of gossypol on oxidoreductases of *T. cruzi*. The $K_m$ values were calculated by Lineweaver-Burk plots. The velocity, *V*, is expressed as a percentage, taking as 100 percent the maximum activity without gossypol. The $K_i$ values were calculated by using various concentrations of substrates at a constant inhibitor concentration.

| Enzyme | Substrate | $K_m$ (m*M*) | *V* | $K_i$ ($\mu M$) |
|---|---|---|---|---|
| α-Hydroxy-acid dehydro-genase | α-Ketoisocaproate | 0.41 | 100 | |
| | α-Ketoisocaproate plus gossypol (0.5 $\mu M$) | 0.41 | 59.57 | 0.73 |
| | NADH | 0.026 | 100 | |
| | NADH plus gossypol (4.0 $\mu M$) | 0.014 | 53.28 | 1.85 |
| Malate dehydro-genase | Oxaloacetate | 0.21 | 100 | |
| | Oxaloacetate plus gossypol (0.3 $\mu M$) | 0.21 | 55.5 | 0.37 |
| | NADH | 0.066 | 100 | |
| | NADH plus gossypol (0.16 $\mu M$) | 0.036 | 52.8 | 0.21 |
| Glutamate dehydro-genase | α-Ketoglutarate | 2.7 | 100 | |
| | α-Ketoglutarate plus gossypol (5 $\mu M$) | 2.7 | 41.35 | 3.5 |
| | NADPH | 0.015 | 100 | |
| | NADPH plus gossypol (11.1 $\mu M$) | 0.0072 | 45.26 | 10.3 |


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