Discussion

This preliminary study in two normal volunteers has shown that LTC and LTD produce moderate bronchoconstriction when inhaled. The two leukotrienes were equipotent and equieffective, as was reported for human airway tissue in vitro [8, 9]. Although pulmonary reactivity to other stimuli was not studied in these subjects, it is unlikely that histamine or methacholine would have produced bronchoconstriction over the same concentration range (0.2–2 × 10⁻⁵ M).

The leukotriene-induced falls in FEV₁ were relatively small. However, it is possible that asthmatic subjects would respond more severely to LTC and LTD, due to inherent hyperreactivity of the airways. Similarly, it is conceivable that LTC or LTD could render the airways hyperreactive to other stimuli.

The ability of leukotrienes C and D to induce coughing suggests that they are able to initiate reflexes following stimulation of irritant receptors. It is possible that the bronchoconstriction produced by LTC and LTD involved a reflex component, but we did not investigate this. Similarly we are unable to comment on the site of action of LTC and LTD within the lungs. As the bronchoconstriction was more apparent when measured as flow at low lung volumes, we suspect that small airways may have been the predominant site affected. However, confirmation of this must depend on appropriate tests such as density-dependent flow volume determinations.

References

Leukotrienes C and D induce aspirin-sensitive bronchoconstriction in the guinea-pig

B. BORIS VARGAFTIG, JEAN LEFORT and ROBERT C. MURPHY
Institut Pasteur, Paris, France, and University of Colorado, USA

Abstract
Bronchoconstriction in the guinea-pig due to leukotrienes C₄ and D₄ in vivo and in vitro was suppressed by aspirin. Since contracting effects of putative mediators of bronchial asthma should be refractory to inhibition of cyclooxygenase, our results indicate that release of leukotrienes in the guinea-pig does not alone account for anaphylactic bronchoconstriction.

Introduction
Prostaglandins (PGs), particularly E₂, were held in the early 70's to be the main cause of acute inflammation [1]. Years before, plasma kinins had the same privilege, and not so long ago, thromboxane A₂ (TXA₂) was considered by some investigators as the final mediator of platelet aggregation, even though it had been clearly established in the late 60's that aspirin only inhibits aggregation due to low amounts of collagen [2]. 'Slow-reacting substance of anaphylaxis' (SRS-A), now identified as one of the leukotrienes (LTs), is released from shocked guinea pig lungs [3], and may be involved in asthma. The arguments put forward are as follows; (1) SRS-A is released from shocked rat, human and guinea-pig lungs and different cell populations; (2) SRS-A contracts smooth muscle in vitro, and a selective inhibitor of its effects does not interfere with contractions due to other spasmogens, such as histamine; (3) LTs are potent pharmacological agents particularly in relation to the pulmonary mechanics of the guinea-pig [4]; (4) aspirin and indomethacin, when inhibiting the cyclooxygenase-dependent metabolism of arachidonic acid (AA), stimulate the formation of SRS-A. There is, nevertheless, experimental evidence against an independent role for SRS-A/LTs in asthma. Thus, it has been shown [5] that bronchoconstriction induced by crude SRS-A in the guinea-pig is inhibited by aspirin, at doses similar to those which block bronchoconstriction due to other agonists (see refs. [6] and [7]). Nevertheless, bronchial asthma in humans, as well as anaphylactic bronchoconstriction in the guinea-pig and passive cutaneous anaphylaxis in the rat (two models used for asthma), are refractory to inhibition by aspirin, raising the problem of the significance of SRS-A/LTs in the aetiology of asthma. This has now led us to investigate the
interaction of aspirin with the LTs in the guinea-pig, and to bring evidence that their bronchoconstrictor activity in the guinea-pig is suppressed when cyclooxygenase is inhibited.

**Materials and methods**

Variations in the pulmonary resistance to inflation, arterial blood pressure, and platelet counts in arterial blood of anaesthetized guinea-pigs were recorded as described [8]. In a few experiments, such as that illustrated in Figure 1, an open tip catheter was introduced cephalad into the trachea, for recording the variations of the intratracheal pressure. After responses to LTC$_4$ or LTD$_4$ had been obtained, aspirin or salicylic acid were injected i.v. at 20 mg/kg, followed within 10 minutes by new injections of the LTs. Previous experiments demonstrated that there was no desensitization to repeated injections of the LTs at hourly intervals. Aerosol sprays were administered with a Marion nebulizer, connected to the output of the respiratory pump. Consistent responses to single 2 min aerosol sprays were obtained with 0.5–1 μg/ml of the LTs in the chamber. As a comparison, bronchoconstriction was induced by PGF$_2$α at similar concentrations. Lung parenchyma strips were prepared according to [9], in 16 ml of Krebs' solution containing antagonists of histamine, serotonin and catecholamines. Aspirin or salicylic acid were added to the organ bath for 10 minutes, after standard contractions of the tissue to 0.1–0.3 nM of the LTs had been obtained. Once these contractions had been inhibited, more LT was added, without removal of the solution, in order to see whether inhibition was surmountable.

**Results**

Intravenous injections of 0.1–0.9 μg/kg of LTC$_4$ or LTD$_4$ increased the pulmonary and bronchial resistances to inflation and induced hypotension. No thrombocytopenia was seen after these injections. All effects of the LTs were suppressed by 20 mg/kg i.v. aspirin (Fig. 1). Since it might be argued that bronchoconstriction by i.v. LTs is a poor model of the endogenous release, they were administered by aerosol. Bronchoconstriction started after a delay of around 2 minutes, and increased steadily for 5–10 minutes, even after disconnecting the aerosol. A new aerosol spray resulted in marked desensitization, even when applied up to 2 hours afterwards. The animals were thus not used as their own controls, and separate guinea-pigs were treated by aerosol once only, 10 minutes after i.v. aspirin. Bronchoconstriction was not fully suppressed, but was clearly reduced when compared to control animals (8.75 ± 6 cm H$_2$O increase for eight control animals vs. 3.27 ± 1.6 cm H$_2$O increase for six aspirin treated).

Leukotriene C4 (Fig. 2) and D$_4$ (not shown) contracted the lung parenchyma strip. The contractions were abolished by aspirin added to the bath at 0.3–1 μg/ml 2–5 minutes beforehand and were slightly surmounted when more LTs were added. Salicylic acid was inactive up to 10 μg/ml.

![Figure 1](image1.png)

**Figure 1**

Inhibition by aspirin of the effects of leukotriene D in the guinea-pig. From above to below: increase in the intratracheal and in the pulmonary resistance to inflation (bronchoconstriction) both in cm H$_2$O and arterial blood pressure (cm Hg). The indicated agonists were injected i.v before and after the injection of 20 mg/kg of aspirin i.v (arrow). Time scale: 10 minutes.

![Figure 2](image2.png)

**Figure 2**

Inhibition by aspirin of the contractile activity of leukotriene C on the lung parenchyma strip. Tracings of the contractions induced by LTc before (upper) and after (lower) the addition to the organ bath of aspirin. Observe partial surmountability of inhibition by adding more LTC, and reversion of inhibition after washing out aspirin (2nd and 3rd cycles). Vertical scale: 100 mg of tissue contraction. Horizontal scale: time, 10 minutes.

**Discussion**

The release of 'rabbit aorta contracting substance' (RCS, identified as TxA$_2$) from AA injected guinea-pig lungs, as well as bronchoconstriction, hypotension and thrombocytopenia due to the i.v. injections of AA, are suppressed by aspirin [8], [10]; see [11] for a review. When cyclooxygenase is inhibited, AA is catabolized mainly to LTs [12] and as a consequence one would expect bronchoconstriction to occur in spite, or even because of aspirin. This is never the case, and inhibition by aspirin of bronchoconstriction due to AA is unsurmountable, until the effects of aspirin fade away [8]. Slow reacting substance of anaphylaxis and LTC release TxA2 from the isolated guinea-pig

![Figure 2](image3.png)

**Figure 2**

Inhibition by aspirin of the contractile activity of leukotriene C on the lung parenchyma strip. Tracings of the contractions induced by LTc before (upper) and after (lower) the addition to the organ bath of aspirin. Observe partial surmountability of inhibition by adding more LTC, and reversion of inhibition after washing out aspirin (2nd and 3rd cycles). Vertical scale: 100 mg of tissue contraction. Horizontal scale: time, 10 minutes.