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Evaluation of Surinamese Medicinal Plants for their Potential Bronchospasmolytic Effects in Isolated Guinea Pig Tracheal Chains

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ABSTRACT

Preparations from the leaves of *Kalanchoe pinnata* (Lam.) Pers. (Crassulaceae), *Asclepias curassavica* L. (Asclepiadaceae), *Amaranthus spinosus* L. (Amaranthaceae), *Bixa orellana* L. (Bixaceae), *Cymbopogon citratus* L. (Poaceae), *Caesalpinia pulcherrima* (L.) Schwartz (Caesalpiniacese) and *Solanum jamaicense* Mill. (Solanaceae), as well as the peels of *Punica granatum* L. (Punicaceae), are popularly used in Suriname to relieve respiratory distress. To verify this claim, aqueous extracts from certain parts of these plants were assessed at 0.001-10 mg mL^{-1} for their ability to reduce the force of smooth muscle contraction of isolated guinea pig trachea chains induced by acetylcholine (3×10^{-5} M) or histamine (10^{-5} M). Experiments were carried out in Tyrode buffer mixed with 5% CO_2 in air and at 37°C. The extract from *K. pinnata* (10 mg mL^{-1}) reduced the force of contraction of the tracheal chains caused by both acetylcholine and histamine by 40-70%. Those from *P. granatum*, *A. spinosus*, *A. curassavica* and *B. orellana* (1 and 10 mg mL^{-1}) counteracted the force of contraction by histamine by 30-70%. The preparations from *C. pulcherrima*, *C. citratus* and *S. jamaicense* did not affect the force of contraction due to either acetylcholine or histamine. These results suggest that preparations from *K. pinnata*, *A. spinosus*, *A. curassavica*, *B. orellana* and *P. granatum* may elicit bronchospasmolytic effects through antagonism of the muscarinic and/or H_1 histamininergic receptor.

Key words: Medicinal plants, guinea pig tracheal chains, muscarinic receptor, H_1 histaminergic receptor, bronchospasmolysis

INTRODUCTION

Airway diseases such as asthma are highly prevalent conditions with a substantial personal and social impact and an increasing economic burden in many parts of the world (Martinez and Vercelli, 2013). According to the WHO (2014), 100-150 million individuals throughout the world are suffering from asthma and this number is rising. In Western Europe, for instance, asthma incidence has increased two-fold in ten years and in the United States of America (USA) the number of patients with asthma has increased by more than 60% since 1980 while the number of deaths has doubled to 5,000 per year (WHO., 2014). As asthma often affects younger individuals, the economic burden associated with this condition is severe, amounting in the USA and Great Britain, among others, to more than US$ 6 billion and US$ 1.8 billion, respectively, per year (WHO., 2014).

Asthma is characterized by excessive responsiveness of trachea and bronchi to allergens such as pollen; cigarette smoke; chemical fumes or dust at the workplace; upper respiratory tract

infections; exercise, particularly in cold and dry weather; as well as strong emotions and stress (Martinez and Vercelli, 2013). The resulting chronic inflammation leads to swelling of the airways and the production of copious amounts of mucus which cause acute, recurrent and chronic bronchoconstriction (Martinez and Vercelli, 2013). During exacerbations, airway inflammation and airway obstruction are aggravated and pulmonary airflow is substantially reduced (Martinez and Vercelli, 2013).

Asthma is not curable but exacerbations can be controlled by rescue medications such as inhaled short-acting $\$_2$ adrenoreceptor agonists like salbutamol, the muscarinic receptor antagonist ipratropium and oral anti-inflammatory corticosteroids like beclomethasone (Rabe *et al*., 2007). Maintenance medicines are indicated to prevent continuous or frequently recurring symptoms of asthma. Examples are inhaled corticosteroids such as beclomethasone, long-acting inhaled $\$_2$ adrenoreceptor agonists such as salmeterol, the phosphodiesterase inhibitor theophylline as slow-release capsules or tablets taken by mouth and oral antihistamines such as ketotifen (Rabe *et al*., 2007). These compounds are among the bestselling drugs in many countries, turning in revenues in the USA of over US$ 4 billion in the last quarter of the year 2013 and emphasizing the distressing prevalence of asthma and other airway disorders (Drugs.com, 2014).

In addition to these allopathic drugs, a host of traditional, plant-derived substances has been used to relieve airway constriction. For instance, in ancient Egypt and India, the fumes from the burnt leaves of the deadly nightshade *Atropa belladonna* L. (Solanaceae) and the thorn apple *Datura stramonium* L. (Solanaceae) which contain potent anticholinergics were inhaled for relief from bronchoconstriction (Jackson, 2010; Moulton and Fryer, 2011) and in traditional Chinese medicine, the theophylline-containing leaves of the tea plant *Camellia sinensis* (L.) Kuntze (Theaceae), as well as ma huang or ephedra, an extract from the bark of the tree *Ephedra sinica* L. (Ephedraceae) and the source of the $\$_2$ adrenoreceptor agonist ephedrine, have been used for over 5,000 years as bronchodilator (Abourashed *et al*., 2003; Wheeler and Wheeler, 2004).

Today, herbal medicines continue to play an important role in the treatment of airway disorders, particularly in low-resource countries (Patil *et al*., 2008; Ogbole *et al*., 2010; Anjaneyulu and Sudarsanam, 2013). This also holds true for the multi-cultural Republic of Suriname (South America), a country with an extensive ethnopharmacological tradition (Van Andel and Ruysschaert, 2011). Chronic disorders of the lower airways are not uncommon in Suriname and accounted in the year 2009 for two male fatalities every five weeks (Punwasi, 2011). Understandably, a considerable number of plant-derived preparations are available for treating such disorders (Van Andel and Ruysschaert, 2011). However, in the majority of cases there is no scientific evidence of therapeutic efficacy of such preparations. For this reason, it was decided to assess aqueous extracts from a number of such plants for their ability to counteract forces of smooth muscle contraction of isolated guinea pig tracheal chains pre-contracted with acetylcholine or histamine.

MATERIALS AND METHODS

Plants and preparation of plant extracts: Leaves from *Kalanchoe pinnata* L., *Asclepias curassavica* L., *Amaranthus spinosus* L., *Bixa orellana* L., *Cymbopogon citratus* L., *Caesalpinia pulcherrima* (L.) Schwartz and *Solanum jamaicense* Mill., as well as peels from *Punica granatum* L., were collected in rural areas around Suriname's capital city Paramaribo that had been free of herbicidal use for at least the preceding six months. The samples were thoroughly washed with distilled water, dried in open air and macerated. Aqueous extracts were prepared with

Table 1: Relevant information about the plants used in the current study

Botanical name (vernacular names)	Plant family	Plant part (used)	Extraction conditions
Kalanchoë pinnata (Lam.) Pers. (Mother of thousands, wonderblad)	Crassulaceae	Leaves	60 min at 45°C
Punica granatum L. (Pomegranate, granaatappel)	Punicaceae	Peels from fruits	60 min at 45°C
Asclepias curassavica L. (Tropical milkweed, koningsbloem)	Asclepiadaceae	Leaves	30 min at 100°C
Amaranthus spinosus L. (Spiny amaranth, makaklarun)	Amaranthaceae	Leaves	60 min at 100°C
Bixa orellana L. (Annato, kusuwe)	Bixaceae	Leaves	30 min at 100°C
Cymbopogon citratus (A. DC.) Stapf. (Lemon grass, citroengras)	Poaceae	Leaves	30 min at 100°C
Ceasalpinia pulcherrima (L.) Schwartz (Peacock flower, krerekrere)	Ceasalpiniaceae	Leaves	30 min at 100°C
Solanum jamaicense Mill. (Jamaican nightshade, makadroifi)	Solanaceae	Leaves	60 min at 100°C

distilled water as indicated in Table 1. The extraction procedures were based on the traditional methods of preparing these plant parts for treating respiratory disorders. The plant extracts were filtered, evaporated to dryness and divided in 3 g aliquots were stored at -20°C until experiments.

Drugs and chemicals: The specific muscarinic receptor agonist acetylcholine, the specific muscarinic receptor antagonist atropine, the histaminic H_1 receptor agonist histamine and the specific histaminic H_1 receptor antagonist chlorpheniramine were obtained from Sigma (St. Louis, MO, USA). Prior to experiments, these agents were dissolved in Tyrode buffer and then diluted with Tyrode buffer to the desired concentrations. The Tyrode buffer consisted of NaCl 8 g LG^1, KCl 0.2 g LG^1, $MgCl_2$ 0.1 g LG^1, $CaCl_2$ 0.2 g LG^1, NaH_2PO_4 0.05 g LG^1, $NaHCO_3$ 1 g LG^1 and glucose 1 g LG^1 and the pH was adjusted to 7.4. All other chemicals used were from the laboratory stock of our institution and were of the highest grade available.

Animals and preparation of tracheal chains: Adult guinea pigs weighing 200-400 g were acquired from the Animal Facility of our institution. The animals were kept under standard conditions and had free access to food and water. For experiments, the guinea pigs were anaesthetized with chloroform, after which the trachea was quickly removed, placed in cold Tyrode buffer and cut transversally between the cartilage segments so as to obtain a number of rings of tracheal smooth muscle. Eight rings were tied together with cotton to form a chain which was placed in an organ bath containing Tyrode buffer kept at a temperature of 37°C and mixed with a constant flow of ambient air. The Bioethics Committee of Anton de Kom University had approved the design of these experiments.

Incubations and assessment of tracheal responses: For experiments, one end of a tracheal chain was attached to a fixed point in the organ bath and the other to an FT-100 force transducer (CB-Sciences, Dover, NH, USA). Next, the tracheal chain was exposed to acetylcholine, atropine, histamine, chlorpheniramine, or a plant extract, either alone or at certain combinations. Incubations were for 5 min, after which the tracheal chains were allowed to recover for 2×5 min

in pre-warmed Tyrode buffer. The forces of smooth muscle contraction were processed with a 256 Bio-amplifier (CB-Sciences, Dover, NH, USA) and a Powerlab 400E series analog/digital converter (ADInstruments, Castle Hill, Australia) and monitored continuously using the Chart 4.2 for Windows software (ADInstruments, Castle Hill, Australia). Forces of smooth muscle contraction were in mg, corrected for basal muscle tone (readings in the presence of Tyrode buffer alone) and expressed relatively to those found with acetylcholine or histamine alone.

Statistical analysis: Each experiment was repeated at least three times in triplicate and results were expressed as Means±SD taking p-values "<0.05" to indicate statistically significant differences according to the two tailed t-test of Student.

RESULTS

Responses of guinea pig tracheal chains to acetylcholine or histamine in the absence or presence of atropine or chlorpheniramine: The responses of the guinea pig tracheal chains to acetylcholine and histamine are shown in Fig. 1a and b. Both compounds caused a dose-dependent increase in the force of smooth muscle contraction of the tracheal chains. Their exposure to acetylcholine 3×10^{-5} M or histamine 10^{-5} M resulted in forces of contraction of approximately 440 and 570 mg, respectively (Table 2) which were in the linear portion of the dose-response curves (Fig. 1a, b). The simultaneous use of atropine 10^{-6} M with acetylcholine

Table 2: Forces of contraction of isolated guinea pig tracheal chains elicited by acetylcholine 3×10^{-5} M or histamine 10^{-5} M alone or in presence of atropine (10^{-6} M) or chlorpheniramine (10^{-5} M), respectively

Agents	Force of contraction (mg)
Acetylcholine	440±189
Acetylcholine+Atropine	120±78
Histamine	570±252
Histamine+Chlorpheniramine	133±69

Data is presented as Mean±SD (n = 3)

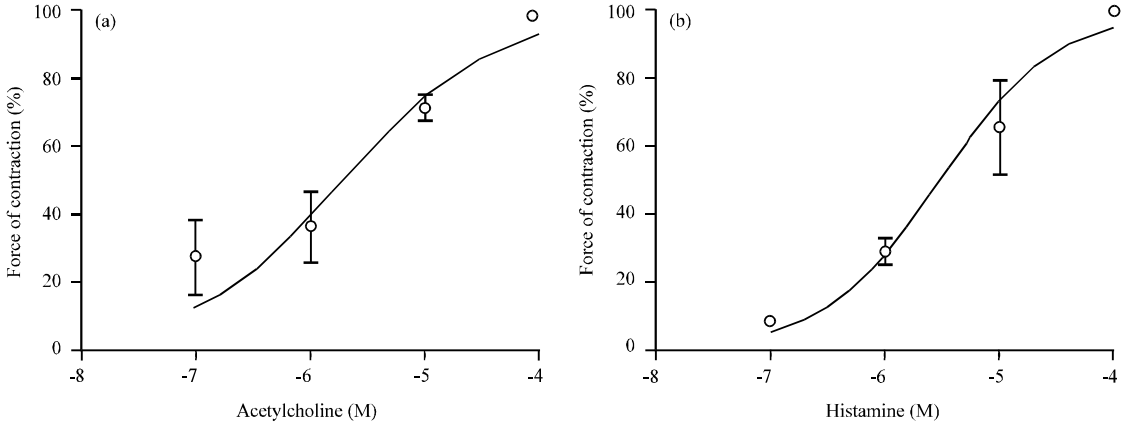

Fig. 1(a-b): Responses of the guinea pig tracheal chains to (a) Acetylcholine and (b) Histamine Isolated tracheal chain was incubated with several doses of acetylcholine (10^{-7} to 10^{-4} M). Data presented are percentages of the highest force of contraction and represent means (data points) ±SD (error bars) of at least three experiments performed in duplicate

$3\times10G^{5}$ M, or chlorpheniramine $10G^{5}$ M with histamine $10G^{5}$ M, led to a decrease in forces of contraction of approximately 75% (Table 2). These observations justified the use of acetylcholine and histamine at these concentrations to pre-contract the tracheal chains and validated the suitability of the experimental set-up to evaluate the plant extracts for their potential bronchospasmolytic effects.

Effects of plant extracts on forces of contraction caused by acetylcholine: Next, the plant extracts were evaluated for their effects on the force of contraction of the guinea pig tracheal chains caused by acetylcholine $3\times10G^{5}$ M. The plant extracts were used at the concentrations of 0.001, 0.01, 0.1, 1 and 10 mg mLG^{1}. The data obtained was expressed relatively to that found for acetylcholine $3\times10G^{5}$ M alone and are given in Table 3.

With the exception of the *K. pinnata* extract, none of the plant extracts altered the force of contraction of the tracheal chains caused by acetylcholine. The effect of the *K. pinnata* extract on the acetylcholine-induced force of contraction was apparent at 10 mg mLG^{1} and involved a decrease of approximately 50% when compared to that produced by acetylcholine $3\times10G^{5}$ M alone.

Effects of plant extracts on forces of contraction caused by histamine: Parallel with the studies described in the preceding paragraph, the plant extracts were evaluated at the same concentrations for their effects on the force of contraction of the guinea pig tracheal chains caused by histamine $10G^{5}$ M. The data obtained have been expressed with respect to that found for histamine $10G^{5}$ M alone and are given in Table 4.

The *P. granatum* extract (10 mg mLG^{1}) led to a decrease of about 50% in the force of contraction of the tracheal chains caused by histamine. The extract from *A. curassavica* reduced the histamine-induced force of contraction by about 25% at 1 mg mLG^{1} and about one-third at 10 mg mLG^{1}. Combination of the *A. spinosus* or the *B. orellana* extract with histamine $10G^{5}$ M also led to a 25-30% decrease in the force of contraction of the tracheal chains when compared to the effect of histamine alone.

The concomitant use of histamine and the extracts from *C. citratus*, *C. pulcherrima*, or *S. jamaicense* did not lead to forces of contraction that differed significantly from that caused by histamine alone.

Table 3: Forces of contraction of isolated guinea pig tracheal chains caused by acetylcholine $3\times10G^{5}$ M in the presence of plant extracts relative to that found for acetylcholine $3\times10G^{5}$ M alone. The latter value was set at 100%

	Relative force of contraction at concentrations of plant extracts of (mg mLG^{1})				
Guinea pig isolates	0.001	0.01	0.1	1	10
K. pinnata	119±15	124±18	125±15	106±25	56±20[1]
P. granatum	111±9	120±14	104±22	100±4	92±14
A. curassavica	128±29	127±40	126±42	105±35	73±25
A. spinosus	102±13	115±21	111±17	118±21	77±56
B. orellana	101±17	93±15	97±14	104±9	103±14
C. citratus	112±15	112±14	117±14	110±29	87±40
C. pulcherrima	106±8	110±14	107±12	109±14	76±38
S. jamaicense	102±7	101±22	106±10	108±15	96±24

[1]Significantly different from the effect of acetylcholine $3\times10G^{5}$ M alone ($p<0.05$, Student's t-test). Data is expressed in percentages and Means±SD of at least three independent experiments performed in triplicate

Table 4: Forces of contraction of isolated guinea pig tracheal chains caused by histamine 10^{-5} M in the presence of plant extracts relative to that found for histamine 10^{-5} M alone. The latter value was set at 100%

	Relative force of contraction at concentrations of plant extracts of (mg mL^{-1})				
Guinea pig isolates	0.001	0.01	0.1	1	10
K. pinnata	102±15	105±17	109±16	103±11	31±53[1]
P. granatum	104±10	103±13	97±16	83±27	49±4[1]
A. curassavica	107±20	91±17	87±14	74±15[1]	67±14[1]
A. spinosus	92±7	100±13	104±21	90±23	71±3[1]
B. orellana	109±18	107±25	95±39	87±31	73±2[1]
C. citratus	99±30	102±34	101±36	96±30	86±22
C. pulcherrima	108±10	118±15	103±31	90±27	54±60
S. jamaicense	97±12	106±6	108±1	108±20	119±19

[1]Significantly different from the effect of histamine 10^{-5} M alone ($p<0.05$, Student's t-test), Data is expressed in percentages and Means±SD of at least three independent experiments performed in triplicate

DISCUSSION

This study presents indications that preparations from *K. pinnata*, *P. granatum*, *A. curassavica*, *A. spinosus* and *B. orellana* but not those from *C. citratus*, *C. pulcherrima* and *S. jamaicense*, may alleviate airway constriction due to asthma. This assumption is based on the observation that aqueous extracts from the former five plants decreased the force of contraction of guinea pig tracheal chains caused by acetylcholine and/or histamine. Thus, these preparations may relieve airway constriction by opposing excessive stimulation of the muscarinic and/or the H_1 histaminergic receptor on the smooth muscle cells of the airways.

The possible involvement of the muscarinic receptor in the apparent bronchospasmolytic effect of the *K. pinnata* leaf extract is in accordance with the decreasing effect of such a preparation on guinea pig tracheal ring contractions caused by carbachol (Ozolua *et al*., 2010). Additional support for an anti-muscarinic effect of the *K. pinnata* preparation is provided by its relaxation of isolated guinea pig ilei and porcine bladder strips pre-contracted with acetylcholine or carbachol (Mans *et al*., 2004b; Schuler *et al*., 2012). The apparent anti-histaminergic effect of the *K. pinnata* preparation noted in the current study is in accordance with its ability to reduce the force of contraction of isolated pig ilei caused by histamine (Nassis *et al*., 1991; Mans *et al*., 2004b) and to protect laboratory rats from vascular permeability responses to and asphyxia by histamine (Nassis *et al*., 1991).

However, the *K. pinnata* leaf extract has also been reported to relax human myometrium contraction caused by electrical pulses or oxytocin (Gwehenberger *et al*., 2004; Plangger *et al*., 2005; Wachter *et al*., 2011) as well as porcine bladder strips contracted by electrical field stimulation (Schuler *et al*., 2012). These observations suggest that this preparation can also affect the intracellular availability of calcium or actin-myosin coupling. Whether interference with these mechanisms is also involved in the bronchospasmolytic effects of the *K. pinnata* preparation is not certain but must be investigated in future studies.

The apparent anti-histaminergic properties of the extract of *P. granatum* peels is supported by the anti-allergic and anti-inflammatory effects of and the significant decrease in the extent of ulcerative colitis and levels of histamine by preparations from several parts of this plant in laboratory animals (Singh *et al*., 2009; Nirmal *et al*., 2011; De Oliveira *et al*., 2013). These observations have partially been attributed to ellagic acid that is present in large amounts in

P. granatum peels (Johanningsmeier and Harris, 2011). However, a randomized, double-blind, placebo-controlled trial concluded that supplementation of pomegranate fruit juice added no benefit to the standard therapy in patients with stable chronic obstructive pulmonary disease (Cerda *et al*., 2005). This warrants more comprehensive studies to assess the precise value of *P. granatum* preparations against airway disorders.

That the *B. orellana* leaf preparation also possessed anti-histaminergic properties is consistent with its ability to oppose smooth muscle contraction of isolated guinea pig ilei caused by histamine (Mans *et al*., 2004a); inhibit the inflammatory response in laboratory rats produced by sub-plantar injection of histamine (Yong *et al*., 2013); relax smooth muscles of laboratory animals (Evans, 2000) and decrease gastric secretions in mice administered castor oil (Gutierrez *et al*., 2007). Notably, the pre-carotenoid bixin (Bouvier *et al*., 2003) that is abundantly present in the seeds of *B. orellana* (Giuliano *et al*., 2003), stimulated the immune response and reduced the skin hypersensitivity reaction to histamine in domestic cats (Park *et al*., 2007).

Partial support for the potential bronchospasmolytic activity of preparations from *A. curassavica* leaves comes from the use of rhizomes and roots of this species as well as those of its family member *A. tuberosa* L. in both powdered and infusion forms against bronchitis and asthma (Hemavani and Thippeswamy, 2012). Furthermore, the traditional use of various parts of *A. curassavica*, among others, South and North America (Hirschmann and de Arias, 1990; Barrett, 1994; Hemavani and Thippeswamy, 2012) and certain regions of India (Anjaneyulu and Sudarsanam, 2013) has been reported to facilitate expectoration and relieve cough and pain. However, whether interference with the H_1 histaminergic receptor is involved in these observations is so far not clear and must be established.

The anti-H_1 activity of the *A. spinosus* extract noted in the current study is in line with the anti-inflammatory and anti-histaminergic activity of preparations from various parts of *A. spinosus* in animal models (Olajide *et al*., 2004; Zeashan *et al*., 2009; Patil *et al*., 2012) and their degranulation of rat peritoneal mast cells (Patil *et al*., 2012). However, *A. spinosus* leaf extracts also inhibited bronchospasm in laboratory rats and contraction of isolated rabbit trachea caused by carbachol (Chaudhary *et al*., 2012). These preparations also induced bronchodilation through $ adrenergic and calcium channel blockade in laboratory animals and isolated organs (Chaudhary *et al*., 2012). These observations, together with the absence of anti-muscarinic effects in the current study, necessitate detailed mechanistic studies with this preparation.

Preparations from *C. citratus* and *C. pulcherrima* leaves were previously found to display substantial anti-cholinergic and anti-histaminergic activities in isolated guinea pig ilei (Mans *et al*., 2004a). Furthermore, extracts from these plants displayed meaningful anti-inflammatory effects in murine alveolar macrophages (Tiwari *et al*., 2010) and in a cotton pellet granuloma model in albino Wistar rats (Sharma and Rajani, 2011). These observations may account for the traditional use of these plants against airway disorders, even though their lack of an effect on constricted guinea pig tracheal chains in the current study cannot be readily explained and must be further assessed.

Scientific data help to explain the absence of an effect of the *S. jamaicense* extract against airway constriction are scant. However, preparations from its family members *S. xanthocarpum* Schradt and Wendl. and *S. nigrum* L. elicited clear anti-histaminergic effects in various laboratory animals (Parmar *et al*., 2010; Nirmal *et al*., 2012). On the other hand, water and methanol extracts from *S. melongena* had no effect on the bronchospasm caused by histamine aerosol in guinea pigs

(Sehgal *et al*., 2013) and even caused bronchoconstriction rather than bronchospasmolysis in isolated guinea pig tracheal chains (Mans *et al*., 2004b). Thus, the potential therapeutic efficacy of *S. jamaicense* preparations in airway disorders remains to be established.

CONCLUSION

The results from this study suggest that aqueous extracts from *K. pinnata*, *A. curassavica*, *A. spinosus* and *B. orellana* leaves as well as those from *P. granatum* peels may elicit bronchospasmolytic effects by antagonizing the muscarinic and/or the H_1 histaminergic receptor in the airways. These findings support the ethnopharmacologal use of such preparations for relieving bronchoconstriction and are particularly relevant to low-resource countries. However, as mentioned above, some of these preparations may affect smooth muscle contraction in organs other than the airways and through distinct mechanisms. This raises the possibility of undesired collateral effects when these preparations are used to alleviate airway constriction. These possibilities are now being evaluated at our institution using a comprehensive series of tests with various laboratory models.

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