These observations suggest the possibility of dividing the *Mycoplasma* into 3 groups according to their effect on mammalian cells growing in tissue culture. The rather general working classification outlined below may prove useful and convenient in distinguishing saprophytic from pathogenic PPLO. Perhaps the first large division might include saprophytic Mycoplasma such as *M. laidlawii* which shows little if any growth or multiplication in tissue cultures. PPLO considered to be primarily endogenous tissue culture contaminants, in that they persist in small numbers and establish an ecological equilibrium with the host, may comprise another large group. The third division could be composed of those PPLO thought to be potentially pathogenic for cell lines when maintained 2 to 3 weeks in tissue culture, particularly when PPLO metabolic processes supplement or alter host cell metabolism. The reported data suggest that the strain of *M. gallisepticum* studied, when growing in FL cells is a rather typical agent according to the third suggested PPLO grouping.

Summary. Infection of FL tissue culture cells with *M. gallisepticum, M. agalactiae* and *M. hominis* was achieved, while the saprophytic PPLO *M. laidlawii* A could not be detected in such cells when cultivated for intervals longer than 2 days following infection. Parasitic PPLO remained in cell cultures for periods up to 3 weeks. After prolonged incubation, cytopathogenic effect could be demonstrated in cultures infected with *M. gallisepticum.* In addition, tissue cultures infected with *M. gallisepticum* showed an accumulation of acetate in growth fluids. The latter observation, supported by appropriate isotope data, strongly suggests that acetate appears as a product of lactate dissimilation by this PPLO.

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Received December 13, 1962. P.S.E.B.M., 1963, v112.

Evidence of Monoamine Oxidase Inhibition by Myristicin and Nutmeg. (28128)

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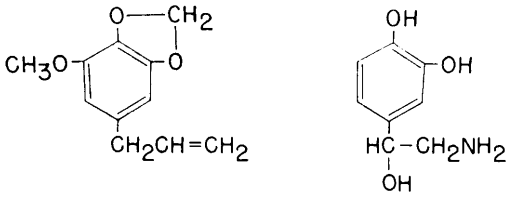
Nutmeg, the seed of *Myristica fragrans,* contains a terpene-like compound, myristicin, which for years has been identified as the principal active ingredient. This early observation of Sir Henry Dale has been recently confirmed using both animal and human tests (1). Nutmeg intoxication produces an unusual syndrome in man involving, first, stimulation and a feeling of euphoria. This may extend into an acute toxic psychosis with an overdose and may be followed by a depressed state during recovery.

There is a degree of structural resemblance between the chemical formula for myristicin and those of certain sympathomimetic amines. This is especially true when the methylenic group of myristicin is considered as isosteric with an amino group. This analogy is shown in Fig. 1. This similarity coupled with the stimulating action of nutmeg prompted a preliminary evaluation of myristicin and crude ground nutmeg for evidence of central monoamine oxidase inhibition.

Materials and methods. Ground East In-



Myristicin Norepinephrine

FIG. 1

dian Nutmeg from a single large batch was given orally in 2% acacia suspension. Chemically synthesized myristicin was dissolved in liquid petrolatum for oral or intraperitoneal administration. A distilled concentrate of oil of nutmeg containing representative amounts of the volatile components was given orally without dilution. Tranylcypromine and iproniazid given orally served as comparative standards.

The method of Tedeschi *et al.*(2) for estimation of monoamine oxidase (MAO) inhibition by measurement of potentiation of tryptamine convulsions was modified and applied to mice. Graded doses of tryptamine HCl 0.5% solution were injected intravenously into 10 mice per dose level. Three seconds or more of clonic jerking, tremors and/or side-to-side head movements were the endpoint criteria used to calculate the CD_{50} from dose-response lines by the method of Litchfield and Wilcoxon(3). Palpebral ptosis was estimated by the method of Rubin *et al.*(6) in rats scoring both eyes on a 5 point scale. Cerebral 5-hydroxytryptamine was measured by the Mead and Finger modification(4) of the method of Bogdanski *et al.*(5).

TABLE I. Tryptamine Convulsion Test for Monoamine Oxidase Inhibition *in vivo*. Summary of control tests.

Species	No.	Vehicle–18 hr prior, cc/kg	CD_{50}, mg/kg	95% confidence limits, mg/kg
Mouse	40	None	25.0	15.4-40.5
"	21	"	17.3	12.1-24.7
"	28	Liq. pet.	24.5	19.9-30.1
"	38	" "	28.0	18.4-42.6
"	37	Acacia–2%	25.8	18.3-36.3
Avg	164		25.0	21.6-29.0
Rat	54	None	18.6	13.6-25.5

Results. No apparent effect was evident from the drug vehicles on the CD_{50} of tryptamine (Table I). When given orally 18 hours in advance, East Indian ground nutmeg gave some evidence of tryptamine potentiation (Fig. 2). The optimum dose was 500 mg/kg. However, a much larger dose, 1000 mg/kg, showed reversal of the activity.

Several samples of synthetic myristicin were tested by the tryptamine potentiation test 18 hours after their oral administration. These results are shown in Fig. 3. Both of these preparations showed considerable activity when the sample was fresh and lemon yellow in color. Later tests (not shown) after the liquid had turned to a light amber color consistently showed a considerable decline in tryptamine potentiation. These deteriorated solutions when studied by gas chromatography showed the appearance of an additional component to the myristicin of unknown identity.

The distilled concentrate of oil of nutmeg was much less active than the synthetic myristicin and, like ground nutmeg, reversed its activity with a large dose (Fig. 3). Gas chromatographic analysis of this oil showed the presence of similar volatile components to ground nutmeg, but no increased concentration of the myristicin as expected from the selected distillation temperature.

In Fig. 4 the slope and activity of the best tryptamine assay for myristicin is compared to tranylcypromine and iproniazid. All 3 drugs were administered orally 18 hours before the test. It may be seen that myristicin is less potent but parallel to the comparative drugs. Safrole, isoborneol and geraniol, which are other volatile components of nutmeg, did not cause potentiation of tryptamine in doses up to 1 g/kg despite obvious signs of hyperactivity and excitement in the mice.

In Fig. 4 the antagonism of reserpine ptosis in rats was used to study variations in dose and time for myristicin activity. Myristicin appears to be less active in the rat. Comparable activity to other MAO inhibitors was obtained only with the largest dose 17 hours after oral administration.

Myristicin treatment of 6 rats increased

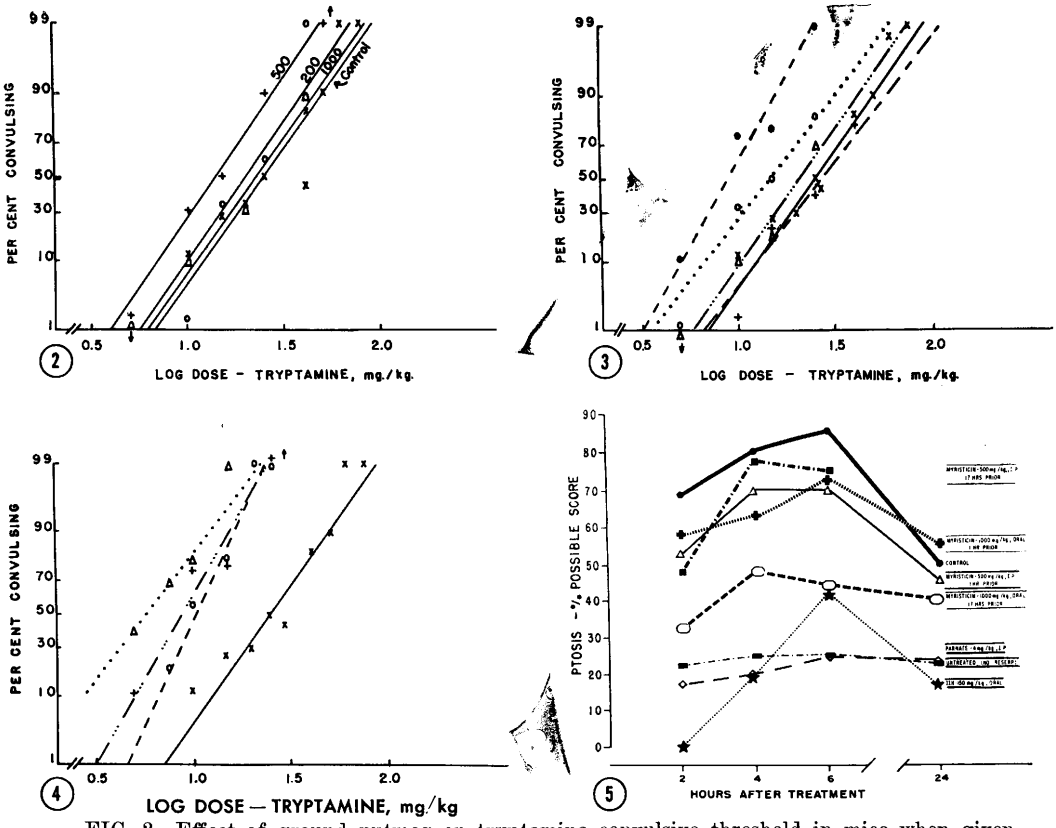


FIG. 2. Effect of ground nutmeg on tryptamine convulsive threshold in mice when given orally in acacia suspension 18 hr before test. ×———× Control, CD_{50} mg/kg (± 95% confidence limits) 25.0 (15.2-41.0); ○—200—○ 200 mg/kg nutmeg, 20.0 (14.2-28.2); +—500—+ 500 mg/kg nutmeg, 14.0 (10.1-19.5); △—1000—△ 1000 mg/kg nutmeg, 23.0 (16.1-32.9).

FIG. 3. Effect of synthetic myristicin samples and oil of nutmeg concentrate on tryptamine convulsive threshold in mice when given orally in acacia suspension 18 hr before test. ×———× Control, CD_{50} mg/kg (± 95% confidence limits) 25.0 (15.2-41.0); ●— —● myristicin sample 1 at 500 mg/kg, 8.7 (5.7-13.4); ○—•••○ myristicin sample 2 at 500 mg/kg, 14.0 (9.3-21.0); • • • • • oil of nutmeg concentrate 500 mg/kg, 20.5 (14.5-28.9); +—— —+ oil of nutmeg concentrate – 1000 mg/kg, 27.0 (19.9-36.7).

FIG. 4. Effect of monoamine oxidase inhibitors and synthetic myristicin on tryptamine convulsive thresholds in mice when given orally in acacia suspension 18 hr before test. ×———× Control, CD_{50} mg/kg (± 95% confidence limits) 25.0 (15.2-41.0); ○— —○ 150 mg/kg iproniazid, 10.4 (8.8-12.2); △ • • • • △ 4 mg/kg tranylcypromine, 5.8 (4.4-7.7); + — • • • + 500 mg/kg, 8.7 (5.7-13.4).

FIG. 5. Effect of monoamine oxidase inhibitors and various schedules of myristicin on reserpine ptosis in rats. Ptosis score: 0 = Eyelid fully open — 5 = Eyelid fully closed. Maximum score = 10/rat (both eyes). Group ptosis score (%) $= \frac{\text{No. rats/group} \times \text{Max score/rat}}{\text{Sum of group eyelid scores}} \times 100$.

brain 5-hydroxytryptamine from control values averaging 0.48 (± 0.05) μg/g to 0.82 (± 0.03) μg/g when given in an oral dose of 1 g/kg and the difference was statistically significant ($p<0.001$). Lower doses were not significantly active.

Discussion. These data may be taken as preliminary evidence of MAO inhibition by nutmeg and myristicin. Further corroboration is necessary by tests of brain MAO kinetics *in vivo* since aqueous solution of myristicin is impractical. These studies are in preparation.

Although the evidence of MAO inhibition through an effect on tryptamine is indirect, Maxwell *et al.*(7) found very good correlation between tryptamine toxicity in mice and *in vivo* MAO inhibition in mouse brain. Res-

erpine antagonism is a general characteristic of both MAO inhibiting and iminodibenzyl types of anti-depressant drugs. The ability of myristicin to increase brain 5-hydroxytryptamine is also circumstantial evidence for enzyme inhibition.

Even though they are not markedly potent inhibitors of MAO, nutmeg and myristicin are relatively safe compounds with respect to human toxicity. Doses of 10-15 g are required to produce acute intoxication in man with ground nutmeg. Oral doses in amounts up to 400 mg of the distilled concentrate of oil of nutmeg have not shown toxicity in human volunteers(1).

A preliminary human trial with nutmeg has been carried out in one depressed and 4 schizophrenic patients by Dr. Albert A. Kurland at Spring Grove State Hospital. Capsules of ground nutmeg containing 500 mg were given 3 times daily for 3 weeks. One patient was markedly improved, 3 showed some improvement and 1 showed no response. A further trial is contemplated in patients showing more depressive symptoms as a part of their mental difficulties.

The instability of synthetic myristicin appears to be a problem in its evaluation and use as the active component of nutmeg. Gas chromatographic evidence showing formation of an additional peak in aged preparations confirm this possibility. However, no precautions other than cold and the exclusion of light were taken with the synthetic compounds.

Summary. Nutmeg and its active component, myristicin, show evidence of central monoamine oxidase (MAO) inhibition by their ability to lower the convulsive dose of intravenous tryptamine in mice and to increase rat brain 5-hydroxytryptamine concentrations. They also show some ability to antagonize reserpine-induced ptosis of the eyelids. Myristicin is chemically unique as a nitrogen-free MAO inhibitor. Although its potency in this respect is not comparable to some of the more potent inhibitors such as tranylcypromine and iproniazid, it seems quite adequate when compared to its low toxicity. Other volatile components of nutmeg such as borneol, geraniol and safrole, do not show tryptamine potentiation, although some appear to cause C.N.S. stimulation in high doses. Further study is recommended for more direct evidence of nutmeg and myristicin as enzyme inhibitors and for their utility as anti-depressant drugs.

The authors gratefully thank Dr. Carl D. Lunsford, A. H. Robins Co., Richmond, Va., for synthetic myristicin; Dr. William K. Stahl, McCormick & Co., Baltimore, Md., for nutmeg and gas chromatographic studies; Magnus, Mabee and Reynard, Inc., New York, for nutmeg oil concentrate; Dr. E. J. Fellows, Smith Kline and French Co., Philadelphia, Pa., for tranylcypromine, Dr. R. D. Phillips, Hoffmann-LaRoche Co., Nutley, N. J. for iproniazid, and Dr. R. M. Burgison of this Department for chemical aid.

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