

DNP and N_2 greatly increased the level of Schiff base and decreased the level of nucleoside in the tissue. These results suggest that the role of ATP in ethylene synthesis may occur after the formation of a methionine-pyridoxal complex.

Oxygen is required for the conversion of methionine to ethylene, but it has been found that the lag period observed in the conversion of methionine to ethylene is oxygen-independent¹⁰. This suggests that conversion of methionine into ethylene proceeds in 2 steps: the first step is the oxygen-independent conversion of methionine to an intermediate and the second step is the oxygen-dependent conversion of the intermediate to ethylene. One may expect that the level of the intermediate would increase under a N_2 atmosphere as compared to air. According to the reaction scheme proposed for the conversion of ethylene⁴ (figure), the oxygen-independent step would be the conversion of a pyridoxal phosphate enzyme into a pyridoxal-methionine Schiff base. If this is correct, it would be expected that the Schiff base would accumulate under a N_2 atmosphere. Since L-canaline binds stoichiometrically with pyridoxal phosphate¹⁵, it would follow that the formation of pyridoxal-methionine Schiff base would be decreased in the presence of canaline (table 2). The importance of monitoring the levels of 5'-methylthioadenosine stems from observations that no volatile sulfur compounds are released during ethylene synthesis^{8,9}, and the conclusion that the methylthio group must be retained and recycled into methionine in order to sustain ethylene

production during fruit ripening⁹. In yeast it has been shown that 5'-methylthioadenosine is first converted to methionine prior to formation of S-adenosyl-L-methionine¹⁶. The present experiments further support the hypothesis that in apple tissue 5'-methylthioadenosine acts as the donor compound in cycling the methylthio group back into methionine^{5,7}. As yet it is not known whether this step is a transmethylation or if transfer of the methyl group occurs independent of the sulfur atom. As well, the acceptor compound remains unknown. We are presently investigating this step as it may regulate the rate of ethylene formation since retention of sulfur in the tissue is necessary for continued synthesis of ethylene from methionine.

Caution must be exercised in the interpretation of this data, since methionine can react with other pyridoxal enzymes and the methylthio group may be transferred into other sulfur amino acids as well as methionine which may not be involved in ethylene synthesis. However, it has been reported that conversion of exogenously supplied methionine into ethylene is the major metabolic pathway for methionine in postclimacteric apple tissue, provided the amount of methionine supplied is very small⁸.

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Identification of thromboxane B_2 in guinea-pig uterine homogenates¹

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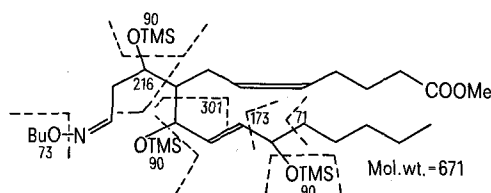
Summary. On the basis of gas chromatographic and mass spectrometric evidence, thromboxane B_2 has been identified in incubates of homogenised guinea-pig uterus.

Homogenates of guinea-pig uterus synthesize prostaglandin $F_{2\alpha}$ (PGF_2) and smaller quantities of prostaglandin E_2 (PGE_2) from endogenous precursors when incubated in vitro⁴. This result has been confirmed using a uterine microsomal enzyme preparation and exogenous precursors². More recently, it has been found that homogenized guinea-pig uteri also synthesise 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo- $PGF_{1\alpha}$) in vitro³. This prostaglandin is the more stable metabolite of prostacyclin (PGI_2). In this study, guinea-pig uterine homogenates have been further investigated to see whether they also produce thromboxanes during incubation.

Material and methods. 9 guinea-pigs in dioestrus (vagina closed) were killed by stunning and incising the neck. Each uterus was removed, homogenised in 15 ml Krebs'

solution, and incubated at 37°C for 90 min, being aerated with 5% carbon dioxide in oxygen. Prostaglandins and any thromboxanes formed were solvent extracted⁴. The 3 samples were pooled into 3 groups of 3 extracts, and further purified by silicic acid column chromatography⁴. Thromboxane B_2 (TXB_2) is eluted in the 'prostaglandin E fraction' from the column. Consequently, this fraction was converted to the methyl ester, trimethylsilyl ether derivative (Me-TMS), methyl ester, butyloxime, trimethylsilyl ether derivative (Me-BuO-TMS) or the methyl ester, methoxime, trimethylsilyl ether derivative (Me-MO-TMS) for analysis by combined gas chromatography and mass spectrometry (GC-MS) by methods described previously⁵.

Results. The Me-TMS derivative of the post-column extract produced a GC peak at carbon value (C value) 24.6, the reported value for this derivative of TXB_2 ⁶.



Methyl ester, butyloxime, trimethylsilyl ether (Me-BuO-TMS) derivative of thromboxane B_2 . Some fragments produced on mass spectrometry.

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The mass spectrum taken of this peak was identical to that reported for TXB_2 (Me-TMS), 2 characteristic ions occurring at m/e 600 (molecular ion, M) and 256 (base peak)⁶. The Me-BuO-TMS derivative of the post-column extract produced a GC peak at C value 26.9. The mass spectrum taken had significant peaks at m/e 671 (M), 656 (M-15), 640 (M-31), 600 (M-71), 598 (M-73), 581 (M-90), 510 (M-90-71), 508 (M-90-73), 491 (M-2 \times 90), 420 (M-2 \times 90-71), 418 (M-2 \times 90-73), 408, 328 (M-3 \times 90-73), 301 (base peak) 216, 211 (301-90), 191, 173 and 142 (α chain). This mass spectrum is consistent with the Me-BuO-TMS derivative of TXB_2 (figure). It is very similar to the mass spectrum of the Me-BuO-TMS derivative of 6-oxo-PGF_{1 α} (C value 26.8, 2nd isomer)⁵, as the 2 compounds are isomers. The major differences are the lack of m/e peaks at 413, 229 and 199 and the addition of m/e peaks at 408, 301, 216, 211 and 142 in the mass spectrum of TXB_2 compared with 6-oxo-PGF_{1 α} (Me-BuO-TMS derivatives). The Me-MO-TMS derivative of the post-column extract produced a GC peak at C value 25.1, the expected value for this derivative of TXB_2 ⁷. The mass spectrum had a molecular ion at m/e 629, 42 mass units lower than the molecular ion of the Me-BuO-TMS derivative due to the differences in mass between a butyl and methyl group. Other peaks produced by fragments containing

the oxime group were also 42 mass units lower than similar peaks occurring in the spectrum of the Me-BuO-TMS derivative e.g. m/e 216 in the Me-MO-TMS derivative had moved to 174 in the Me-MO-TMS derivative. However, the base peak in the latter derivative was still at m/e 301, as expected, since this fragment does not contain the oxime group. The mass spectrum obtained was consistent with the Me-MO-TMS derivative of TXB_2 . It was similar but not identical to the Me-MO-TMS derivative of 6-oxo-PGF_{1 α} (C value 25.3).

No quantitation of the amounts of TXB_2 produced was attempted due to the lack of authentic TXB_2 for assay purposes. It is very unlikely that the TXB_2 was produced by the small quantity of blood trapped in the uterine tissue since TXB_2 production by guinea-pig whole blood is very low⁸, and the amounts produced by the residual blood alone would be below detection limits. Consequently, this study has shown that homogenates of guinea-pig uterine tissue produce TXB_2 , in addition to PGF_{2 α} , PGE₂ and 6-oxo-PGF_{1 α} , when incubated in vitro.

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Analyse d'une population de *Cannabis sativa* L. originaire du Mexique et cultivé en France¹

Analysis of a population of *Cannabis sativa* L. originating from Mexico and cultivated in France

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Summary. Cannabinoid content of a population of *Cannabis sativa* L. originating from Mexico and cultivated in France is studied. The statistical analysis of results shows how difficult it is to obtain homogenous and representative samples. This problem is correlated to genetic heterogeneity of seeds.

Au cours de nos travaux sur le *Cannabis sativa* L. nous avons été souvent confrontés au problème de l'hétérogénéité des échantillons représentatifs d'une population de Chanvre³.

Des cultures expérimentales sont réalisées aux Etats-Unis⁴, au Canada⁵ et en Tchécoslovaquie⁶ notamment; les analyses portent toujours sur un échantillon moyen établi à partir d'un mélange de plusieurs plantes.

Il nous a paru intéressant d'effectuer une étude statistique de la variabilité d'une population de Chanvre, d'origine

géographique déterminée. Nos observations concernent les quatre lots de *Cannabis* originaire du Mexique⁷ et cultivé en plein champ, en France, à l'abri des fertilisations croisées. Le climat est tempéré et diffère des conditions d'environnement d'origine.

Matériel et méthodes. Les semis sont réalisés en pleine terre, à partir des graines contenues dans de la marijuana mexicaine en provenance des 3 localités:

Durango: lot 1A et 1B,
Michoacan: lot 2,
Oaxaca: lot 3.

Les sommités des plantes mâles et femelles sont prélevées individuellement, de façon standardisée⁸ au stade de la floraison et de la fructification c'est-à-dire environ 5 et 6 mois après la date du semis. La hauteur moyenne des

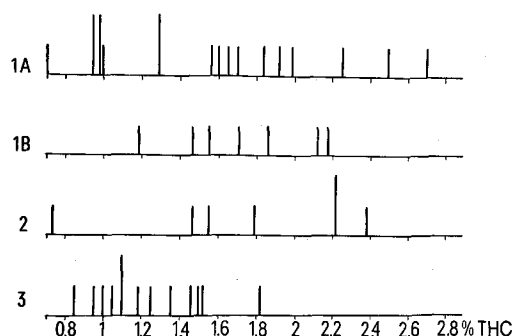


Fig. 1. Distribution du tétrahydrocannabinol chez les 4 lots de plantes mâles.

1 Remerciements. Les cultures ont été effectuées à l'Arboretum de Chèvreloup, France.

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