konnten dagegen zeigen, dass sowohl ein oxidativer Abbau zum Cotinin oder Nikotin-N-oxid als auch ein demethylierender zum Nornikotin erfolgt.

Die Untersuchungen wurden am weiblichen Göttinger Miniaturschwein durchgeführt. Pro Tier wurden 1,8 mg Nikotin in 0,9%iger NaCl-Lösung i.v. oder i.p. appliziert. Mit Hilfe von Dauerkathetern wurde der Urin über 6 h gesammelt und wie folgt aufgearbeitet: Nach Zusatz von 40 ml 30%iger NaOH pro 250 ml Urin Extraktion des Nikotins mit 3×200 ml Äther, Reduktion des Nikotin-N-oxid mit 20 ml 14–15%iger $TiCl_3$-Lösung unter Zusatz von 100 ml 5 *N* HCl und 5 ml 7,3 *M* NH_4 SCN-Lösung bei Zimmertemperatur während 15 h, Extraktion des Nikotins mit 3×200 ml Äther nach Zusatz von 100 ml 30%iger NaOH, Extraktion des Cotinins mit 3×200 ml $CHCl_3$, Bestimmung der Substanzen auf photometrischem Weg nach vorheriger dünnschichtchromatographischer Reinigung[8]. Auf diese Weise konnten eindeutig Nikotin, Nikotin-N-oxid und Cotinin sowie Nornikotin und Norcotinin nachgewiesen werden.

Auf Grund der bisher vorliegenden Versuchsergebnisse kann man offenbar davon ausgehen, dass etwa 5% des applizierten Nikotins unverändert neben etwa 10% Nikotin-N-oxid und je 3% Cotinin, Nornikotin und Norcotinin im Urin ausgeschieden werden. Genaue quantitative Aussagen sollen in weiteren Untersuchungen ermittelt werden.

Summary. In contrast to earlier experimental results, it could be demonstrated that swine can also enzymatically metabolize nicotine. An oxidative metabolism to cotinine or nicotine-N-oxide as well as a demethylation to nornicotine or norcotinine could be observed.

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A Base-Pairing Hypothesis for t-RNA Methylation

Recently, we elaborated synthetic inhibitors of methyl transferases[1–3]. While studying their action on the transmethylation of t-RNAs, we came to the conclusion that the adenyl moiety of S-adenosyl methionine (SAM) might play a role in the recognition process between the methyl transferase and its corresponding site on the t-RNA. A study of the sequence of 42 t-RNAs (including 2 alternative sequences) showed that 128 methylated nucleosides out of about 155 are found next to a uridine, a pseudouridine, a dihydrouridine or, in some minor cases, another derivative of uridine.

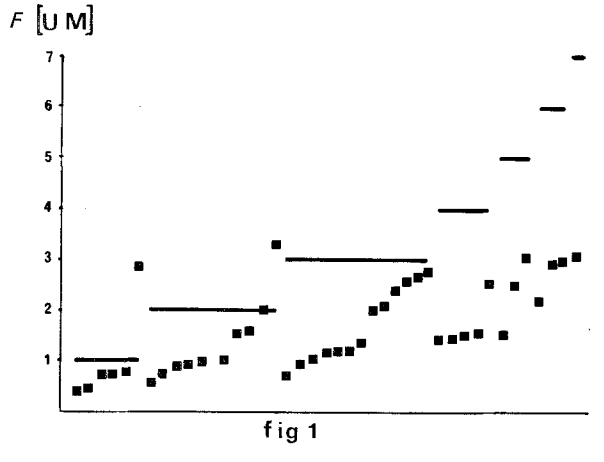

Fig. 1. Comparison between the observed number of methylated nucleosides [M] adjacent to uridine or a derivative [U] (—) and the corresponding calculated frequency (○) within each t-RNA.

In this communication, we propose a hypothesis implying the pairing of the adenyle moiety of SAM to a uridylic type residue (U, Ψ, hU ...) [U] of the t-RNA. The next nucleoside to a [U] being methylated.

Methods. The sequences of the t-RNAs containing methylated nucleosides studied in this communication were reviewed up to 1971[4]. The sequence of the other t-RNAs studied are the following: $t\text{-RNA}^{Arg}$ *E. coli*[5] $t\text{-RNA}^{Tyr}{}_{Su}III$ mutant A_1 *E. coli*[6] $t\text{-RNA}^{GlnI}$ *E. coli*[7], $t\text{-RNA}^{GlnII}$ *E. coli*[7] $t\text{-RNA}^{ArgII}$ yeast[8] $t\text{-RNA}^{SerIII}$ *E. coli*[9] $t\text{-RNA}^{Gly}$ yeast[10] $t\text{-RNA}_{Suf\ D}$[11] $t\text{-RNA}^{met}_{f}$ yeast[12].

For 2 adjacent nucleosides X and M (M standing for any methylated nucleoside), within a given sequence, the probable frequency XM or MX is given by the relation: $F = \frac{2mx}{(N-1)}$ where m stands for the number of methylated

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nucleosides (M). x stands for the number of nucleosides (X), N stands for the total number of nucleosides including x and m. This analysis was performed on each t-RNA studied and the results were compared to the cases XM or MX actually observed. The results are plotted in Figures 1–5.

Results and discussion. Figure 1 shows that generally a methylated nucleoside (M) is more often adjacent to [U] than predicted by the F calculated values. For guanosine, cytidine and adenosine (not shown here), the results fall within or below these values.

Direct observation of the clover leaf structures of the t-RNAs shows that generally methylated nucleosides are located in non-paired regions. They are also found at the frontier of paired region and a loop, with one exception so far reported[10]: yeast t-RNA^{Gly} shows a Cm at the 4th position from the pG end, right in the middle of the stem. Therefore the analyses were repeated by taking into account only those sequences of nucleotides found in non-paired regions of the t-RNAs (where methylated nucleosides are usually found) and containing at least 1 M, including the base paired nucleosides at the extremities of these sequences (where methylated nucleosides are sometimes found). This different sampling leads to similar conclusions to those resulting from the sampling performed on the whole t-RNA sequences (Figures 2–5). The above analysis suffers, however, from some uncertainties concerning the published sequences in some t-RNAs, for instance, the corrected nucleotide sequence of yeast t-RNA^{Gly} has recently been reported by CHANG et al.[13]: m^{5}C adjacent [U] instead of C was found in the anti-codon loop.

The most frequent methylated nucleoside, thymine, is generally situated in the GTΨ loop, which is in agreement with the hypothesis. On the other hand, and although it was included in our standard calculations, N^2-methyl guanosine is always found adjacent to a cytidine residu, a [U] being found generally at a β-position. We can not yet explain this apparent exception.

The hypothesis which we propose concerning a possible mechanism of the methylation of t-RNAs implies the pairing of the adenine moiety of SAM to a uridylic acid residue of the t-RNA. It is improbable that this binding would be sufficient to produce a recognition for the methylase to its site. It could represent an additional link between the enzyme and the t-RNA substrate through the SAM molecule.

A model of SAM-enzyme-t-RNA ternary complex. The above hypothesis is also supported by some of our previous studies on the inhibition of t-RNA methyl transferases by synthetic analogs of S-adenosyl homocysteine[14]. We have shown recently that S-(N^6-methyl adenosyl) homocysteine is a good competitive inhibitor of t-RNA methyl transferases of rabbit liver, whereas S-inosyl homocysteine lacking the 6-amino function is virtually devoid of inhibitory activity. These facts suggest that only one of the protons of the N^6 adenyl of SAM is linked to the enzyme, the other being involved in the pairing of a [U] as shown in Figure 6.

The representation of the SAM bound enzyme in Figure 6 with the adenine lying above the plane of the ribofuranose (*syn* conformation) is justified by NMR studies and calculations of charge densities in purine systems[15,16]. The resulting electron delocalization would be stabilized when the N^3 charge solves the sulfonium pole, facilitating thus the pairing with [U]. This (N^3) $\rightarrow$ (S$^+$) interaction would lower the energy level of the transition state during the demethylation process of the sulfonium. Interestingly enough, when the sulfonium pole is interacting with the N^3 atom, the resulting conformation with

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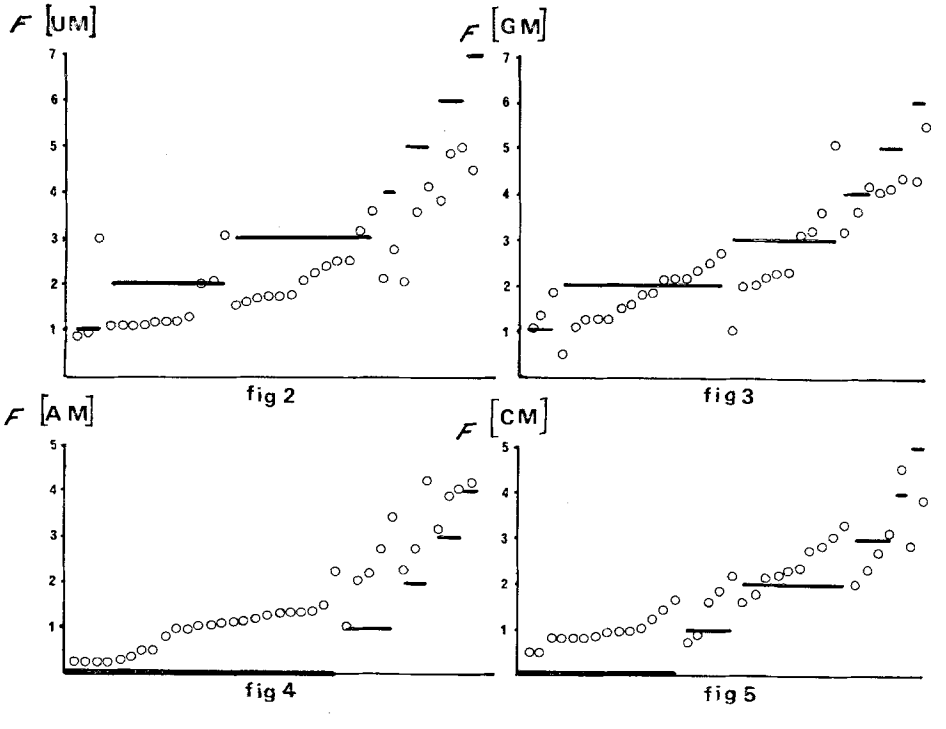

Fig. 2–5. Comparison between the observed number of methylated nucleosides [M] (—) adjacent to [U] (Figure 2), to [G] (Figure 3), to [A] (Figure 4) and to [C] Figure 5) and the corresponding calculated frequencies (○) within each t-RNA. The calculations were performed by taking into account only these portions of t-RNA which contain at least one [M] as defined in the text.

respect to the C(5′)-(S⁺) bond would be the so-called 'gauche, gauche', a conformation known to be predominant in free nucleosides[17].

Previous experiments using synthetic inhibitors[3] have shown that the terminal amino group of S-adenosyl homocysteine is important for binding to a t-RNA methyl transferase, whereas the carboxylate produces only a small contribution to this binding.

Careful observation of a molecular model of the proposed ternary complex suggests that the methyl group bound to sulphonium of SAM should point outwards from the enzyme in order the meet the t-RNA site of methylation. Preliminary experiments which will be reported later, have shown that chemical replacement of the methyl group in SAM by groupments such as allyl, methanoic or benzyl, led to compounds displaying affinity for a t-RNA methyl transferase from rabbit liver, comparable to SAM or close to the affinity displayed by SAM.

Finally, it would be of interest to check the validity of this base-pairing hypothesis by studying the sequences found in 'hypermethylated t-RNA'. This type of study was undertaken only in a few cases, therefore not allowing any statistical observations.

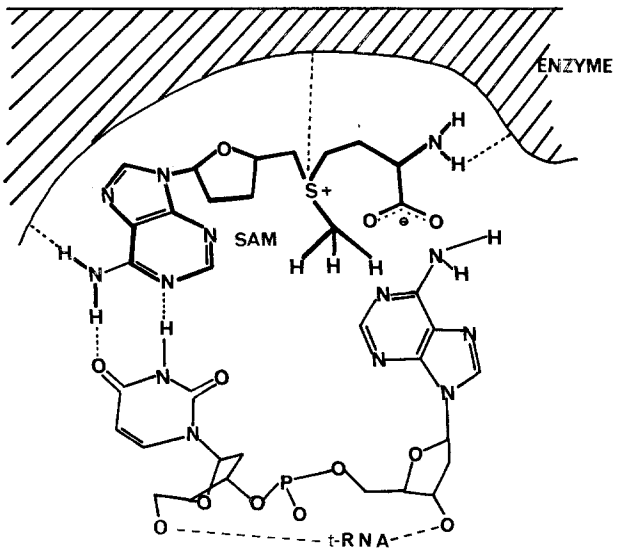

fig 6

Fig. 6. Hypothetical model of the interaction between a uridine of t-RNA and the S-adenosyl methionine-enzyme complex. The adenosyl methionine molecule is drawn with heavy lines. For the sake of clarity, neither the oxygen functions of ribose or phosphate are represented, nor the various hydrogen atoms attached to carbon or phosphorus.

Résumé. L'examen statistique des séquences des t-ARN indique qu'un nucléoside méthylé est généralement adjacent à une uridine ou à un analogue. Ces observations suggèrent que le processus de méthylation du t-ARN se ferait par l'intermédiaire d'un complexe ternaire dont les implications conformationnelles sont discutées.

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Cis-10-Tetradecenyl Acetate, an Attractant Component in the Sex Pheromone of the Oak Leaf Roller Moth (*Archips semiferanus* Walker)

The oak leaf roller, *Archips semiferanus* Walker (Lepidoptera: Tortricidae), is a destructive forest insect which has recently reached damaging population levels in the Northeastern United States. In Pennsylvania alone, the oak leaf roller infestation has spread to more than 1 million acres, and tree mortality due to the pest has climbed as high as 90%. The use of pesticides to control the oak leaf roller has not been economically feasible nor environmentally advisable; this has prompted our search for a natural chemical alternative.

Adult oak leaf roller males have been shown to respond to a sex pheromone produced by the female moth[1]. Moreover, preliminary separation of the active principles of the pheromone has revealed that two chromatographically isolable fractions are involved in the female's sexual message[2]. Each fraction elicits a separate behavioral response from male moths in laboratory and field assays, namely, sexual excitation and sexual attraction. We wish to report here on the identification, synthesis and activity of the major attractant.

Crude pheromone extracts were obtained from 2-day-old, adult, virgin female oak leaf rollers by excising the last abdominal segments and homogenizing them in a tissue grinder with redistilled spectrograde methylene chloride. The homogenate was filtered through a medium porosity fritted glass filter and concentrated on a rotary evaporator. Initial separation was achieved by thin layer chromatography on silica gel (Brinkmann Silplate F-22) using a 50:50 methylene chloride-hexane solvent system. An active region was observed at Rf 0.46–0.67 using a laboratory flight chamber bioassay[1]. The active TLC band was analyzed by gas-liquid chromatography (GC): 5% SE 30 on 80/100 mesh chromosorb Q; 6′, 2 mm I.D. glass U-tube column; oven, programmed from 125 °C to 200 °C at 1°/min.; helium carrier gas, 40 ml/min. The gas chromatograph was equipped with a flame ionization detector and an effluent splitter adjusted to a 25:75 detector:effluent split ratio. Small fractions were arbitrarily collected from the GC in glass capillary tubes cooled in liquid nitrogen. All fractions were laboratory and field bioassayed[1,2] and activity was found in only 1 fraction which had a retention time of 10.1 min. The

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