was recorded for 1 min and subsequently 0.2 ml glucose (0.5 *M*) and 0.025 ml exocinase (Sigma; 15 µg prot./ml) were added. The activity was recorded for a further 15 min: at this time 0.025 ml of exocinase were added. The protein content of the samples was determined by GORNALL's et al.[14] method.

The oxidative activity of the preparation in absence of exogenous substrates is greater in the supernatants (Table I). In the presence of glucose and G-6-P, the oxygen uptake increases greatly in preparations containing bacteria and mitochondria (P); a higher rate of activity is to be found when supernatants and precipitates are pooled (P + S); corresponding increments in aposymbiotic preparations (P′) and (P′ + S′) are very high, but the absolute values for oxygen consumption are nevertheless very small. These results agree with the experimental data previously obtained[15,16] which showed greater aerobic utilization of glucose by the symbiotic fat body than by the aposymbiotic one. The comparison of the data of symbiotic and aposymbiotic preparations could indicate that the bacteria also possess the enzymatic pool for the anaerobic metabolism of glucose. In fact (P), which contains mitochondria and bacteria, is able to utilize aerobically glucose and G-6-P, while (P′), on the contrary, is only just able to oxidize these substrates. Furthermore (P′ + S′) containing fat body mitochondria and extramitochondrial enzymes of the glycolithic pathway, utilizes aerobically the two above-mentioned substrates. Thus the higher concentration of G-6-P (Table II) found in symbiotic fat bodies, in comparison to aposymbiotic ones, could be interpreted in terms of the high efficiency of the glycolithic pathway of the bacteria.

The addition of α-ketoglutarate or of isocitrate, causes a remarkable increment in the oxygen uptake of (P) and (P′), and the absolute values obtained with symbiotic preparations are several times greater than those obtained with homologous aposymbiotic preparations. These results may suggest a higher quantitative efficiency of the tricarboxylic acid cycle in normal fat bodies, owing to the presence of bacteria; this metabolic pathway is also present, however, in the aposymbiotic fat body, which does in fact possess in both trophocites and bacteriocites its own mitochondrial complement. In presence of α-ketoglutarate and isocitrate, the oxygen uptake of (P + S) and (P′ + S′) is lower than that of P and P′ respectively; the lower mitochondrial concentration is probably the cause of this decrement. (P + S) shows far greater activity than (P′ + S′). The activity of (S) and (S′) appears to be constant with all the substrates experimented.

The activity values of succinic oxidase and of cytochrome oxidase (Table II), are many times higher in the symbiotic preparations, thus confirming the hypothesis of the mitochondrial function of the symbionts. The high enzymatic activity is correlated with a greater concentration of ATP in the symbiotic fat body (Table II).

From the data of TARVER and PIERRE[5], of DUBOWSKY and PIERRE[6] and from the data here described, emerges the general consideration that symbiont bacteria, besides carrying out glycogen metabolism[16], are able to perform some fundamental mitochondrial functions.

The absence of endosymbionts not only deprives the insect of the enzymatic pool and of the metabolites of the bacteria themselves, but has a negative influence on the fundamental metabolic processes of the host-insect, which, in the absence of exogenous supply from the bacteria, is no longer able to satisfy the energetic needs of its own organism. The considerable pathological modifications of the aposymbiotic fat body and of the aposymbiotic individuals themselves could therefore possibly be related to the difficulty of the tissue to maintain an adequate metabolic level.

Table II

	Symbiotic fat body	Aposymbiotic fat body
Succinate oxidase [a]	8.9×10^{-2}	7.2×10^{-3}
Cytochrome oxidase [b]	6.1×10^{-2}	8.2×10^{-3}
ATP [c]	3.6×10^{-3}	1.6×10^{-3}
G-6-P [d]	1.3×10^{-2}	7.4×10^{-3}

[a] Activity in µ*M* O_2/mg prot./min, in presence of Sørensen phosphate buffer, 48 µ*M*, pH 7.6; EDTA, 300 µ*M*; Na succinate, 27 µ*M*; cytochrome C, 1 mg; (P) or (P′) 0.5 ml. (prot. conc. ≃ 20 mg/ml). Volume of liquid = 3.0 ml. [b] Activity in µ*M* cytochrome C oxidized per min/mg of protein. [c] µ*M* ATP/mg protein. [d] µ*M* G-6-P/mg protein.

Riassunto. I batteri simbionti endocellulari dei Blattoidei svolgono attività proprie dei mitocondri ossidando attivamente l'α-ketoglutarato, l'isocitrato, incrementando l'attività succino-ossidasica, citocromo-C ossidasica e la concentrazione di ATP nel tessuo ospite rispetto a quello aposimbiotico. Il metabolismo del glucoso e del glucoso-6-fosfato è efficiente solo in presenza dei simbionti. La concentrazione di glucoso-6-fosfato è maggiore nel tessuto simbiotico.

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Über den Nikotinstoffwechsel beim Schwein

Das starke Interesse an der pharmakologischen Wirkung von Nikotin bzw. Tabakrauch hat zu zahlreichen Untersuchungen über den Stoffwechsel des Nikotins geführt (Hamster, Ratte[1,2], Meerschweinchen, Maus, Kaninchen[2], Hund[3], Katze[4] und Mensch[5]). Rückschlüsse aus der pharmakologischen Wirkung von Substanzen am Tier auf den Menschen scheinen in mancher Hinsicht beim Schwein besonders gut möglich zu sein[6]. Es ist daher wünschenswert, auch die Wirkung von Tabakrauch am Schwein zu prüfen. Dies erschien bisher nicht sinnvoll, da nach Untersuchungen von WERLE und MÜLLER[7] das Schwein nicht zum Abbau von Nikotin in der Lage sein soll. Wir

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.

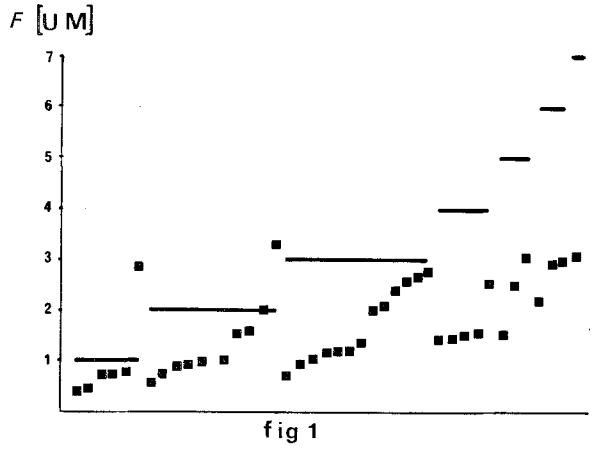

Fig. 1. Comparison between the observed number of methylated nucleosides [M] adjacent to uridine or a derivative [U] (—) and the corresponding calculated frequency (○) within each t-RNA.

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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