The Function of the Endosymbiotic Bacteria of *Blattoidea*

Some aspects of the function of the endosymbiotic bacteria of *Blattoidea* are already known, since it is possible to deprive the insects of their own symbionts[1-3], and to cultivate the microorganisms[4], and thus to study separately both the metabolism of the bacteria, and of the host. It was shown that the symbionts possess two enzymes of the tricarboxylic acid cycle: L-malate: NAD: oxydoreductase (1.1.1.37) and threo-D_S-isocitrate: NADP oxydoreductase (1.1.1.42)[5,6] which are utilized by the fat body its own metabolism. Two other enzymes of the endosymbionts, urate: oxygen oxydoreductase (1.7.3.3.) and guanine aminohydrolase (3.5.4.3.) may be considered useful to the insect during the larval stages[7,8].

In this work, the oxydative activity of normal and aposymbiotic fat bodies was assayed in presence of different substrates. The activities of the succinate oxidase complex and ferrocytochrome C: oxygen oxydoreductase (1.9.3.1) were also determined. Moreover the concentration of ATP and glucose-6-phosphate (G-6-P) were valued. The experiments were carried out on *Nauphoeta cinerea* Oliver.

In order to produce aposymbiotic strains, 200 fertilized females were injected with 1 mg of methoxyphenylpenicillinate of tetracycline[9]. All broods born between the 50th and the 100th day of the antibiotic treatment were aposymbiotic; the control and the aposymbiotic strains were fed daily with chopped lettuce mixed with Meritene (Farber Ltd.) and bran (3:1:1 w/w). The experiments were carried out on female nymphs only. The abdominal fat body was removed by ventral incision. The insects had previously been blocked in their motorial activity by refrigeration at +4 °C for 20 min. All the operations were carried out in a cold room. The samples used for the tests of concentration of ATP and of G-6-P were kept at −80 °C.

The homogenization was carried out (glass-teflon Potter) in sucrose 0.25 *M* containing K_2HPO_4 10.6 m*M* (pH 7.4). The homogenates were centrifuged at $121 \times g \times 10$ min at 0 °C; the fat in the upper part of test-tube and the precipitate were removed. The remaining supernatant was centrifuged at $5090 \times g$ for 20 min at 0 °C: mitochondria and bacteria were found to be present in the precipitate (P) of the normal insects: in the aposymbiotic preparations (P′) were found only mitochondria as proved from microscopic examination, after fixation and staining[10]. Moreover the precipitates were incubated with a solution (1:1:2 v/v) of phosphate buffer (0.2 *M*; pH 7.6), Na succinate (0.2 *M*) and neotetrazolium cloride (1 mg/ml); the bacteria showed one or more polar spots due to the formation of microcrystals of formazan[11]; the mitochondria were uniformly stained. Examination of the supernatants (S) and (S′) carried out by phase contrast showed only a few drops of fat.

Oxygen uptake in presence of different substrates and the succinate oxidase activity were tested manometrically in a Warburg apparatus at +37 °C (Tables I and II). The substrates were added to the side arm of the flasks. After temperature equilibration, the reaction was started by tipping the contents by side arm. Cytochrome oxidase activity was tested by SMITH's[12] spectrophotometric method; 0.30 ml of buffer phosphate were placed in a cuvette with 0.21 ml of ferrocytochrome C 1% (Sigma – Type III) and 2.49 ml of water; after equilibration at 37 °C, 30 µl of (P) or (P′) were added. The cytochrome C was dissolved in 0.01 *M* potassium phosphate buffer pH 7.0 and reduced with potassium ascorbate; the excess ascorbate was removed by dialysis against 0.01 *M* phosphate buffer pH 7.0. The readings of the decrement of the O.D. (at 550 nm) were taken every 15 sec for 2 min: in this lapse of time the decrements were found to be constant. Lastly the cytochrome C solution was oxidized with 30 µl of potassium ferricyanide 0.1 *M*. The protein concentration, in cuvette, was approximately 2 mg/ml. For determination of ATP and G-6-P, the fat body was ground in a mortar with triethanolammine 0.05 *M* (pH 7.6; 3 ml/g fat body); the homogenate was centrifuged for 1 h at $200{,}000 \times g$; the supernatant was deproteinized with $HClO_4$ (6%), centrifuged for 10 min at $13{,}300 \times g$ and neutralized with 5 *M* K_2CO_3. ATP and G-6-P concentration were tested by a modification of the LAMPRECHT and TRAUTSCHOLD[13] method, recording O.D. variation (at 366 nm) indicating reduction of TPN^+ to TPNH in a reaction system containing: triethanolammine 0.05 *M* (2.000 ml); β-TPN^+ 7×10^{-3} *M* (0.1 ml); $MgCl_2$ 0.1 *M* (0.175 ml); 0.5 ml of supernatant (prot. conc. ≃ 10 mg/ml). 3 min after addition of the sample, 0.01 ml G-6-P-D (Boehringer; 20 µg prot./ml) were added, and after a further 5 min 0.01 ml of G-6-P-D. The activity

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Table I

	P	P′	S	S′	P + S	P′ + S′[a]
Endogenous O_2 uptake	0.6×10^{-4}	0.5×10^{-4}	2.2×10^{-4}	1.9×10^{-4}	0.8×10^{-4}	1.1×10^{-4}
Glucose (15.0 µ*M*)	3.8×10^{-3}	1.2×10^{-4}	2.1×10^{-4}	2.4×10^{-4}	4.2×10^{-3}	8.8×10^{-4}
Glucose-6-P (18.0 µ*M*)	3.1×10^{-3}	1.6×10^{-4}	2.1×10^{-4}	1.9×10^{-4}	4.6×10^{-3}	9.9×10^{-4}
α-ketoglutarate (23.0 µ*M*)	7.6×10^{-2}	4.8×10^{-3}	1.9×10^{-4}	1.5×10^{-4}	4.1×10^{-2}	3.0×10^{-3}
Isocitrate (16.0 µ*M*)	5.9×10^{-2}	3.6×10^{-3}	1.7×10^{-4}	1.5×10^{-4}	3.1×10^{-2}	2.6×10^{-3}

P, precipitate of normal fat body homogenate, containing endosymbiotic bacteria and mitochondria; P′, precipitate of aposymbiotic fat body homogenate containing only mitochondria; S, supernatant of normal fat body homogenate; S′, supernatant of aposymbiotic fat body homogenate. µ*M* O_2/mg prot./min, in presence of $MgCl_2$, 10 µ*M*; KCl, 200 µ*M*; Sørensen phosphate buffer, 48 µ*M*, pH = 7.2; homogenate, 0.5 ml (prot. conc. ≃ 20 mg/ml). Volume of liquid = 2.8 ml. [a] P:S and P′:S′ = 1:1 v/v.

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