Metabolism of the Sulphur Amino-Acids in *Stegobium paniceum* L. and *Lasioderma serricorne* F.

That the intracellular yeast symbionts of the drug-store beetle, *Stegobium paniceum* L., supply the host with vitamins of the B-complex was reported by KOCH in 1933[1]. Since then his findings in this Anobiid have been enlarged upon by his students and others[2–5]. Recent reviews of the literature describe the vital role of intracellular symbionts in many other host organisms[6,7]. HENRY and BLOCK[8] showed that the cockroach, *Blatella germanica*, infected with its bacterial symbionts, is capable of utilizing inorganic sulphate for the synthesis of methionine and cystine, while the aposymbiotic roach cannot do so. The following investigations were carried out to determine the role of the symbionts of *S. paniceum* L. and of the closely related tobacco beetle, *Lasioderma serricorne* F., in the sulphur amino-acid metabolism of the hosts.

Both aposymbiotic and normally infected *Stegobium* and *Lasioderma* larvae, freshly hatched, were placed on a diet consisting of wheat grit and yeast extract (90:10 v/v), which was labelled with $Na_2S^{35}O_4$. After 3 weeks the larvae were homogenized and extracted, and the proteinaceous residues were hydrolysed. The extracts and hydrolysates were chromatographed in 2 directions on paper. The methionine and cystine spots were eluted from the paper and the radioactivity was measured in a liquid scintillation counter.

The results are listed in the Table. In *Stegobium* the specific activity for methionine and cystine was very low in the extracts and hydrolysates of both aposymbiotic and normally infected larvae. The specific activity of extracts from aposymbiotic and normal *Lasioderma* larvae was also low. However, in hydrolysates of normally infected larvae the specific activity of both methionine and cystine was found to be much greater than in aposymbiotic larvae.

These results show that larvae of *Lasioderma serricorne* F., infected with their normal yeast symbionts, are capable of utilizing inorganic sulphate in the synthesis of methionine and cystine. Larvae, artificially freed of their symbionts, are not able to synthetize these sulphur amino-acids.

Additional experiments are being carried out on *Stegobium* and *Lasioderma* with glucose-C^{14}-U to determine the role of the yeast symbionts in the carbon synthesis of these and other amino acids, and will be reported later[9].

Specific activity of S^{35}-amino-acids from larvae of *Stegobium paniceum* and *Lasioderma serricorne* after 3 weeks on diet labelled with $Na_2S^{35}O_4$

Amino acid	*Stegobium paniceum*[a]				*Lasioderma serricorne*[a]			
	Aposymbiotic		Normal		Aposymbiotic		Normal	
	Extract	Hydrolysate	Extract	Hydrolysate	Extract	Hydrolysate	Extract	Hydrolysate
Cystine	8	20	16	26	12	71	32	598
Methionine	0	10	4	18	16	62	23	3663

[a] Counts per minute per μmole of sulphur.

Résumé. Les larves de *Lasioderma serricorne* F., infectées avec leurs symbiontes intracellulaires normales, ont pu utiliser du sulfate inorganique dans la synthèse de méthiomine et cystine. Les larves libérées artificiellement de leurs symbiontes ne furent pas capables de synthétiser ces acides aminés sulfuriques. Les larves de *Stegobium paniceum* L. infectées normalement et les larves de *Stegobium paniceum* L. libérées des symbiontes n'utilisèrent pas de sulfate inorganique dans la synthèse de méthionine et cystine.

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Biochemical Characteristics of Yeast Respiration-Deficient Mutants Differing in Buoyant Densities of Mitochondrial DNA

Mitochondrial DNA (M-DNA) has been assumed to represent the extrachromosomal genetic factor whose modification determines cytoplasmatically inherited respiratory deficiency in yeast[1]. A number of cytoplasmic (ϱ^-) mutants have been prepared[2] which differed from each other and also from the original wildtype strain in buoyant densities of their respective M-DNA reflecting considerable differences in base composition[3].

It will be shown in this paper that despite these differences in the M-DNA all the mutants display uniform biochemical deficiencies lacking cytochromes $a.a_3$, b and c_1, oligomycin-sensitivity of mitochondrial ATPase, and the ability to incorporate amino acids by isolated mitochondria. In addition they show a uniform pattern of mitochondrial 'structural protein' in disc electrophoresis.

Material and methods. Haploid strains of cytoplasmic respiration-deficient mutants of *Saccharomyces cerevisiae*, listed in the Table, were kindly provided by Dr. H. Jakob (Centre de genétique moléculaire, Gif-sur-Yvette, France). Methods of culture, of isolation of mitochondria and of ATPase determination[5] and assay of amino acids incorporation by isolated mitochondria[6] have been described previously. Cytochrome spectra were measured in a SF 10 spectrophotometer equipped with an integrating sphere. Mitochondrial 'structural protein' was isolated[7] and its electrophoresis in polyacrylamide gel performed[8] according to published procedures.

Results and discussion. As observed with cytoplasmic mutants of unknown buoyant densities of M-DNA[9,10] all these mutants studied lack in their absolute or difference (reduced minus oxidized) spectra cytochromes $a.a_3$ and b. In addition, no evidence for the presence of cytochrome c_1 could be found by low-temperature spectroscopy.

Unlike the case of wild-type strains[5], mitochondrial ATPase from all the cytoplasmic mutants was found to be insensitive to oligomycin. This extends the previous finding obtained with single mutants[11,12].

Although the nature of mitochondrial 'structural protein', as well as its modification accompanying the cytoplasmic mutation in yeast, has become the matter of controversy[13–19], its properties have been examined. As shown in the Figure, the electrophoretic pattern of mitochondrial 'structural protein' has been found to be uniform in all the mutants.

Extending the previous finding obtained with a neutral and a highly suppressive cytoplasmic respiration-deficient strains[6], mitochondria isolated from all the strains studied were not able to incorporate ^{14}C-leucine into trichloroacetic acid-insoluble fraction under conditions in which wild-type mitochondria showed active incorporation.

The pleiotropic nature of the cytoplasmic mutation for respiratory deficiency may reflect either miscoding of several proteins or complete loss of some essential function of yeast M-DNA. The observation that all the cytoplasmic mutants examined exhibited the same biochemical characteristics independently of how profoundly the base composition of their respective M-DNA had been modified favours the latter possibility. Intact M-DNA may be a prerequisite for some complex mitochondrial function, e.g. synthesis or functioning of mitochondrial ribosomes or derepression of nuclear genes coding for some mitochondrial components. A certain change in base composition of M-DNA which results in respiratory deficiency in yeast may completely eliminate this function. Any further modifications exceeding this minimal limit would then not bring about any additional alteration of mitochondria[20].

Strains of cytoplasmic respiratory-deficient mutants employed in the study

Strain	Degree of suppressiveness[a] (%)[3]	Buoyant density in CsCl (g/cm^3)[3]
C 982-19 dA_1	0 (neutral)	1.682
D 243-2B-R_1-No6	0 (neutral)	?
D 243-2B-g	0 (neutral)	1.682
D 243-2B-120	51	1.684
D 243-2B-106	78	1.685
D 243-2B-116	95	1.689

[a] As defined by EPHUSSI and GRANDCHAMP[4].

Zusammenfassung. Es wurde nachgewiesen, dass, obwohl die mitochondriale DNS verschiedener, atmungsdefekter Hefemutanten sowohl untereinander als auch von den Wildstämmen unterschieden werden können, diese uniforme biochemische Charakteristika aufweisen: Fehlen von spektroskopisch nachweisbarem Zytochrom $a.a_3$, b und c_1, gegen Oligomyzin unempfindliche ATPase und die Unfähigkeit, Aminosäuren in Proteine zu inkorporieren.

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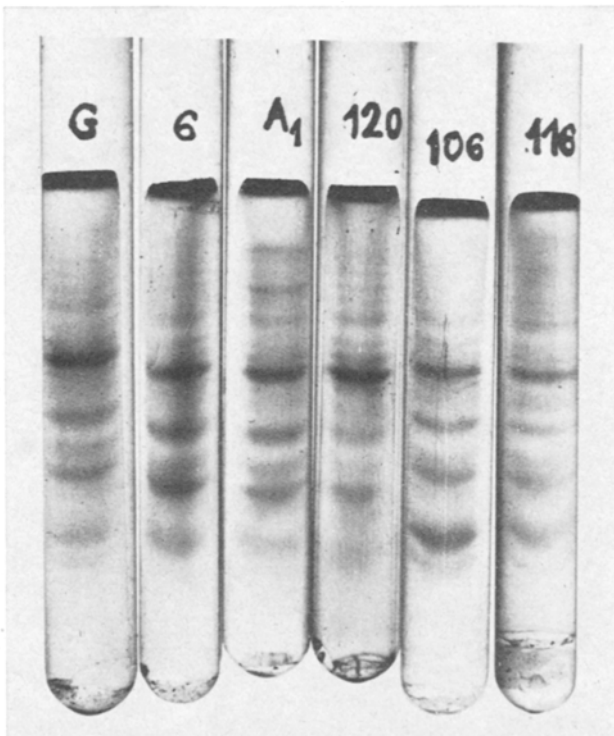

Electrophoretic pattern of 'structural protein' isolated from mitochondria of different cytoplasmic mutants. Abbreviated symbols of the strains used are indicated.

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