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The 'Spitzenkörper', centre of the reducing power in the growing hyphal apices of two septomycetous fungi[1]

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Summary. Redox dyes, such as methylene blue and neutral red, are stably reduced to their leucobase in the 'Spitzenkörper' of the growing hyphal tips of *Neurospora crassa* and *Monilia fructigena.*

The ultimate tip of vegetatively growing hyphae of Septomycetes contains a centrally located apical body, the so-called Spitzenkörper[2-4]. This spherical organelle, now known to be composed of microvesicles and membranous tubules[5], was first characterized by its high stainability with haemotoxylin dye[2], contrasting, however, with its absence of affinity for nucleic acid stains[6]. Practically nothing is known of its functional role except that it temporarily disintegrates when irradiated with strong light[6], and that it has been assumed to play a crucial role in the maintenance of the apical polar growth[5].

As we have recently found that the ultimate tips of vegetative hyphae of both the chytridiomycete *Allomyces* and the septomycete *Neurospora crassa* are highly reducing to a few selected semi-vital redox stains[7], we thought it would be interesting to try to localize more precisely the site of that apical reducing power in representatives of those fungi, the Septomycetes, all known to present a 'Spitzenkörper' in their hyphal tips[5].

Vegetative hyphae of *Neurospora crassa* (Lindegren strain A) were harvested either from the progressing front of mycelium growing for 16-20 h at 25 °C from the lower part of slants of malt agar inoculated with macroconidia, or from young mycelia developed from conidia germinating in liquid Vogel's medium[8]. The bunches of parallely elongating hyphae were bathed on glass slides in 10^{-4}-10^{-3} freshly prepared solutions of redox dyes (analytical grade) in distilled water below coverslips which were only momentarily lifted off to permit reoxidation of the leucobases in the mitochondrially-rich subapical zones of the hyphae. A similar method was applied to the pH indicators used and the lipid reagent (Sudan III in alcoholic solution) applied after pumping off excess water. Similarly, vegetative hyphae of *Monilia = Sclerotinia fructigena* Aden and Ruhl were grown on membranes aseptically placed on the surface of malt + casamino-acids agar plates[9]. Bits of mycelial margin were removed after 3 days growth at 25 °C and treated as the *Neurospora* materials. Microphotographs were made with Ilford pan F 120 on a Wild M 20-EB microscope.

Topological ultrastructural controls were obtained from current electron microscope studies in our laboratory using both *N. crassa*[10] and *M. fructigena*[9] and the technique described for *Allomyces*[11] to obtain longitudinally oriented thin sections of hyphae.

We first repeated our previous experiments with neutral red on *N. crassa* and extended them to the hyphal apices of *Monilia fructigena.* Using stepwise vertical focussing on the ultimate tip of *Neurospora* hyphae, we got a first glance at the densest location of the switch from red to yellow in a corpuscle corresponding in its size and location to the apical 'Spitzenkörper' (figure 1a). In an especially favourable case, in which the stain was vitally still faintly colouring in red the whole apical cytoplasm, the yellow tinge was restricted to the only apical granule detectable (figure 1b) which corresponded positionally to the 'Spitzenkörper', as checked by electron microscopy (figure 2b). In apices of *Monilia,* the relatively extensive yellow 'capping' of otherwise uniformly red coloured hyphae could also be located by adequate focussing in the spherical centre of the ultimate hyphal tips (figure 1c).

To decide whether the switch from red to yellow of neutral red was really due to the highly reducing power (lower than the $E'_o = -0{,}30$ of this stain at pH 7.0)[7] of the hyphal tips, especially of their 'Spitzenkörper', and not to local alkalinisation (an unexpectedly needed pH 8), we devised a few pH determinations using indicators overlapping for their colour changes: bromothymol blue stained yellow (pH 6.0) the apices (vivid yellow in the tip granule) with a subapical slightly greenish hue (pH 6.2) in a few relatively wide hyphae; with bromocresol green, all apices were mostly greenish ('Spitzenkörper' whitish) and never blue (pH 5.5), bromocresol purple turned to reddish orange (pH around 5.0) while Congo red provided a generalized reddish staining (pH at least pH 5.0); all these tests confirm the average of pH 5.0-6.0 (closer to 5.0) preliminarily visualized from the yellow colour obtained in bathing vegetative hyphal tips in an extracted water solution of the universal Merck indicator. In any case, such acidic pH, apparently reinforced in the ultimate tip as also suggested by the yellow staining by alizarin (sulfonated dioxy-anthraquinone) of the 'Spitzenkörper' compared to an orange tinge developing farther back in the hyphae, should exclude an alkaline-due yellow switch and confirm our previous assumption of a reductive reaction of neutral red at the level of the hyphal tip.

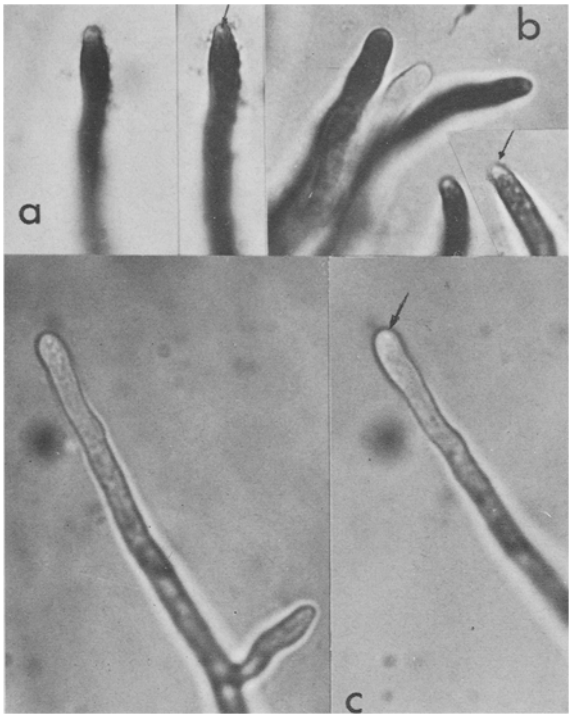

Fig. 1. Neutral red stainings of vegetative hyphae of *Neurospora crassa: a* red content (10^{-3}) with yellow apices and more vivid staining in a corpuscle (arrow); *b* faintly reddish cytoplasm (10^{-4}) with 2 levels of focussing on the yellow apical granule (arrow); *c Monilia fructigena* hyphae red (10^{-3}) but with yellow tips and apical granule (arrow). × 700.

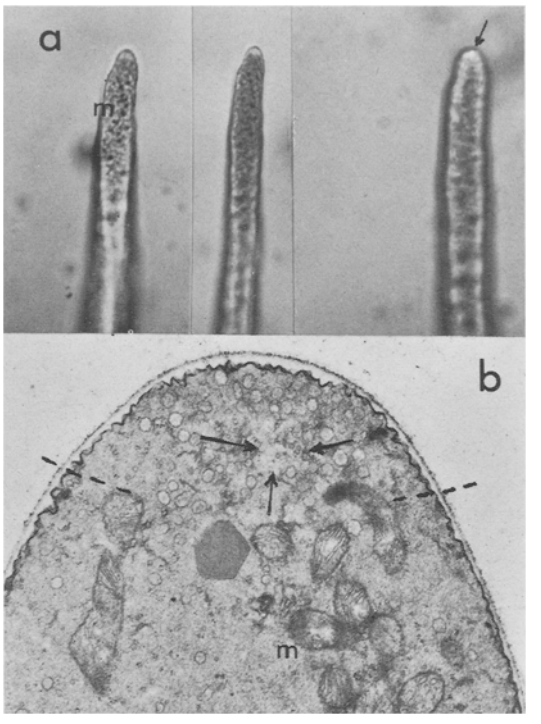

Fig. 2. Methylene blue stainings (10^{-3}) of growing hyphae of *N. crassa: a* more surface focussing on the blue recoloured mitochondria (m) and bluish-white apex; *b* more centrally focussed on the brilliant white 'Spitzenkörper' (arrow); × 700. *c* Submedian longitudinal thin section showing the delimitation of the microvesicula-containing 'Spitzenkörper' (arrows) in the exclusive apical zone rich in vesicula but deprived of mitochondria (m). × 18,000.

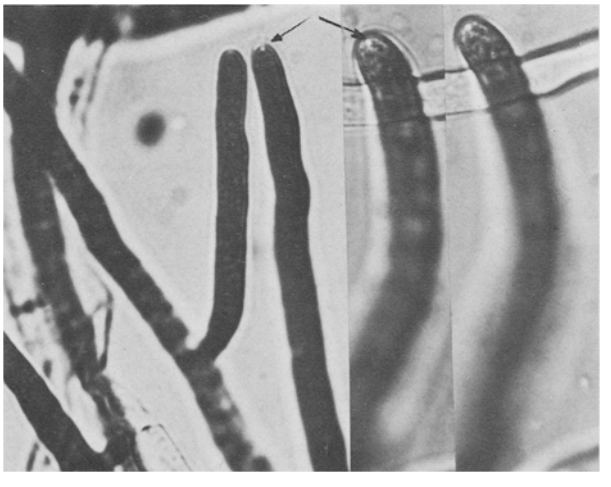

Fig. 3. Growing hyphae of *Monilia fructigena* fully stained with methylene blue (10^{-3}) except in the 'Spitzenkörper' (arrows; left × 700, right × 1000).

Methylene blue is the most currently used redox indicator dye ($E'_o = +0{,}011$ at pH 7.0)[12]. In cells submitted to semi-anaerobic conditions, it is discoloured to its leucobase and, when newly exposed to oxygen, it first recovers its blue colour at the level of oxidative structures. We have confirmed this in hyphae of *Neurospora* which recolour most vividly in the subpical, mitochondrially-rich zone following the momentary lift-off of the coverslip. The apical zone regains only an apparently uniform bluish-white hue (figure 2a) but remains fully colourless at the ultimate tip in which optimal focussing reveals on the fore-front the brilliantly white granule of the 'Spitzenkörper'. Such positioning of this organelle appears to be that of fast growing hyphae with narrow tips. Those which are apparently slower in growth and present a more dome-like tip can show the white granule in a relatively sub-frontal position as confirmed by electron microscopy (figure 2b).

In the wide hyphae (around 15 µm) of *Monilia* harvested from the surface of the nutrient medium, the methylene blue reaction was less contrasted and, in their almost colourless wide apical dome, a small whitish spherical zone could be vitally observed after repeated focussing observations. However, such hyphae when left for a few h in the mixture of methylene blue (around 10^{-4}) and nutrients dissolved from a bit of splashed medium in the aqueous preparation reinitiated elongating hyphal growth. Such secondarily, fast growing, narrower hyphae first remained more or less colourless even after draining fresh methylene blue solution (10^{-3}) below the coverslip but, following momentary removal of the coverslip for a few min of aeration, they took on a frank blue colour in all their cytoplasm except in a welldefined spherical zone of their tips which remained pure white (figure 3a). Such a colourless corpuscle is exactly positioned as the 'Spitzenkörper' of *Monilia* hyphal apices viewed on thin sections and found to be especially rich in microvesicula[9].

That the 'Spitzenkörper' concentrates the reducing power of the hyphal tips at least in Septomycetes is further shown by the fact that the contrast between its white, colourless state and its blue cytoplasmic environment tends to intensify with the duration of semi-anaerobic non-lethal maintenance below the coverslip (in humid chamber). This also

clearly argues against a possibility of restrictive entrance of the oxidized stain inside the relatively dense, microvesicular substructure of the apical corpuscle. Significantly, in the initial phase of the experiments, the whole tip of vegetative hyphae first takes on a bluish tint showing, as above with the pH indicators, that the stains can freely penetrate inside the spherical organelle, in which they either switch in their colour for pH reasons or, as with neutral red or methylene blue and additionally with Janus green B (white to pinkish 'Spitzenkörper' contrasting with the densely green mitochondria), as a consequence of their local reduction. To further ascertain whether the 'Spitzenkörper' is well differentiated in its permeability characteristics from the lipid granules, which can occasionally migrate close to the hyphal tips, especially in those of *Monilia*[9], vegetative hyphae were counterstained in Sudan III. Conclusively, in all actively elongating hyphae, a discrete white corpuscle could be seen in the ultimate tips, in clear contrast with the orange stained subapical lipid granules. Only in vacuolated hyphae, somewhat arrested in their growth, one stained granule could occasionally reach the tip.

A final question is what sustains the reducing power among the microvesicula of the 'Spitzenkörper'? It might be either NADH overproduced by apical glycolysis, further excreted as reducing equivalents of ethanol by vegetative hyphae at least in *Neurospora*[13] and/or -SH groups associated with the enzymatic proteins of this alcohol glycolysis[7]. In favour of the local accumulation of both types of these reducing compounds, we can make the following preliminary observations: a) a positive blue reduction of a direct acceptor of the NADH electrons[12], phenazine methosulfate (PMS), visibly expressed by a generalized greenish blue tinge in the apices of *Neurospora* hyphae ('Spitzenkörper' dispersed by the lethal, 10^{-4}, concentration of the yellow water solution of PMS) while a faint but more frankly blue reaction had been obtained with the *Allomyces* hyphal tips[7]; b) a positive reaction of Na nitroprusside revealed by a few dark reddish-violet deposits on the central part of apices elongating from wide hyphae of *Monilia* bathed in a 10^{-4} solution of this classical[14] and normally not too sensitive reagent for -SH groups.

1 We acknowledge the support of the 'Fonds National Suisse de la Recherche Scientifique' and thank both Mrs Ton-That for her electron micrograph and Miss E. Herz for her technical help.
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RNA polymerisation capacity and permeability to ribonucleoside triphosphates of nuclei from livers of whole-body X-irradiated rats

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Summary. For 4–18 h following whole-body X-irradiation of rats, liver nuclei showed a progressive increase in the permeability to ribonucleoside triphosphates (as assessed in vitro using tritiated uridine triphosphate (UTP)) and in the capacity to polymerise RNA in vitro (Mg^{++}-containing and $Mn^{++}/(NH_4)_2SO_4$-containing assay systems).

Whole-body radiation exposure of animals has been shown to stimulate the rate of RNA synthesis in the liver[1-3]. The stimulus in RNA synthesis correlates well with the increased capacity of liver nuclei to incorporate radioactive nucleoside triphosphates into RNA in vitro[1,4-6]. We have reported earlier that template efficiency of liver chromatin (though not activity of RNA polymerase as isolated free of template DNA) was significantly stimulated as a result of total-body radiation exposure[1]. Since the ability of liver nuclei to incorporate radioactive precursors into RNA was increased to a much higher extent (187% over non-irradiated control) than the increase in template efficiency of chromatin (about 51% over non-irradiated control)[1], it appeared that some other factors may be involved in the activation of nuclei. We have now obtained evidence which indicates that the liver nuclei could have become more permeable to nucleoside triphosphate precursors as a result of whole-body radiation exposure. Such change in permeability was not discerned if nuclei were irradiated in vitro.

Materials and methods. Wistar strain rats, each weighing between 150 and 160 g fed on a laboratory diet, were used. Rats were given a single whole-body dose of 1000 R of X-irradiation by housing them in groups of 3, in a 3-place perspex container located at the distance of 50 cm from 250 kV X-ray generator (Seimen's Stabilipan). The latter was operated at 15 mA tube current with added filter 2 mm Al. The dose rate was 100 R/min.

The procedures for isolation and purification of liver nuclei and assay for Mg^{++}-dependent and $Mn^{++}/(NH_4)_2SO_4$-dependent RNA polymerase reactions were carried out according to Widnell and Tata[7]. For in vitro irradiation of liver nuclei, isolated nuclei were suspended in 0.9% saline (≡3 mg DNA/ml) and irradiated by the X-ray generator at 0–2 °C.

Uptake of ^{3}H-UTP by isolated nuclei in vitro was studied by incubating the nuclear suspension (≡0.3 mg DNA) separately in the reaction mixtures (in 0.5 ml final volume) for assays of Mg^{++}-dependent and $Mn^{++}/(NH_4)_2SO_4$-dependent RNA polymerase activities with the following changes: Instead of the 4 nucleoside triphosphates, 0.3 µmoles of ^{3}H-UTP (7.5 µCi/mmole) and 15 µg actinomycin D were included in each reaction mixture. Actinomycin D was included to suppress synthesis of RNA, if any, from endogenous ribonucleoside triphosphates. The incubations were stopped by placing the assay tubes immediate-