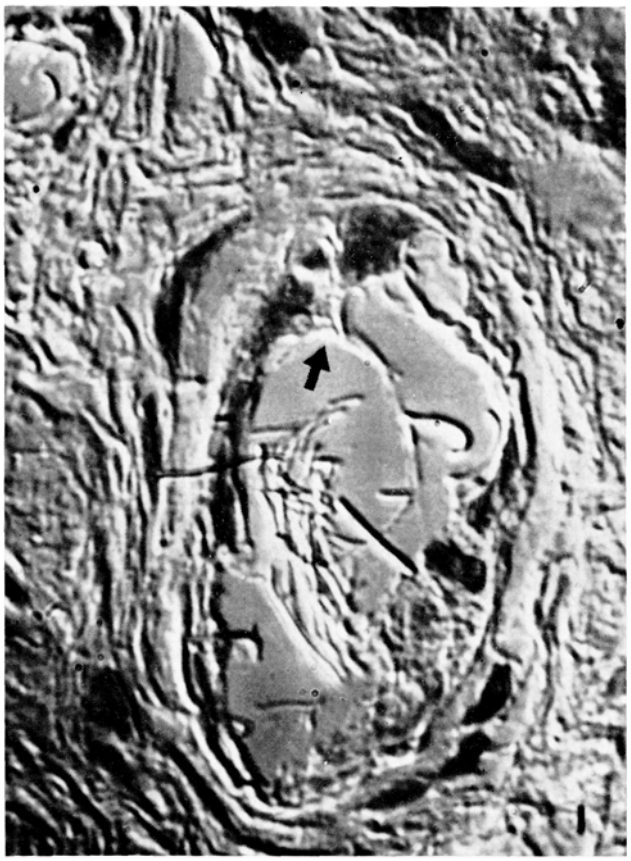
It is the intent of this report to present an observation that may shed light on one possible mechanism involved in sperm storage.

Methods. Female newts were killed by decapitation following anesthetization with MS-222 (Sandoz) and their cloacal regions were fixed in either 10% neutral buffered formalin or Bouin's fluid. These tissues were processed in the usual manner to obtain paraffin sections and the sections were stained with azure A (0.1% in 30% ethanol) or in a Feulgen-Naphthol yellow sequence.



Section through a spermathecal tubule containing several spermatozoa. The arrow indicates the site of sperm-epithelial cell contact. × 500.

Observations. It was seen that the sperm were accumulated in the proximal ends of the spermathecal tubules and were, for the most part, highly coiled. In order to study the positioning of the sperm in the tubules it was necessary to use animals which had only a small amount of sperm since it was rather difficult to trace the sperm from section to section when the concentration was high.

Within the spermathecal tubules the sperm heads appeared to be in contact with the epithelium (Figure) tubules. This condition was seen in animals killed at all seasons of the year. With the light microscope, the contact appeared to be in the region of the acrosome, however, a definitive statement on the sperm-epithelial cell contact must await further investigation with the electron microscope.

Discussion. An observation of the type reported is very suggestive of a nutritive or maintanance role on the part of the spermathecal epithelium. Although the sperm appear inhibited in the spermatophore it is doubtful that the polysaccharide material thought to be responsible for this inhibition could keep them in an inactive yet viable condition for a period of several months[6]. Histochemical studies on the spermatheca reveal that none of the polysaccharides of the spermatophore persist. Therefore, it is not unreasonable to suggest that the sperm of the urodele are maintained by the tubule epithelium in a relationship similar to that which exists between the Sertoli cells and sperm of the mammalian testis.

Zusammenfassung. Die Spermatheca des Weibchens zeigt bei den untersuchten Urodelen eine innige Beziehung zu den Spermien, so dass eine Ernährungsfunktion der Spermatheca wahrscheinlich gemacht werden kann. Es wird auf die Sertoli-Zellen der Mammalia verwiesen.

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The Cytochemical Demonstration of Dehydrogenases and Oxidases in the Cells of Fungi

In human pathology, methods for the intracellular detection of dehydrogenases and oxidases by means of more or less specific staining procedures have been developed and successfully used in the study of many organs. Extended investigations have shown that some of these methods are useful for the demonstration of enzymes in the fungi *Neurospora crassa, Oospora lactis* and *Saccharomyces cerevisiae.*

(1) *Dehydrogenases.* Dehydrogenases can be demonstrated with tetrazolium salts as indicators that are reduced to more or less insoluble, intensively coloured formazan granula[1]. Out of a great number of available tetrazolium salts, whose usefulness for fungi has been studied, Nitro-BT proved to be the best indicator[2]: after an incubation period of 30 min, optimal for nearly all investigated enzymes, the cells contained blue-black formazan deposits that were equally distributed in the nearly colourless cytoplasm. MTT, frequently used in medical histochemistry, was unsuitable for the detection of several dehydrogenases because the solution of $CoCl_2$, necessary for the stabilization of the formazan, proved to be toxic for many fungal enzymes.

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Table I. *Saccharomyces cerevisiae*: Cytochemically detectable dehydrogenases and their inhibitors

Dehydrogenase	Substrate	Pyridine nucleotide	Inhibitors (active concentration in *M*)	Literature
Alcohol	Ethanol	NAD	PCMB: 10^{-3}; PM: 10^{-1}	3
Glycerol-phosphate	Na-α-glycerophosphate	NAD	PCMB: 10^{-4}; PM: 10^{-2}	3
Glucose-6-phosphate	Na-glucose-6-phosphate	NADP	PCMB: 10^{-1}	3
6-phosphogluconate	Ba-6-phosphogluconate	NADP	PCMB: 10^{-3}; $CuSO_4$: 10^{-2}	3
Isocitrate	Na-DL-isocitrate	NAD NADP	PCMB: 10^{-3}; PM: 10^{-1}; $CuSO_4$: 10^{-3} PCMB: 10^{-3}; PM: 10^{-1}	3
Glutamate	Na-L-glutamate	NAD NADP	PCMB: 10^{-4}; PM: 10^{-2} PCMB: 10^{-3}; PM: 10^{-1}	3
Succinate	Na-succinate	–	PCMB: 10^{-4}; PM: 10^{-2}; malonic acid: 10^{-2}; oxaloacetic acid: 10^{-3}	3
Malate	Na-DL-malate	NAD	PCMB: 10^{-2}; PM: 10^{-1}; oxaloacetic acid: 10^{-1}	3
NAD-H_2-Cytochrome *c* reductase	NAD-H_2	–	(No inhibition with 10^{-2} *M* PCMB)	3
Pyridoxol	Pyridoxol-HCl	NADP	PCMB: 10^{-4}; $CuSO_4$: 10^{-3}	6
Glutathione reductase	Reduced glutathione	NAD NADP	Preincubation (30 min at 37 °C) in 10^{-5} *M* PCMB and 10^{-4} *M* PM	7
Proline	L-proline	NAD	PCMB: 10^{-4}; $CuSO_4$: 10^{-3}; PM: 10^{-2}	8
Aldehyde	Acetaldehyde	NAD, NADP	PCMB: 10^{-5}; PM: 10^{-3}	8
Dihydrolipoic	Dihydrolipoamide	NAD	Preincubation (30 min at 37 °C) in 10^{-2} *M* PCMB and 10^{-1} *M* $CuSO_4$	8

Abbreviations: PCMB, *p*-chloromercuribenzoate; PM, potassium monoiodate

Table II. *Saccharomyces cerevisiae*: Cytochemically detectable oxidases and their inhibitors

Oxidase	Inhibitors (active concentration in *M*)	Literature
Cytochrome oxidase	KCN: 10^{-2}; NaN_3: 10^{-2}; Hydroxylamine: 10^{-2}	10
Peroxidase	KCN: 10^{-2}; NaN_3: 10^{-2}	10

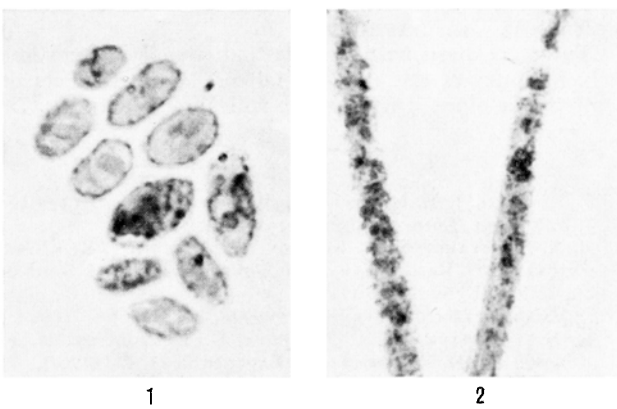

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Fig. 1. *Saccharomyces cerevisiae*: Demonstration of succinate dehydrogenase with Nitro-BT. × 2000.

Fig. 2. *Neurospora crassa*: Demonstration of cytochrome oxidase. × 1000.

After the demonstration of cytoplasmic dehydrogenases, the formazan deposits were round-shaped in *N. crassa*, *O. lactis* and *S. cerevisiae*; but when detecting mitochondrial dehydrogenases, the cells of *S. cerevisiae* additionally contained long, filamentous structures that are probably identical with mitochondria (Figure 1).

In the cells of *N. crassa*, *O. lactis* and *S. cerevisiae* the following dehydrogenases could be detected cytochemically:

(a) Glycolysis[3]: L-glycerol-3-phosphate: NAD oxidoreductase (E.C. 1.1.1.8), alcohol: NAD oxidoreductase (E.C. 1.1.1.1), D-lactate: cytochrome *c* oxidoreductase (E.C. 1.1.2.4), L-lactate: cytochrome *c* oxidoreductase (E.C. 1.1.2.3). Additionally, in *N. crassa* D-lactate: NAD oxidoreductase (E.C. 1.1.1.28) could be demonstrated.

(b) Hexose monophosphate pathway[3]: D-glucose-6-phosphate: NADP oxidoreductase (E.C. 1.1.1.49) and 6-phosphogluconate: NADP oxidoreductase (E.C. 1.1.1.44).

(c) Citric acid cycle[3]: Isocitrate: NAD oxidoreductase (E.C. 1.1.1.41), isocitrate: NADP oxidoreductase (E.C. 1.1.1.42), L-glutamate: NAD oxidoreductase (E.C. 1.4.1.2), L-glutamate: NADP oxidoreductase (E.C. 1.4.1.4), succinate: (acceptor) oxidoreductase (E.C. 1.3.99.1) and two Na-DL-malate dehydrogenating enzymes, one specific for NAD, the other for NADP.

(d) Cytochrome *c* reductases[3]: NAD-H_2: cytochrome *c* reductase (E.C. 1.6.2.1) and NADP-H_2: cytochrome *c* reductase (E.C. 1.6.2.3).

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Some further dehydrogenases could be demonstrated in the cells of different fungi: (e) Pentose metabolism[4]: aldopentose reductase in *Penicillium expansum* and *O. lactis* and pentitol dehydrogenase in *P. expansum* and *O. lactis*. (f) Other dehydrogenases: L-amino acid: O_2 oxidoreductase (E.C. 1.4.3.2) in *N. crassa*[3], D-glucose: O_2 oxidoreductase (E.C. 1.1.3.4) in *Aspergillus niger*[5], pyridoxol: NADP oxidoreductase (E.C. 1.1.1.1) in *S. cerevisiae*[6], glutathione reductase (E.C. 1.6.4.2) in *S. cerevisiae*[7], as well as NAD- and NADP-linked proline dehydrogenases, aldehyde dehydrogenases and dihydrolipoic dehydrogenase (E.C. 1.6.4.3) in *S. cerevisiae*[8].

The specificity of the different staining reactions could be confirmed by means of control reactions[1]. Table I shows the inhibitors employed and their concentrations which inhibited enzyme activity and thus formazan production in the cells of *S. cerevisiae*.

(2) *Oxidases.* Cytochrome oxidase and peroxidase can be demonstrated cytochemically in the cells by various indicators that are changed by oxidation to more or less stable, coloured reaction products[9].

(a) Cytochrome oxidase: After testing the well-known indicators, the combination of the 2 amines *p*-aminodiphenylamine and *p*-methoxy-*p'*-aminodiphenylamine proved to be the best method for the intracellular demonstration of this enzyme in *N. crassa*, *O. lactis* and *S. cerevisiae*[10]. After an incubation period of 30 min, the uncoloured cytoplasm contained brown-red, round-shaped deposits (Figure 2).

(b) Peroxidase: Different methods (amines, phenols, zinc-leuco) were tested, but only the indicator 3-amino-9-ethylcarbazole produced a useful reaction picture (incubation period for all fungi: 60 min): small, round, red-brown, distinct granula, equally distributed in a lightly tinged cytoplasm[10].

The results of control reactions (inhibitors with their active concentration in the medium) are summarized in Table II:

The reaction pictures of all cytochemical enzyme demonstrations showed an equal distribution of the different deposits in the cytoplasm. In the hyphae-producing fungus *N. crassa* the reactions were weaker in older parts of the mycelium than in the younger, growing hyphal tips. In the stained preparations of *O. lactis* and *S. cerevisiae*, many cells were only weakly stained or not at all. Probably, this is due to a different enzyme equipment of the single cells[11].

The practical value of the cytochemical demonstration of dehydrogenases and oxidases in fungi could be demonstrated when studying the influence of phenylboric acid on the cells of *O. lactis*[12].

Zusammenfassung. Die Ergebnisse der bisherigen Untersuchungen zum zytochemischen Nachweis verschiedener Dehydrogenasen und Oxydasen in den Zellen von Pilzen (*Neurospora crassa, Oospora lactis, Saccharomyces cerevisiae* u.a.) werden beschrieben.

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DISPUTANDUM

Relationship Between the Structure of Chloramphenicol and its Action upon Peptide Synthetase

The antibiotic chloramphenicol (CM) is a growth inhibitor and a widely used biochemical research tool because it inhibits specifically protein biosynthesis. Hundreds of chemical derivatives of CM have been tested for antibacterial activity and a set of empirical structure-activity rules has been derived from this body of chemical and microbiological information[1,2]. A few growth-inhibitory derivatives of CM have more recently been tested in vitro in ribosomal amino acid incorporation systems[3,4].

Evidence accumulating during the past years[5-7] and especially recent studies of GOTTESMAN[8] suggest that CM interferes with the action of ribosomal peptide synthetase(s), i.e. with the enzymatic transfer of growing peptides to the α-amino groups of incoming amino acids. The presence of at least 1 peptide or amide bond, such as that of acetyl-phenylalanyl-tRNA, is required for recognition and transfer of a peptide by the enzyme[8]; peptidyl synthetase appears to be an integral constituent of ribosomes[8,9].

This report suggests that CM, a substituted amide reminiscent of a dipeptide[10] (Figure 1), attaches vicariously to the peptidyl recognition site of peptide synthetase(s) and inhibits peptidyl transfer, viz. the elongation reaction in protein synthesis.

This hypothesis first predicts that chemical alterations which eliminate the amide bond of CM or affect its reactivity or steric environment will abolish activity. In-

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