an insignificant variation is observed when the substrate is incubated with CoQ_{10} without cytochrome c. In the case of mitochondria from fatty liver, on the other hand, significant increase in mitochondrial respiration is found when mitochondria are incubated with CoQ_{10} in presence of cytochrome c, while the increase is not statistically significant when cytochrome c is not added.

CRANE et al.[14] found that succinoxidase activity of electron transport particles prepared by isooctane and deoxycholate extraction was restored by addition of both CoQ_{10} and cytochrome c to the medium. The same behaviour is also observed in CCl_4 liver injury: while the addition of only CoQ_{10} to mitochondria of fatty liver does not stimulate respiration, the addition of CoQ_{10} in presence of cytochrome c stimulates significantly the O_2 uptake (the two values marked with * in the Table). The fact that those two values differ significantly might be interpreted in terms of an increased mitochondrial membrane permeability, as observed by several authors (DIANZANI[5,15]). This would bring about an easier access to the coenzymes, which are in excess in the reaction medium, to the sites suitable to their activity.

Riassunto. È stato studiato il comportamento dell'attività succinossidasica dei mitocondri di fegato di ratto normale e intossicato con CCl_4 in presenza di CoQ_{10}. Si è notato che l'aggiunta di CoQ_{10} in presenza di citocromo c ai mitocondri di fegato grasso stimula la respirazione, fatto che può essere interpretato come aumento di permeabilità della membrana mitocondriale.

ANNA CASU

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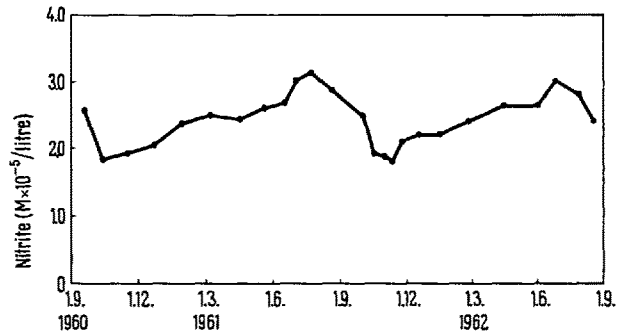
Seasonal Changes in the Nitrate-Reducing Activity of a Green Alga[1]

Nitrate reduction *in vivo* by the green alga, *Ankistrodesmus braunii*, has been studied in some detail (for a summary, see [2]). In the course of this work we observed that, under constant experimental and growth conditions, the algae appeared to have a lower nitrate-reducing capacity in late autumn and early winter than in the rest of the year. A series of quantitative determinations of the activity *in vivo* of the systems reducing nitrate and nitrite, carried out during the past two years, has revealed the existence of pronounced seasonal changes in enzymatic activity. The results shown in the Figure for the reduction of nitrate have been obtained in the presence of $2 \times 10^{-3} M$ 2,4-dinitrophenol (DNP) at pH 6.5 in the dark. This concentration of DNP completely inhibits the further reduction of nitrite and has no influence on the reaction nitrate→nitrite[3]. Under constant conditions, a minimum of activity can be observed in October to November. In the course of winter and spring, the activity slowly rises to a maximum in June to July which, in turn, is followed by a rather sharp decline. During the summer maximum, the activity of the nitrate-reducing system is about 75% higher than in late autumn. The capacity of the system responsible for the reduction of nitrite was found to show changes quite closely resembling those of nitrate reduction. A similar behaviour with even greater seasonal changes has recently been found in the hydrogenase activity of intact cells of a strain of *Chlorella pyrenoidosa*[4]. Therefore it seems that periodic changes of enzymatic activities in the course of the year are quite a common phenomenon with green algae of those genera that are widely used for physiological and biochemical research.



Nitrate-reducing activity of intact cells of *Ankistrodesmus braunii* at different times of the year. Formation of nitrite in 2 h under standard conditions in the dark. Cell concentration, 0.8 mg dry weight/ml; phosphate buffer, pH 6.5; nitrate concentration, $1.7 \times 10^{-2} M$; DNP concentration, $2 \times 10^{-3} M$; temperature, 22°C.

Investigations on the reduction of nitrate and nitrite have also been carried out with cell-free extracts from *Ankistrodesmus*. For this work, *Ankistrodesmus braunii* (strain Marburg) was grown under continuous illumination (light intensity, 4000 lux) in a medium containing nitrate[5]. The cells were centrifuged, taken up in phosphate buffer of pH 6.5, and then broken by violent shaking with small glass beads in a Merkenschlager homogeniser (Type MSK; B. Braun, Melsungen) at 3–8°C. Cell walls and larger fragments were removed in a refrigerated centrifuge (Type Omikron; M. Christ, Osterode) at 2000 *g* and 4°C. The supernatant was found to contain both nitrate and nitrite-reducing enzymes. The nitrite reductase activity of this preparation could be inhibited by $2 \times 10^{-3} M$ DNP at pH 6.5. Thus the cell-free extracts were found to have the same sensitivity towards this inhibitor as was observed with intact cells. In addition, a separation of nitrate and nitrite reductases could be achieved by high-speed centrifugation at 4°C. After 4 h at 104000 *g*, the nitrite reductase activity was found in the sediment, whereas the nitrate reductase activity remained in the supernatant. Some other properties of the nitrate and nitrite reducing systems of *Ankistrodesmus* are summarized in the Table. They are in good agreement with those of the corresponding enzymes obtained from fungi, higher plants, and

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Some properties of the nitrate and nitrite-reducing systems from *Ankistrodesmus braunii*

	Localization	Hydrogen donor	Flavin requirement	Effect of heavy metal poisons	Effect of 2,4-dinitrophenol	Effect of *p*-chloromercuribenzoate
Nitrate reductase	soluble	DPNH	FAD	inhibition	no effect	inhibition
Nitrite reductase	particle-bound	TPNH (DPNH)	FAD or FMN	inhibition	inhibition	inhibition

Chlorella[6–8]. In accordance with the results reported for higher plants and microorganisms[6,7], the nitrate reductase of *Ankistrodesmus* was found to be an adaptive enzyme formed only in the presence of nitrate or nitrite. The nitrite reductase of *Ankistrodesmus*, on the other hand, seems to be constitutive since appreciable nitrite-reducing activity could be observed in extracts of algae grown on urea or other organic nitrogen compounds.

Experiments with cell-free extracts from *Ankistrodesmus* have shown that seasonal changes in the activity of nitrate and nitrite reduction, very similar to those described above for intact cells, can also be observed *in vitro*.

Zusammenfassung. Trotz konstanter Kultur- und Versuchsbedingungen zeigt die Nitratreduktion intakter Zellen und zellfreier Extrakte der Grünalge *Ankistrodesmus braunii* ausgeprägte jahresperiodische Schwankungen. Das Aktivitätsmaximum liegt in den Monaten Juni/Juli, das Minimum im Oktober/November.

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'Chromatoid Body' in the Spermatogenesis of Scorpions

An insight into the current cytochemical literature forces us to reconsider, in the light of what we know to-day, the various old definitions of different cellular organelles which had been given from time to time by old workers, and which at present are retained just because of convenience of common usage. This practice of indiscriminate and loose use of specific nomenclature is unfortunate and causes confusion. It is for this reason that SUD[1], while giving a review of the 'chromatoid body' in spermatogenesis in general, has significantly remarked, '... morphological, physical and chemical nature, behaviour during division stages, origin and fate of chromatoid bodies in different species or even in the same species as given by different authors are in most cases quite divergent'. He has therefore advised the analysis of this enigmatic structure cytochemically for its correct identification.

Keeping this in view, a detailed cytochemical analysis of the so-called 'chromatoid body', encountered in the male germ cells of *Palamnaeus bengalensis* and *P. fulvipes*, has been made and the various tests employed during the course of the present investigations include current techniques (a) for lipids[2–5], (b) for proteins[6–9], (c) for carbohydrates[10,11], and (d) for nucleic acids[12].

After extensive and exhaustive studies on male germ cells, SUD[13–15] has been able to establish the identity of 'chromatoid bodies' based on their cytochemical nature—the 'chromatoid body' is that which is deeply stained with acid and basic dyes and dye lakes. It is osmiophobe and argentophobe. It consists mainly of RNA and basic proteins—rich in arginine. There is little or no tyrosine. 'Chromatoid bodies' are free from lipids, carbohydrates and DNA.

The structure described as the 'chromatoid body' by GATENBY and BHATTACHARYA[16] in *P. bengalensis*, on the other hand, when cytochemically examined, reveals the presence of lipids in addition to proteins. It is distinctly osmiophil and is not at all stained with basic dyes and dye lakes. Above all, it lacks RNA—the most essential component of the true 'chromatoid body'. The 'chromatoid body' encountered in the male germ cells of *P. fulvipes* is also histochemically exactly like that of *P. bengalensis*.

It is therefore inferred and rightly too that this particular structure labelled as 'chromatoid body' in the spermatids of *P. bengalensis* by GATENBY and BHATTACHARYA[16] is definitely not a true 'chromatoid body'. It is just an accessory organelle having lipoproteinous nature and no RNA. Since it is observed only during the stages when the acroblast is degenerating and shrivelling up, it is very likely that it is also one of the acroblast remnants, which

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