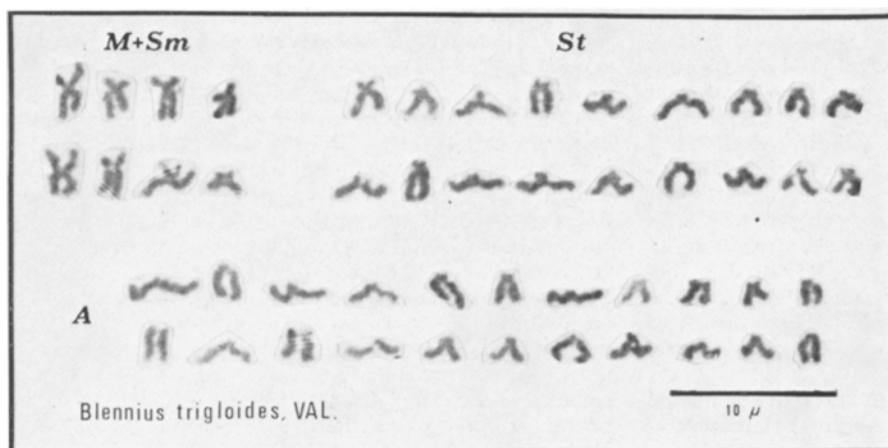

Fig. 4. Metaphase plate and karyotype of *Blennius trigloides*. M + SM, metacentric and submetacentric chromosomes; St, subtelocentric chromosomes and A, acrocentric chromosomes.

into the subgenera *Salaria* and *Lipophrys* is based, does not seem to be sufficient ground for such a subdivision. On the other hand, this cytotaxonomic feature shows up the peculiarity of the *B. trigloides* karyotype, thus calling for an accurate taxonomic review of this species and a more extensive analysis of all the biological characters in order to ascertain its natural taxonomic position in the *Blennius* L. genus[7].

Riassunto. È stato descritto il cariotipo di 7 specie di Blennidi Mediterranei appartenenti al genere *Blennius* L.: *B. sanguinolentus*, *B. pavo*, *B. sphinx*, *B. trigloides*, *B. canevae*, *B. fluviatilis* e *B. incognitus*. Il numero diploide di tutte le specie è $2n = 48$ tuttavia la morfologia del cariotipo presenta alcune differenze tra le specie; *Blennius trigloides* si distingue infatti nettamente da tutti gli altri *Blennius* poichè il suo cariotipo mostra un alto numero di metacentrici e sub-metacentrici mentre il cariotipo delle altre specie studiate è formato fondamentalmente da acrocentrici tra i quali fanno spicco quattro coppie (*sanguinolentus*, *pavo* e *canevae*) o una coppia (*incognitus* e *sphinx*) di subtelocentrici.

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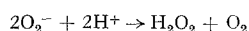
The Generation of the Superoxide Radical by 2-Amino-4-Hydroxy-6,7-Dimethyl-5,6,7,8-Tetrahydropteridine ($DMPH_4$)

Superoxide dismutase (SOD) is a copper-zinc containing protein of molecular weight 33,000 whose enzymatic activity was first described by McCORD and FRIDOVICH[1]. SOD catalyzes the dismutation of the superoxide anion radical (O_2^-) to form hydrogen peroxide and oxygen:



$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

Recently, PETRACK and CHERTOCK[2] reported that SOD partially protected the labile activated state of tyrosine hydroxylase. This effect of SOD suggested that O_2^- was inactivating tyrosine hydroxylase. PETRACK and CHERTOCK[2] did not identify a source of the O_2^- in their experimental system. A likely origin of O_2^- was the reduced pteridine cofactor $DMPH_4$, a compound known to generate H_2O_2[3]. Many compounds which autoxidize to form H_2O_2 (the two-electron reduction product of oxygen) also generate O_2^- (the one-electron reduction product of oxygen) as an intermediate[4].

Some autoxidations are catalyzed by O_2^- which is generated during the reaction. SOD can diminish the overall rate of such autoxidation reactions. For example, it has been shown that SOD inhibits both the base catalyzed and the metal ion catalyzed autoxidation of epinephrine[5], and the spontaneous autoxidation of 6-hydroxydopamine at pH 7.4[6]. We now present evidence that $DMPH_4$ generates O_2^- which catalyzes the autoxidation of $DMPH_4$.

Materials and methods. Experiments were run on a Biological Oxygen Monitor (Clark Oxygen Electrode, Yellow Springs Instruments, Yellow Springs, Ohio) connected to a Honeywell Electronik 19 Recorder at 37 °C in 1 ml of a modified Krebs-Ringer phosphate buffer at pH 7.4[6] or a 0.05 M sodium acetate buffer at pH 6.5. The $DMPH_4$ was dissolved in O_2 free water and was added to the oxygen electrode by means of an Oxford automatic pipette. In some experiments 100 μg SOD (frozen liquid, 3,000 units per mg, Truett Laboratories, Dallas, Texas) was added prior to the addition of $DMPH_4$.

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Results. $DMPH_4$ (10^{-3} *M*) consumed O_2 at both pH 6.5 and 7.4 (Table, note the higher initial rate of O_2 consumption at pH 7.4). The addition of SOD caused a significant ($p < 0.005$) inhibition in the rate of O_2 consumption at both pH 6.5 and 7.4. Boiled SOD had no effect on the rate of O_2 consumption. These data indicated that O_2^- was formed from $DMPH_4$ and that it catalyzed the autoxidation of $DMPH_4$.

It is known that O_2^- can reduce cytochrome c[1]. In separate experiments at pH 7.4, we found that 10 µg/ml SOD caused a 33% inhibition in the initial rate of reduction of 10^{-5} *M* cytochrome c by 5×10^{-6} *M* $DMPH_4$ (data based on absorbance change at 550 nm between 15 and 30 sec). This result confirmed that O_2^- was generated from $DMPH_4$.

Discussion. In a recent report, NISHIKIMI[7] indicated that SOD inhibited the $DMPH_4$ mediated reduction of nitro blue tetrazolium as well as the rate of autoxidation of $DMPH_4$ as measured spectrophotometrically at 330 nm. These data further confirm the formation of O_2^- from $DMPH_4$. NISHIKIMI[7] also reported that the naturally occurring pteridine cofactor, tetrahydrobiopterin, generated O_2^-.

The absolute requirement for pteridine cofactors in tyrosine hydroxylase systems, as well as the known capacity of these cofactors to autoxidize suggest that some species generated during the autoxidation of pteridines is essential for tyrosine hydroxylase activity. In spite of the failure of SOD to inhibit tyrosine hydroxylase activity[2], it is still possible that O_2^- is essential for hydroxylation. Failure of SOD to inhibit might be explained by an inability of SOD to come into contact with O_2^- generated at the active site of tyrosine hydroxylase. The data of PETRACK and CHERTOCK[2] indicate that O_2^- (or some species derived from O_2^- like the hydroxyl radical or singlet oxygen) can inactivate tyrosine hydroxylase. Perhaps therefore, the well known substrate inhibition seen with excess $DMPH_4$ in tyrosine hydroxylase assay systems[8] is due at least partially to O_2^- generated from $DMPH_4$.

The effect of superoxide dismutase (SOD, 100 µg) on the initial rate of oxygen consumption by $DMPH_4$ (10^{-3} *M*)

pH	O_2 consumption (nmoles O_2/min)		Inhibition (%)
	– SOD	+ SOD	
6.5	47 ± 8 (5)	30 ± 1 (5)	36
7.4	62 ± 9 (5)	36 ± 6 (5)	42

Data are the mean ± S.D. of the initial rate of O_2 consumption (nmoles O_2/min); the number of determinations are in brackets. Experiments were run in 1 ml of Krebs-Ringer phosphate buffer at pH 7.4 or 0.05 *M* sodium acetate buffer at pH 6.5.

Zusammenfassung. Es werden neue Befunde beigebracht, die für die Bildung von Superoxyd-Radikal durch $DMPH_4$, ein biologisch wichtiges Pterinderivat, sprechen.

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Sur l'effet multipolarisant de la vinblastine dans la division cellulaire d'*Allium cepa* L.

La vinblastine (vincaleucoblastine ou VLB), alcaloïde isolé de la *Vinca rosea* L. (*Catharanthus roseus* G.) est un agent cytostatique connu et utilisé dans la thérapie de diverses tumeurs[1,2]. Les études cytologiques réalisées sur des cultures de tissus animaux[3,4] et des cellules végétales[5] exposées à la VLB, aboutirent à la conclusion que cet alcaloïde, aussi bien in vivo qu'in vitro, exerçait son action sur le processus mitotique, à la manière de la colchicine, en bloquant la division cellulaire au niveau de la métaphase, par interférence avec la formation du fuseau mitotique.

Dans cette étude, nous avons analysé l'effet de la VLB sur le fonctionnement du fuseau de cellules végétales en division, en suivant la cinétique de production des figures multipolaires, après le traitement opéré avec cet agent mitostatique.

Nous avons utilisé des méristèmes radiculaires d'*Allium cepa* L. Les bulbes sont mis à germer dans l'eau aérée, à 25 °C et à l'obscurité. Les racines sont traitées par une solution aqueuse de sulfate de vinblastine (Lilly) à 0,02%, pendant 15 min, et remises ensuite dans l'eau. Les observations sont effectuées sur plusieurs racines fixées

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Action de la vinblastine sur l'activité mitotique des cellules méristématiques d'*Allium cepa* L.

Heures après le traitement	0	1	2	3	4	5	6	7	8
Index mitotique[a]	9,7	13,0	11,5	13,8	12,1	11,0	12,9	11,2	10,2

[a] mitoses pour 100 cellules.