Biochemical Pharmacology, Vol. 30, pp. 673.

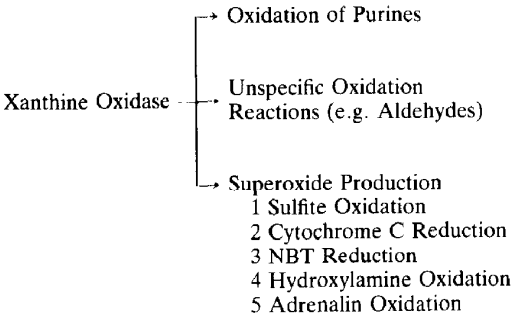
0006-2952/81/060673-01 $02.00/0

Concerning the determination of xanthine oxidase in biological material via its ability to produce superoxide

(*Received* 12 *September* 1980; *accepted* 24 *October* 1980)

The determination of many enzymes in crude biological material is not always an easy task to fulfil, especially with regard to the assay method used. To assay an enzymic activity, a specific method should be used to avoid interfering reactions. Recently, Tubaro *et al.* [1, 2] reported an increase of xanthine oxidase in liver as well as in polymorphonuclear (PMN) leukocytes of mice in three pathological situations, namely during bacterial or protozoal infection or inoculation with Ehrlich solid carcinoma. The assay method used to determine xanthine oxidase based upon the reduction of a tetrazolium salt by the enzyme [3].

Xanthine oxidase is a flavoprotein which contains molybdenum and iron. It is multifunctional (Scheme 1) in that



Scheme 1. Enzymic activities of xanthine oxidase.

it can oxidize a variety of substrates, including the 'specific' substrates, i.e. the purines xanthine and hypoxanthine; several aldehydes, pterines and certain drugs [4]. In the presence of an oxidizable substrate and oxygen, xanthine oxidase had been found capable of initiating the oxidation of sulfite [5] and of catalyzing the reduction of cytochrome *c* [6]. In both cases, the agent responsible for these reactions was shown to be superoxide (O_2^-) generated by the enzyme [7]. The ability of some protein preparations to inhibit these reactions led finally to the discovery of the superoxide dismutase activity by McCord and Fridovich [8]. Several methods were developed to assay superoxide dismutase using xanthine oxidase as a source of reducing radicals, including the reduction of nitro blue tetrazolium [9] as well as the oxidation of adrenalin [8] and hydroxylamine [10]. In all cases, the original enzymic function, i.e., the oxidation of purines to urate, remains unaffected in the presence of superoxide dismutase. In so far, the two processes are independent. The release of superoxide ion is not restricted to xanthine oxidase (see [11] for a review). Several enzymes produce O_2^- during activity, many autoxidizable biological substances, including hemoglobin, release superoxide, and, most prominent of all, phagocytes 'synthesize' $\cdot O_2^-$ by an NADPH oxidase located in the plasma membrane as an aid in the killing of ingested microorganisms [12]. As superoxide, regardless of its origin, will reduce tetrazolium salts, the use of such a reaction to determine one single enzyme, namely xanthine oxidase [1, 2], will most probably lead to erroneous results. In the pathological models of Tubaro *et al.* [1, 2], at least the leukocytes will produce additional $\cdot O_2^-$ which will lead to an overestimation of the amount of enzyme present.

From another point of view, the reactions of xanthine oxidase attributed to $\cdot O_2^-$ are by no means very specific indicators of superoxide [13, 14]. Therefore, care should be taken when determining superoxide dismutase this way, too.

In summary, one should be cautious when assaying an enzyme in crude biological material. In the case of xanthine oxidase, the use of any method basing on the determination of the superoxide anion radicals released by the enzyme might lead to error, as several other sources of this radical will most probably interfere.

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