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SHORT COMMUNICATIONS

Increase of xanthine oxidase activity in infected mice: a second look by a specific assay method

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In a recent communication, Younes [1] has suggested caution in the use of methods of determining xanthine oxidase activity in biological materials dependent upon the ability to produce superoxide. Most concern arises from the occurrence in biological materials of other systems capable of producing superoxide and to these we add the almost ubiquitous presence in tissues of superoxide dismutase [2] which, by catalytically intercepting superoxide, will decrease the reaction with the chromophoric molecules (e.g. NBT, cytochrome *c*) conventionally used to monitor its flux. Therefore, a word of caution in relation to the use of these methods for the determination of xanthine oxidase activity is justified.

The concern expressed by Younes relates to reports made by us [3–6] of an increase in xanthine oxidase activity in mouse tissues (and particularly in polymorphonuclear (PMN) leucocytes) in three pathological models of infection. In fact, the method [7] used to determine the increase in xanthine oxidase activity is based on the reduction of INT by superoxide produced during the action of the enzyme. In view of the comments of Younes, it seemed important to repeat the assay in at least one of the pathological conditions, using an additional method, namely the direct determination of the product urate, after addition to the biological material tested of the specific substrate, hypoxanthine. The method described by Kalckar [8], follows the production of urate through its specific absorption at 290 nm, and seems fully appropriate for our purpose.

The results obtained for PMNs in the case of *Staphylococcus aureus* infection in mice are reported in Table 1, where data obtained by our original method (Fried) and the alternative method (Kalckar) on the same specimens are directly compared. It is evident that a similar increase of xanthine oxidase activity was measured by both methods.

Therefore, the conclusion that an increase of xanthine oxidase in PMNs of bacterially infected mice, previously reported [6] seems justified and is a real phenomenon confirmed by the use of two independent methods of determining the enzyme. Although the caution expressed by Younes is valid from a general standpoint, it does not seem to affect the conclusions of our investigations.

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Table 1. Xanthine oxidase determination using a colorimetric and u.v. method in elicited PMN of non-infected and infected mice

Groups	Fried's colorimetric method at 550 nm: μg Iodoformazan/ 20′/1 × 10^6 PMN at 37° (×10^{-5})	Percentage increase over controls	Kalckar's u.v. absorption method at 290 nm: Kalckar U/1 × 10^6 PMN (×10^{-5})	Percentage increase over controls
Controls	4.36 ± 0.06	—	4.23 ± 0.08	—
Infected animals	8.33 ± 0.05	91	8.43 ± 0.09	99

Each value is the mean ± S.E.M. of three separate determinations in triplicate.
Student's *t*-test: P = 0.08; P ≥ 94%, not significant.

Charles River CD1 male albino mice were infected i.p. with 0.25 ml/10 g of a suspension of *S. aureus* CN 6538 containing 1 × 10^9/cells/ml. Four days later PMNs were elicited in both groups with 1.5 ml of 12% (w/v) natrium caseinate solution in saline i.p. PMNs were harvested 18 hr later by a double washing of the peritoneum with 2 ml of heparinised saline. Peritoneal cells were counted, centrifuged and resuspended in HBSS, pH 7.4, and the suspension adjusted to 1.8 × 10^7 PMN/ml.