ADIPOCYTES DO NOT FALSIFY THE SUPEROXIDE THEORY OF OXYGEN TOXICITY

William H. BANNISTER and Joe V. BANNISTER*

Nuffield Dept. of Clinical Biochemistry, University of Oxford, Radcliffe Infirmary, Oxford OX2 6HE and **Inorganic Chemistry Laboratory, University of Oxford, Oxford OX1 3QR, England*

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1. Introduction

The superoxide theory of oxygen toxicity [1] considers the enzyme superoxide dismutase (EC 1.15.1.1) as an important detoxification enzyme of oxygen metabolism and absence of the enzyme in aerobic cells is an embarassment to the theory [2]. This absence has been reported for 3 aerobic microorganisms, namely, *Lactobacillus plantarum* [2], *Neisseria gonorrhoeae* [3] and *Mycoplasma pneumoniae* [4,5] and for rat adipose tissue [6]. There is also a report of absence of copper/zinc superoxide dismutase in human bone marrow [7]. We have re-examined rat adipose tissue and find that it contains superoxide dismutase identifiable as both copper/zinc and manganese superoxide dismutase.

2. Materials and methods

White adipose tissue was obtained from the epididymal fat pad and brown adipose tissue from the interscapular fat of male Wistar rats. A 10% (w/v) homogenate of the tissue in 0.1 M phosphate buffer (pH 7.8) containing 10^{-4} M EDTA was prepared and sonicated for 30 s. The supernatant obtained after centrifugation at 48 000 × *g* for 15 min was used for the investigation. Copper/zinc superoxide dismutase was recovered by passage through ethanol–chloroform [8]. The extract was freeze-dried and the residue dissolved in phosphate/EDTA buffer. The enzyme was similarly recovered from a hemolysate of rat red blood cells. Cyanide was also used to differentiate between the copper/zinc and the manganese superoxide dismutase [9] at 2 mM or 10 mM with equivalent results. Superoxide dismutase was assayed by the xanthine oxidase–cytochrome *c* method in [10]. Haemoglobin was measured according to [11] and protein was estimated by the Folin method [12].

3. Results and discussion

The average haemoglobin concentration of the supernatant was 83 μg/ml from a homogenate of white or brown adipose tissue. Since the average superoxide dismutase content of rat red blood cells was found to be 1830 U/g Hb, contamination of the homogenates with superoxide dismutase from red blood cells was considered negligible (0.1–0.2 U/mg protein) was estimated. Table 1 gives the superoxide dismutase activity found in adipose tissue. Total superoxide dismutase activity is higher in brown than in white adipose tissue. This is mainly due to the higher activity of the cyanide-insensitive (or manganese) superoxide dismutase. Brown adipose tissue has a higher content of mitochondria and about twice the rate of oxygen consumption of white adipose tissue [13]. This appears to have a parallel in the superoxide dismutase and superoxide dismutase pattern of the tissue.

The earlier report of absence of superoxide dismutase in rat adipose tissue [6] appears to have been a methodological error. It is evident that this tissue contains superoxide dismutase and does not falsify the superoxide theory of oxygen toxicity. In its most general form this has 4 postulates:

(i) Living cells produce oxygen toxicity;
(ii) Superoxide is the starting-point of oxygen toxicity;
(iii) Living cells contain superoxide dismutase to detoxify superoxide;
(iv) Superoxide dismutase is inducible.

Postulate (i), the one-electron reduction of oxygen in biological systems, is not disputed although quantitative information is sparse [14]. Whether or not superoxide is itself toxic is debatable on present evidence [15]. However, it cannot be considered innocuous since it appears to produce hydroxyl radicals

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Table 1
Superoxide dismutase activity in rat adipose tissue[a]

Adipose tissue	Chloroform–ethanol extractable (U/mg protein)	Cyanide-insensitive (U/mg protein)	Total[b] (U/mg protein)
White	7.2 ± 0.2	3.2 ± 0.1	11.2 ± 0.6 (10.2)
Brown	8.7 ± 0.2	10.2 ± 0.2	20.4 ± 0.8 (18.9)

[a] Values are mean ± SEM of triplicate measurements
[b] Values in parentheses are chloroform–ethanol-extractable + cyanide-insensitive superoxide dismutase

from hydrogen peroxide in the presence of catalytic iron complexes [16]. The three-electron reduction of oxygen has become the basis of postulate (ii). It is a feasible postulate for biological systems with transferrin [17], lactoferrin [18,19] or iron picolinate [20] as catalyst. It is postulate (iii) that has created difficulties for the superoxide theory of oxygen toxicity since absence of superoxide dismutase in aerobic cells appears to falsify the theory. Whereas the reported absence of superoxide dismutase in adipose tissue [6] (and probably its reported absence in bone marrow while present in blood cells [7]) can be rationalised as methodological error, the absence of the enzyme in certain aerobic microorganisms cannot be so explained. One of these theory-breaking microorganisms namely, *L. plantarum*, may scavenge superoxide by means of manganese(II) which it actively accumulates and may inhibit the three-electron reduction of oxygen by excluding iron [21]. It remains to be seen how *N. gonorrhoeae* and *M. pneumoniae* deal with superoxide. In a recent criticism [2] the type of evidence which falsifies the superoxide theory of oxygen toxicity has not been clearly distinguished. Thus consideration of the logical structure of the theory (postulates (i)–(iv)) shows that lack of proof of postulate (iv), the induction of superoxide dismutase, still debatable, does not falsify the theory.

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