tubers of this species when grazing in its natural habitat. In literature, there are 2 other wild Manihot species which had been reported to have high protein content; M. melanobasis[10] and M. saxicola[11], but there is no reference to their HCN content; consequently, the authors have no idea how much the hydrolytic products of glucosides interfers with the total estimated crude protein. From the first instance, it seems logical to find wild cassava with high protein content, since human selection has aimed continually to select for tuber size and less fibre, without paying attention to protein content. This could lead to discarding protein-producing genes from the cultivated varieties.

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Hydrogen peroxide generation in *Trypanosoma cruzi*[1]

A. Boveris[2] and A. O. M. Stoppani[2]

Universidad de Buenos Aires, Facultad de Medicina, Instituto de Química Biológica, Paraguay 2155, 1121 CF, Buenos Aires (Argentina), 31 March 1977

Summary. Homogenates from T. cruzi epimastigotes produced 3.4 pmoles H_2O_2/min 10^6 cells, as detected by the cytochrome c peroxidase assay. Addition of NADH or NADPH increased H_2O_2 production by a factor of 3 and 5, respectively. When supplemented with NADH and NADPH, the mitochondrial, microsomal and supernatant fractions produced H_2O_2, the soluble fraction and the mitochondrial membranes being apparently the main generators of H_2O_2. The epimastigote homogenates showed cyanide-sensitive superoxide dismutase activity, equivalent to 0.28 μg bovine superoxide dismutase per mg homogenate protein.

Production of potentially toxic intermediates of oxygen reduction in cells and tissues was early suggested by Gerschman et al.[3,4]. Experimental confirmation of the biological formation of O_2^- and H_2O_2 was later provided by Chance et al.[5] and Fridovich[6]. The epimastigote (culture form) of Trypanosoma cruzi, the agent of Chagas disease, contains peroxidase[7,8], thus suggesting that H_2O_2 is a normal metabolite in this organism, but so far no direct evidence of H_2O_2 formation has been offered. Hydrogen peroxide is toxic for trypanosomes and, consequently, the study of H_2O_2 metabolism holds potential pharmacological interest, since an increased intracellular level of H_2O_2 may be lethal for T. cruzi[9,10].

Materials and methods. The Tulahuen strain of T. cruzi was grown as described before[8]. Epimastigotes were disrupted by freezing (at −16 °C) and thawing 3 times. The cell suspension was homogenized by several passages through a hypodermic needle, gauge No. 24, attached to a syringe. The homogenates were suspended in 0.23 M mannitol, 0.07 M sucrose, 1 mM EDTA, 10 mM Tris HCl, pH 7.2, at 8.0 mg protein/ml and fractionated in the Sorvall RC-2B centrifuge at 4 °C. The fractions obtained were: a) the nuclear-flagellar fraction (sedimented at 480 × g for 10 min; the fluffy layer was reincorporated to the supernatant); b) the mitochondrial fraction (sedimented at 12,000 × g for 10 min); c) the microsomal fraction (sedimented at 105,000 × g for 45 min); d) the supernatant.

NADH, NADPH, xanthine, horseradish peroxidase (EC 1.11.1.7; HRP) type VI, xanthine oxidase, bovine superoxide dismutase and D-glucose oxidase were purchased from Sigma Chemical Company. Cytochrome c peroxidase (EC 1.11.1.5; CCP) was prepared as described by Yonetani[11].

The rate of H_2O_2 generation was determined spectrophotometrically, by measuring the formation of the CCP-H_2O_2/complex (reaction 1)

$$CCP + H_2O_2 \rightarrow CCP\text{-}H_2O_2 \quad (1)$$

and the HRP-H_2O_2 complex (reaction 2)

$$HRP + H_2O_2 \rightarrow HRP\text{-}H_2O_2 \quad (2)$$

as described previously[12,13]. Superoxide dismutase was determined on the basis of its ability to inhibit the O_2^- dependent adrenochrome formation from epinephrine, using the xanthine oxidase reaction as source of O_2^-, as described in Cadenas et al.[14]. Production of O_2^- by beef heart submitochondrial particles was measured as described by Cadenas et al.[14].

Protein content of cell suspensions and fractions was determined by the biuret method[15] in the presence of 0.2% sodium deoxycholate. One mg of epimastigote total protein corresponded to 1.7 mg dry weight, to 12.3 mg wet weight and to 77×10^6 cells.

Generation of hydrogen peroxide by T. cruzi fractions*

Fraction (percent of the homogenate total protein)	Substrate	H_2O_2 generation (nmoles/min mg protein)	
		Antimycin omitted	Antimycin added
Nuclear-flagellar (20)			
	NADH	0.77	–
	NADPH	0.14	–
Mitochondrial (40)			
	NADH	2.35	2.30
	NADPH	0.75	0.70
	Succinate	0.00	0.00
Microsomal (7)			
	NADH	1.18	–
	NADPH	0.45	–
Supernatant (33)			
	NADH	4.7	–
	NADPH	6.0	–

*All samples were made of 130 mM KCl, 20 mM phosphate buffer (pH 7.2), 0.6 μM HRP and 0.2–1.0 mg protein/ml. 40 μM NADH (or NADPH), 7 mM succinate and 1–2 μM antimycin were added where indicated.

Results. Trace A in figure 1 shows that addition of CCP to respiring epimastigotes suspended in saline medium did not reveal formation of H_2O_2, despite the fact that limiting amounts of H_2O_2 formed by an extracellular H_2O_2

generator (D-glucose oxidase plus D-glucose) were easily detected. In contrast to this negative result with living epimastigotes, formation of H_2O_2 by an equivalent amount of disrupted cells could be demonstrated, as shown by the upward deflection of trace B in figure 1, from which a generation rate of 3.4 pmoles H_2O_2/min mg protein could be calculated. Addition of NADH or NADPH significantly increased the rate of H_2O_2 production by the homogenate (figure 1, trace C). Under similar experimental conditions, 5 mM D-glucose, L-lactate, pyruvate, succinate, glycolate, D-alanine, L-leucine, 3 μM urate and 35 μM xanthine failed to increase the rate of H_2O_2 production, in all probability because of the dilution of cofactors and enzymes involved in the oxidation of these substrates. Homogenates of epimastigotes made dyskinetoplastic by culturing in the presence of 5 μg/ml ethidium bromide[16] generated H_2O_2 just as the normal ones.

The accompanying table shows the production of H_2O_2 by subcellular fractions. It is to be seen that with the mitochondrial fraction, addition of NADH or NADPH was essential in order to have measurable rates of H_2O_2 generation, NADH being about 3 times as effective as NADPH. It is worth noting that neither succinate nor antimycin stimulated H_2O_2 production, despite the presence of succinate dehydrogenase and an antimycin-sensitive respiratory chain in T. cruzi

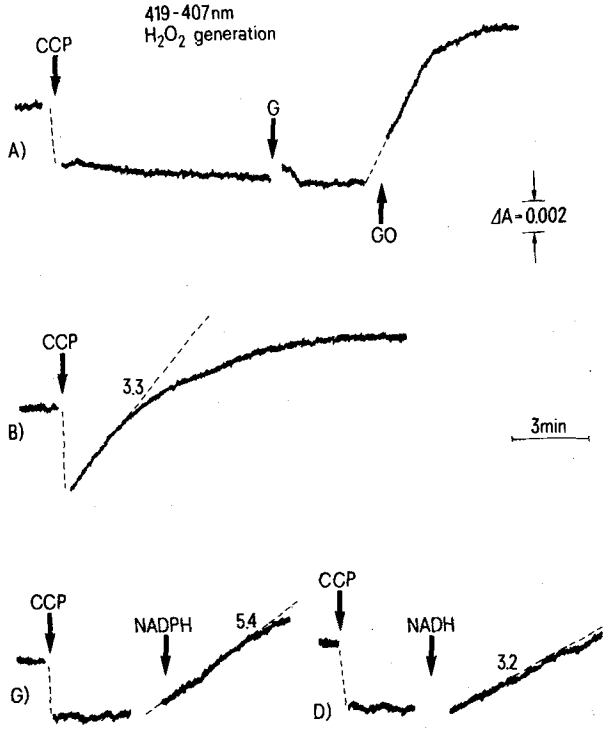

Fig. 1. Generation of H_2O_2 by T. cruzi epimastigotes. *A* Normal epimastigotes (0.5 mg protein/ml) suspended in 130 mM NaCl, 20 mM Tris-HCl buffer (pH 7.2). Where indicated, 0.25 μM CCP, 5 mM D-glucose (G) and 40 ng/ml D-glucose oxidase (GO) were added. *B* Epimastigote homogenate (0.5 mg protein/ml) in 130 mM KCl, 20 mM phosphate buffer (pH 7.2); 0.35 μM CCP. *C* and *D* Homogenate, 0.16 mg protein/ml; where indicated, 0.25 μM CCP, NADPH or NADH 40 μM; other conditions were as in *B*. The numbers near the traces indicate H_2O_2 production in nmoles/min 10^6 cells. The downward deflection after CCP addition is due to this latter own absorbance.

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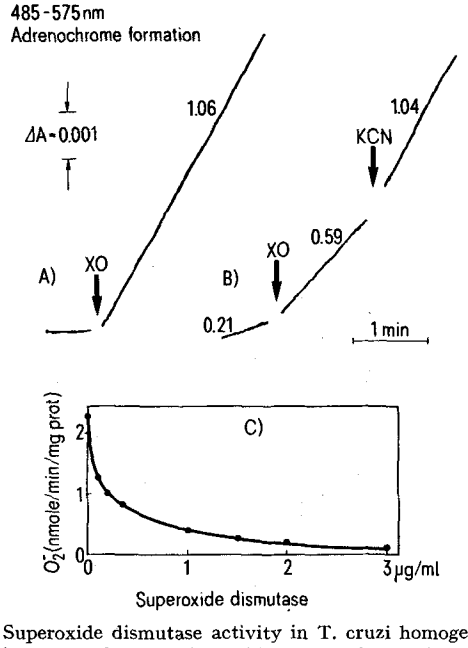

Fig. 2. Superoxide dismutase activity in T. cruzi homogenates. *A* Generation of O^-_2 by xanthine oxidase. Reaction medium: 0.23 M mannitol, 0.07 M sucrose, Tris-morpholinopropane sulfonate buffer 30 mM (pH 7.9), 1 mM epinephrine, 0.5 mM xanthine. Where indicated, xanthine oxidase (XO) was added (1 μg/ml; specific activity: 0.61 mU/mg protein. *B* Inhibition of adrenochrome formation by T. cruzi homogenate. Experimental conditions as in *A*; homogenate, 1.1 mg protein/ml; xanthine oxidase (XO) and 1 mM KCN where indicated. The numbers neat the traces indicate adrenochrome formation (μM/min). *C* Inhibition of adrenochrome formation by bovine superoxide dismutase. Heart muscle submitochondrial particles (0.2 mg protein/ml) in the presence of 7 mM succinate and 0.2 μM antimycin were utilized as source of O^-_2.

epimastigotes[17,18]. Similar results were obtained with the microsomal fraction, although specific H_2O_2 production rates were about 50% of those with the mitochondrial fraction. The highest rates of H_2O_2 generation were observed with the supernatant, but at variance with the other assayed fractions, NADPH was the more effective electron donor. The high H_2O_2 production rates with the soluble fraction suggests the presence of autoxidizable flavoproteins of the flavodoxin[19] type in that fraction. Taking into account the role of superoxide dismutase in H_2O_2 generation[6] (reaction 3),

$$O^-_2 + O^-_2 + 2H^+ \rightarrow H_2O_2 + O_2. \quad (3)$$

T. cruzi homogenates were investigated for this enzyme. The experimental results are presented in figure 2. Trace A shows the control activity, while trace B shows the inhibition of adrenochrome formation by the homogenate. Addition of cyanide abolished the inhibition, thus indicating the presence of a cyanide-sensitive cupro-zinc superoxide dismutase in the homogenate. The quantitative data in figure 2 (lower graph) enable one to calculate the dismutase concentration in the homogenate, which was equivalent to 0.28 µg bovine superoxide dismutase per mg homogenate protein.

Discussion. Measurement of H_2O_2 generation by T. cruzi homogenates accounts for about 4% of the endogenous oxygen uptake by the epimastigotes (see De Boiso et al.[17] for rates of respiration). This may be a minimum value considering the dilution of endogenous substrates and coenzymes presumably involved in peroxide generation under physiological conditions. On the other hand, the antimycin and cyanide-insensitive respiration of T. cruzi is about 15% of the overall aerobic respiration[17], a value from which one can infer that a large proportion of the antimycin- and cyanide-insensitive oxygen consumption is due to H_2O_2 formation.

Distribution of protein in the subcellular fractions as described in the table, as well as the specific values for H_2O_2 generation by those fractions, allow one to calculate the relative contribution of fractions to total cellular generation of H_2O_2. This contribution is as follows: supernatant, 63%; mitochondrial membranes, 31%, and endoplasmic reticulum, 6%. It must be noted, however, that this calculation is based on the assumption that in physiological conditions the peroxide generators are fully saturated with reductant.

In contrast to the results obtained with homogenates, the CCP assay failed to demonstrate H_2O_2 production by epimastigotes. This failure is presumably due to the inaccesibility of the extracellular CCP to the intracellular H_2O_2, as well as to the negligible diffusion of intracellular H_2O_2 to the extracellular medium.

Since T. cruzi epimastigotes contain peroxidase (not catalase) as H_2O_2 metabolizing enzyme[8], the intracellular steady state level of H_2O_2 must depend on both a) the rate of H_2O_2 generation and b) the supply of hydrogen donors for the peroxidase reaction. Unbalance of these processes may prove lethal for the parasite since H_2O_2 is toxic for Trypanosomatidae[9,10]. Consequently, drugs that should either prevent H_2O_2 utilization or stimulate H_2O_2 generation may be regarded as possible trypanocide agents.

The control of melanin dispersion in the hypomere of *Xenopus* larvae[1]

G. J. MacMillan[2,3]

Department of Zoology, University of Glasgow, Glasgow (Scotland), 11 February 1977

Summary. Studies on melanophores cultured in vesicles derived from Xenopus hypomeric tissues suggest production of a diffusible melanin-concentrating substance by ventral hypomeric tissues and melanin-dispersing properties in lateral hypomeric tissues.

During normal development and under normal levels of incident illumination the reticulate melanophores in the lateral tissues of the hypomere of early larval Xenopus undergo a progressive concentration of melanin granules which begins at stage 40 in the most ventrally situated melanophores then spreads dorsally, culminating in a dorsoventral gradation of reticulate, stellate and punctate melanophores. The dispersion of melanin in melanophores is controlled principally by the level of MSH (melanophore stimulating hormone) in the tissues[4,5]. However, Pehlemann[6] found that the dispersion of melanin in melanophores on the peritoneum (formerly hypomeric melanophores) of Xenopus larvae is largely independent of MSH and suggested some control by environmental tissues. Recent work[7] has suggested the possibility that the dispersion of melanin in hypomeric melanophores might be influenced by a melanin-concentrating substance produced by the ventral tissues of this region. In the present study the dispersion of melanin in melanophores isolated in vesicles derived from tissues of various regions of the hypomere was examined in order to investigate this possibility.

Methods. Eggs of Xenopus laevis were obtained by standard methods[8]. The techniques used in preparing and culturing vesicles have been described previously[9]. Uniform portions of neural crest and underlying dorsal sector of neural tube were excised from the anterior trunk of stage 22 embryos (staging according to Nieuwkoop and Faber[10]). Such explants were cultured in vesicles derived from sheets of a) lateral, b) ventral and c) lateral and ventral hypomeric epidermis and all subadjacent mesoderm obtained from stage 22 embryos. 50 vesicles of lateral, 50 vesicles of ventral and 46 vesicles of lateral and ventral hypomeric tissues (composite vesicles) were cultured for up to 2 weeks under normal indoor illumination and the dispersion of melanin in melanophores which

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2 Present address: Department of Developmental Biology, University of Aberdeen, Aberdeen, AB9 1AS, Scotland.

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