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METABOLISM AND MODE OF ACTION OF GENTIAN VIOLET

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Transmission of Chagas'disease by blood transfusion in endemic and non-endemic areas often has been reported (Bruce-Chwatt, 1985; Schmuñis, 1985). The epidemiologic importance of this mechanism of transmission is increasing because of the growing urbanization of the countries where this disease is endemic, the extensive migratory flow of rural populations to urban areas, and the large scale use of whole blood and its derivaties (Bruce-Chwatt, 1985; Schmuñis, 1985).

Considering the prevalence of *T. cruzi* in blood banks of different areas (Schmuñis, 1985) it can be estimated that more than 20,000 blood samples would be potentially capable of transmitting the disease each year (Docampo and Moreno,1985).

A novel procedure has been employed against transmission of Chagas disease by blood transfusion in areas where the incidence of the disease is very high, i. e. the use of blood additives which kill the trypomastigotes of *T. cruzi*. This procedure is essential in such cases since hospitals in those areas do not usually have the facilities to carry out serological tests, end even if such tests were possible, discarding of seropositive blood would severely compromise the blood transfusion servies (Anonimous, 1984).

Nussenzweig et al.(Nussenzweig et al.,1953) first reported the in vitro activity of the tryphenylmethane dye gentian violet (crystal violet) against the trypomastigote forms of *T. cruzi* and proposed its use in blood banks to prevent this form of transmisslon.

Gentian violet is added routinely at a concentration of 250 µg/ml (0.6 mM) and the blood is stored at 4ºC for 24 hours before use (Rezende et al.,1965). No serious toxic side effects have been observed in the patients transfused with this blood (Rezende et al.,1965).

The main disadvantages of treating blood with gentian violet are the necessity to store the blood for at least 24 hours and the colouring of the blood. This is because hospitals in some endemic regions do not have the facilities to store blood and practice immediate transfusions and because the colouring of the blood causes adverse, psychological reactions in both patients and physicians. Any method that could eliminate these disadvantages may improve the chemoprophylactic value of gentian violet. Recent work in our Laboratory has been done in this direction (Docampo et al., 1988).

Several hypothesis have been provided to explain the selective toxicity of gentian violet in microorganisms: (1) poising of the redox potential by the dye; (2) formation of an unionized complex of the microorganisms with dye; (3) inhibition of protein synthesis because the ribosomes to which the dye is bound function less efficiently (4) inhibition of glutamine synthesis; and (5) inhibition of the formation of the bacterial wall at a point different from that affected by penicillin (Docampo and Moreno, 1985). The formation of a carbon-centered radical metabolite of gentian violet by intact epimastigote and trypomastigote stages of

T. cruzi has been described (Docampo et al.,1983). Incubation of *T. cruzi* homogenates in the presence of an NADH-generating system also generates this free radical in the dark (Docampo et al.,1983). However, this free radical formation is very slow and very high concentrations of gentian violet (5 mM) are necessary to detect the radical (Docampo et al.,1983). We then investigated the earlier ultrastructural alterations that occur upon incubation of *T. cruzi* different stages in the presence of gentian violet to try to identify its possible target. Incubation of *T. cruzi* epimastigotes or trypomastigotes with low concentrations (5-50 μM) of gentian violet for short periods of time (15-30 min) resulted in mitochondrial swelling (De Souza et al.,1987). At these concentrations of gentian violet there was no apparent increase in the permeability of the cells, no ultrastructural alterations as observed in freeze-fracture replicas of *T. cruzi* plasma membrane and no changes in the plasma membrane ATPase activity as detected cytochemically. Lysosomal damage as measured cytochemically by the release of acid phosphatase content was not detected (De Souza et al.,1987). Taken together, these results indicate that *T. cruzi* mitochondrion is the main target of gentian violet toxicity. Accordingly, since rat liver mitochondria is the better studied and more useful system for the study of a mitochondrial toxin, we studied the effect of gentian violet on rat liver mitochondria. These studies indicated that gentian

violet is a potent uncoupler of oxidative phosphorylation in rat liver mitochondria. Gentian violet released respiratory control, hindered ATP synthesis, enhanced ATPase activity, produced swelling of isolated rat liver mitochondria, and released the inhibition of state 3 respiration by oligomycin. Maximal stimulation of respiration, ATPase activity, and swelling was observed at a concentration of 40 μM. Higher concentrations of gentian violet had an inhibitory effect on mitochondrial respiration. The uncoupling action of gentian violet was not mediated by a free radical metabolism of the dye and was light-insensitive. Gentian violet induced stimulation of state 4 respiration and swelling of mitochondria in the presence of phosphate. It was postulated that the membrane-bound dye causes perturbation of the integrity of the membrane structure in cooperation with phosphate, and that this perturbation stimulates phosphate translocation via the phosphate/H symporter, associated with penetration of cations from the incubation medium into the mitochondria. The transported phosphate then returns to the positively charged outer side of the mitochondria, probably via the dycarboxylate-phosphate carrier. The cation intrusion accompanied by phosphate translocation causes swelling and dissipation of the membrane potential which leads to uncoupling of oxidative phosphorylation in mitochondria (Moreno et al., 1988) (Figure 1).

Using digitonin to permeabilize *T. cruzi* plasma membrane (Docampo and Vercesi, 1988) we were able to demonstrate

an uncoupling action of gentian violet on *T. cruzi* mitochondrion since this method allowed the study of oxidative phosphorylation in mitochondria in situ. Using ADP as phosphate acceptor and succinate as oxidizable substrate, respiratory control values in the range of 2.0-2.5 were obtained. Low concentrations of gentian violet (20-50 μM) or FCCP (0.5 μM) uncoupled the respiratory control mechanism. The inhibition of state 3 respiration by oligomycin was released by gentian violet or FCCP (Figure 2). Respiration of gentian violet-treated, digitonin-permeabilized *T. cruzi* remained sensitive to cyanide and antimycin A. There was no indication that gentian violet underwent reversible oxidation-reduction or diverted the normal pathway of the electron transport system. In addition, gentian violet stimulated the Mg^{2+}-ATPase of digitonin-permeabilized cells (Moreno et al., 1988).

In conclusion, as other cationic dyes (Oseroff et al.,1986), gentian violet appears to concentrate in mitochondria and exert a disruptive effect on those organelles.

It is interesting to note that, despite its potent uncoupling action, gentian violet is devoid of acute toxic side effects when used in blood at high concentrations (0.6 mM) to prevent blood transmission of Chagas'disease. On the other hand, gentian violet is active against *T. cruzi* in the micromolar concentration range (Docampo et al.,1983). The presence of an active demethylation activity in liver microsomes (Mc Donald et al.,1984) could partially explain this selective

toxicity. Differences in accumulation and retention of gentian violet by *T. cruzi* and mammalian cells mitochondria as those that have been described for other cationic dyes between normal and carcinoma cells' mitochondria (Davis et al.,1985) could also account for this different toxicity and is presently under investigation.

Acknowledgements

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Figure 1. Mechanism of uncoupling of oxidative phosphorylation by gentian violet. The dye is not permeant but binds to the mitochondrial membrane causing a perturbation of the integrity of the membrane structure tnat stimulates phosphate translocation via the phosphate/H symporter (P) associated with penetration of cations (C^+) from the incubation medium into the mitochondria. The transported phosphate then returns to the positively charged outer side of the mitochondria, probably via the dicarboxylate-phosphate carrier (DP). The cation intrusion accompanied by phosphate translocation causes swelling and disipation of the membrane potential which leads to uncoupling of oxidative phosphorylation in mitochondria (Moreno et al., 1988).

Figure 2. Oxygen consumption by digitonin-permeabilized epimastigotes. The reaction mixture contained 200 mM sucrose, 2 mM $MgCl_2$, 1 mM EDTA, 10 mM potassium phosphate buffer (pH 7.4), 5 mM succinate, and 0.066% digitonin in a total volume of 1.5 ml. Additions: epimastigotes (E), 1.1 mg protein/ml, 0.066 mM ADP, 1 µg oligomycin/ml (OLIG), 40 µM gentian violet (CV), 0.35 µM FCCP, 1 µg antimycin/ml (ANT).

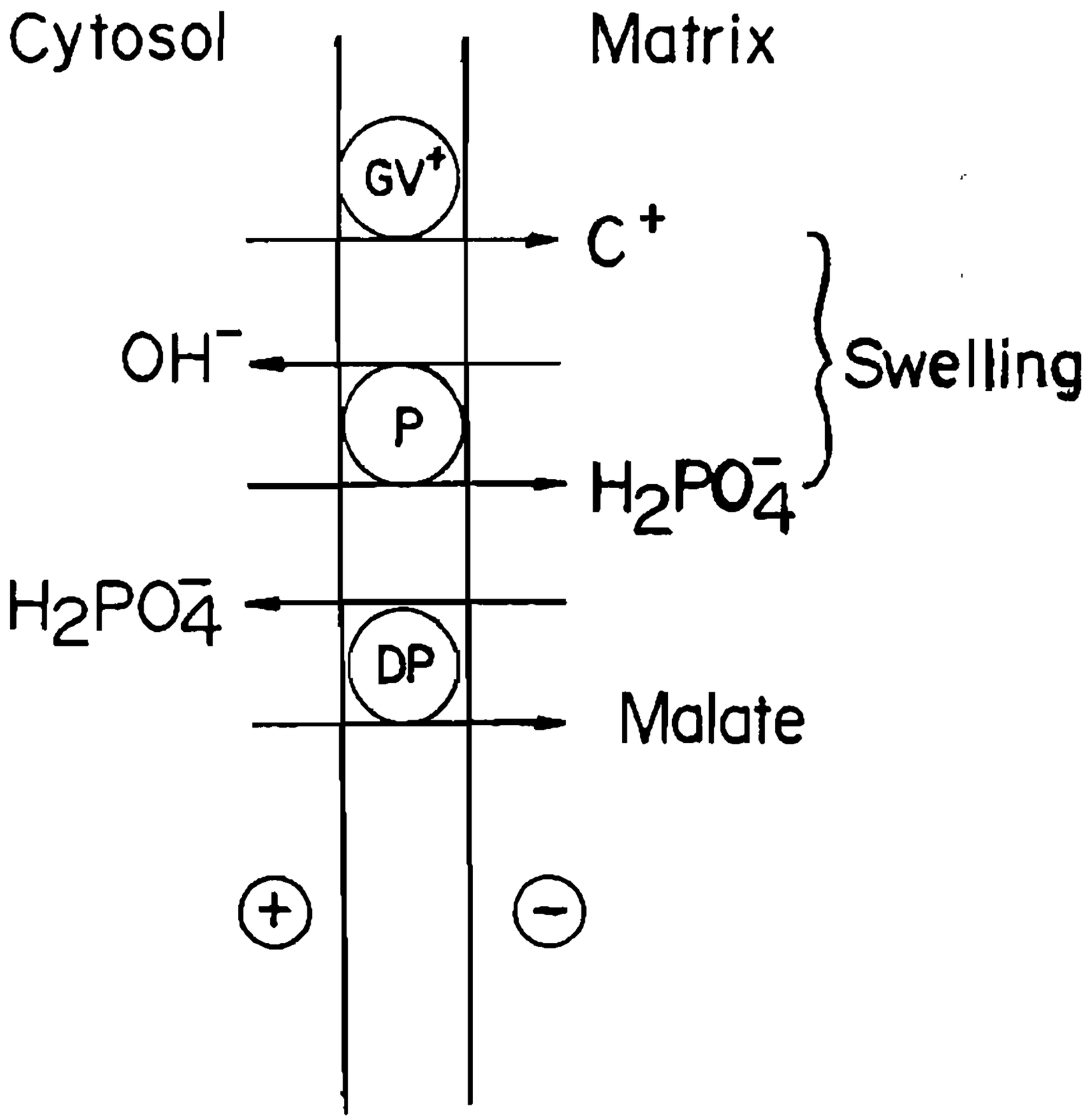

Figure 1. Moreno, 1988.

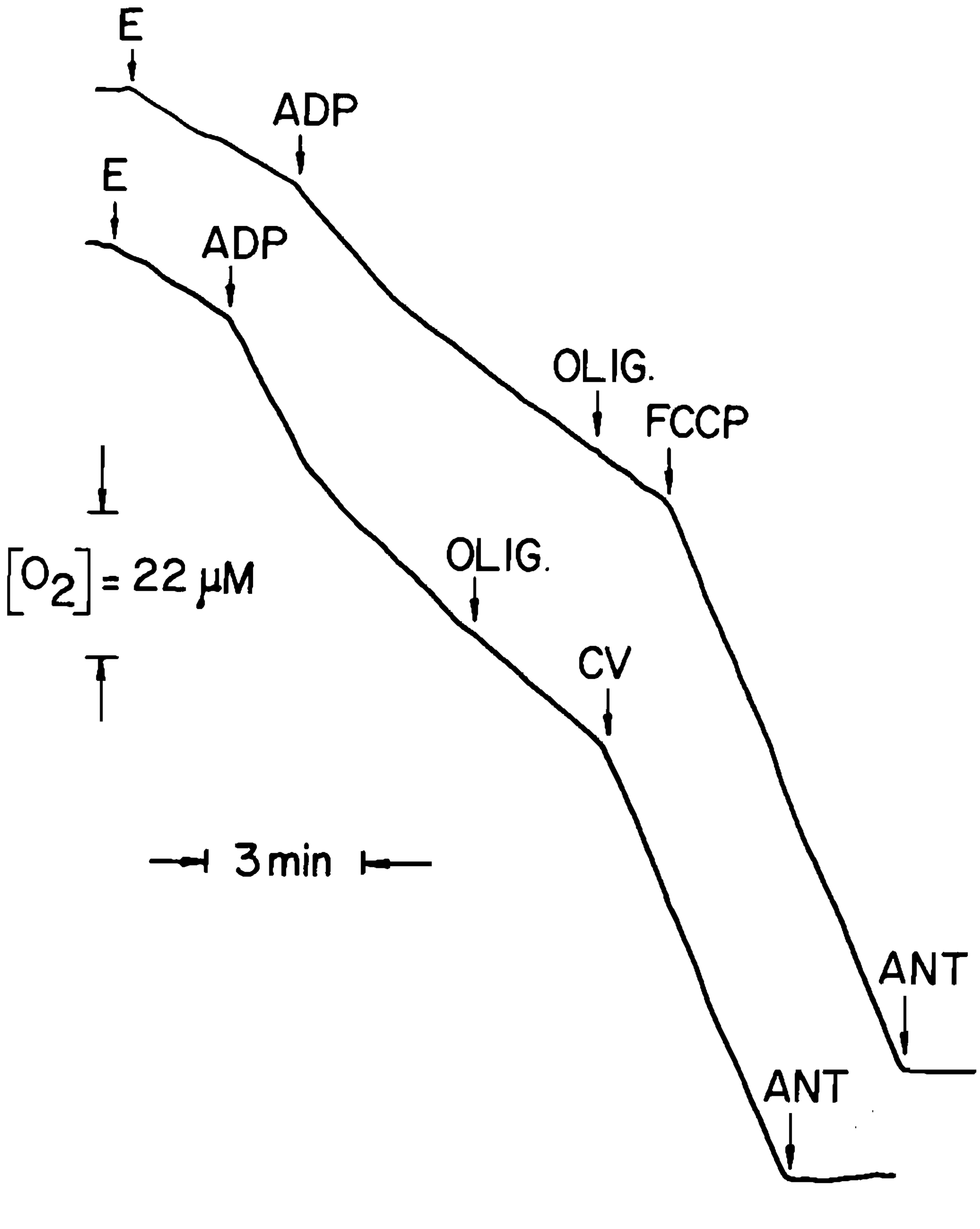

Figure 2. Moreno, 1988.