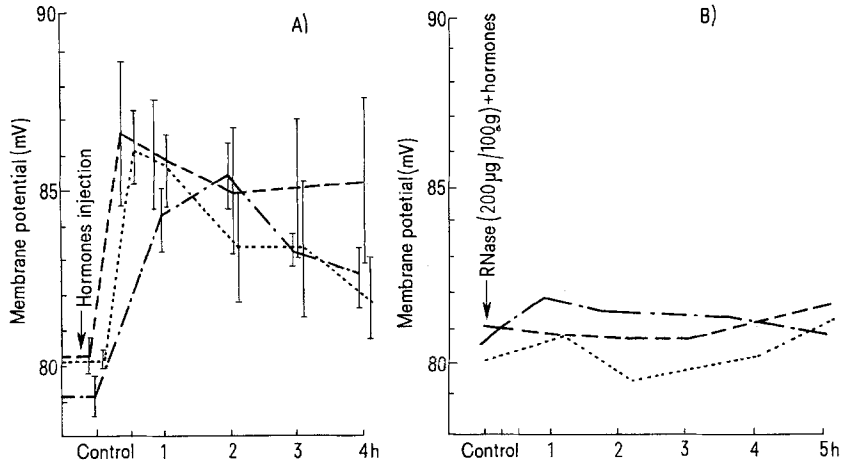

Fig. 4. Effect of ribonuclease on membrane potential level of single muscle fibres. (A) Change of membrane potential level of single muscle fibres induced by hormones. (B) Effect of ribonuclease on the change in membrane potential level caused by hormones. Broken line, insulin (0.16 units/100 g); Dotted line, estradioldipropionate (0.1 mg/100 g); Broken-dotted line, 1 dehydromethyltestosterone (0.2 mg/100 g).

3. Inhibitors of protein biosynthesis (actinomycin D, ribonuclease) prevent the activation of RNA synthesis in liver cells occurring after bloodletting and with insulin injection. Thus, 24 h after bloodletting there was an increased RNA renovation rate from 928 ± 84 imp/min/mg RNA to 1730 ± 104 imp/min/mg RNA. With a prior actinomycin D injection, the inclusion was found to increase insignificantly (1260 ± 160 imp/min/mg RNA).

Thus, with the activation of protein biosynthesis, the hyperpolarization of a cell developes. Inhibitors of protein biosynthesis prevent the development of hyperpolarization. It can be stated that a relationship between protein biosynthesis and cellular function plays an important role in the development of hyperpolarization and regulation of membrane potential level.

ВЫВОДЫ. Активация биосинтеза белка в клетке ведет к развитию ее гиперполяризации.

V. V. FROLKIS

Laboratory of Physiology, Institute of Gerontology, Academy of Medical Sciences USSR, Vyshgorodskaya 67, USSR-252655 Kiev (USSR),
29 January 1973.

Oxidative Phosphorylation in *Trypanosoma cruzi*[1]

The occurence of oxidative phosphorylation in Trypanosomatidae has been demonstrated with *Crithidia fasciculata*[2–5] and *Trypanosoma mega*[6]. In this communication we describe experiments that prove the operation of oxidative phosphorylation in *T. cruzi*, the agent of Chagas' disease. The demonstration is based on the effect of specific inhibitors on: 1. the redox state of the parasite cytochrome 'b'; 2. respiration; and 3. the intracellular concentration of high-energy phosphate and P_i.

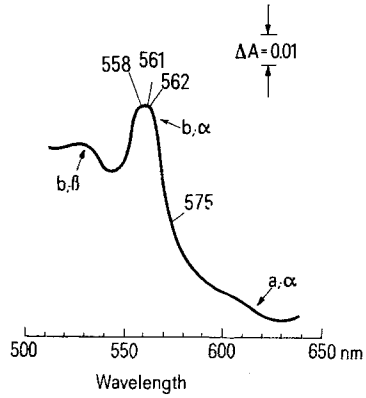

Fig. 1. Spectrum of *T. cruzi*. 27 mg epimastigotes suspended in 1.75 ml of a 30% (v/v) glycerol-standard saline medium mixture. To the measuring cuvette was added dithionite; flat oiled paper was placed in the reference cuvette[20]. 5 mm Light path. For other conditions see Methods.

Materials. *T. cruzi* epimastigotes were grown at 28 °C as described earlier[7,8]. The medium (solid phase) contained 5% (v/v) sheep blood. Unless stated otherwise the concentration of cell suspensions was measured by the weight obtained after drying washed epimastigotes at 100–104 °C for 24 h (the dry-weight was about 15% of

[1] Abbreviations. In addition to standard abbreviations, the following are used: PCP, pentachlorophenol; CCP, carbonylcyanide-*m*-chlorophenyl hydrazone; DCCD, N,N'-dicyclohexylcarbodiimide; P_i, inorganic orthophosphate; P_7, heat and acid labile phosphate; P_{as}, total acid-soluble phosphate; $P_0 = P_{as}$ less ($P_i + P_7$).

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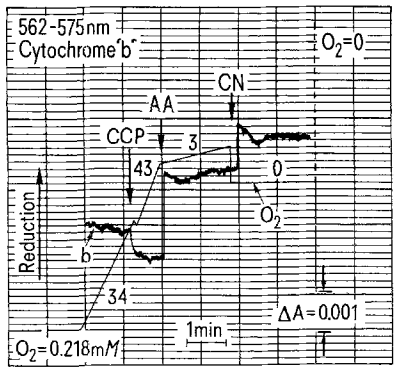

Fig. 2. Effects of CCP, antimycin A and cyanide on the redox state of cytochrome 'b' and respiration. 6.2 mg epimastigotes suspended in 1.75 ml of standard saline medium (pH 7.2); endogenous substrates; CCP, 5.7 μM; antimycin A, 0.34 μg/ml; cyanide, 0.4 mM; temperature, 37 °C; light-path 5.0 mm. Oxygen concentration was measured with the platinum vibrating electrode. Where indicated by the arrows, oxygen was introduced into the anaerobic suspension by means of mechanical agitation. The number at the oxygen trace indicates the rate of respiration (ng atom O/min/mg of cells).

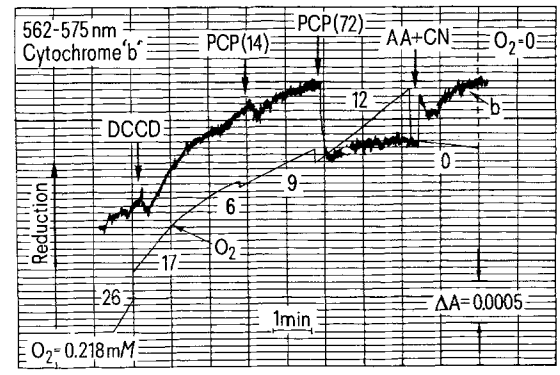

Fig. 3. Effects of DCCD on the redox state of cytochrome 'b' and respiration. Reversal of DCCD effects by PCP. 6.5 mg epimastigotes; DCCD, 590 μM; cyanide (CN), 0.3 mM; antimycin A (AA), 0.34 μg/ml; PCP (μM) as indicated above. DCCD was added in 50 μl of dimethyl sulfoxide (the solvent had no effect). For other conditions see Figure 2. In the absence of DCCD, respiration continued at the initial rate and 'b' maintained its initial redox state until exhaustion of oxygen. In the absence of PCP, the inhibition of respiration and the reduction of 'b' continued to increase.

the fresh-weight[9]). Reagents were purchased from the following sources: kits for measuring ATP, from Böhringer und Söhne; DCCD, from Koch-Light & Co; antimycin A, from Sigma Chemical Co. and PCP, from E. Kodak Co. CCP was a gift from Dr. P.G. HEYTLER, E.I. Dupont de Nemours and Co.

Methods. The spectrum of epimastigotes was obtained with a Johnson Research Foundation split-beam spectrophotometer. Changes in absorbance at selected wavelength pairs were followed in an Aminco-Chance dual wavelength spectrophotometer. Respiration was measured with the vibrating platinum electrode or with Warburg direct method. ATP was measured as described in reference[10]; P_i and incorporation of ^{32}P in organic phosphates were measured as described in reference[8].

Incubation conditions were as described in Results, using a medium (henceforth standard saline medium) consisting of 110 mM K^+, 45 mM *Tris*, 6.8 mM Na^+; 6.8 mM phosphate buffer and 157 mM Cl^-; pH 7.2.

Results and discussion. Figure 1 shows the spectrum of *T. cruzi* in the 650–500 nm range. In agreement with earlier reports[7,11,12], the α-band of cytochrome b was the main peak of the spectrum; the α-band of cytochrome a was very small and a typical c_{550} was undetectable. The α-band of cytochrome b showed a broad maximum at 558–562 nm and it is assumed that it could involve contributions from several cytochrome species, including c_{558} (ref.[3]). Therefore, absorbance at 558–562 nm will be provisionally termed cytochrome 'b' until the constituent species have been identified.

Mammalian cytochrome b is a suitable test of the energy state of the inner membrane because the redox state of at least one b species (b_T) is dependent on the phosphorylation potential[13]. On this basis, the response of *T. cruzi* cytochrome 'b' to inhibitors of oxidative phosphorylation was investigated. Figure 2 shows the effects of CCP (an uncoupler), antimycin A and cyanide (both inhibitors of electron transfer). Absorbance changes were measured at 562 nm (with 575 as reference) to minimize interference by c_{558} (ref.[3]). It can be seen that 1. addition of CCP caused a shift of 'b' towards the oxidized state and a 26% increase of the rate of respiration; 2. addition of antimycin A caused a reduction of 'b' and 89% inhibition of respiration; 3. final addition of cyanide caused a further increase in absorbance and complete inhibition of respiration. These results strongly suggest that 'b' included at least one energy-transducing cytochrome on the substrate side of the antimycin sensitive site (the b-c_1 segment of the respiratory chain[14]). Concerning the effect of cyanide, an inhibitor of *T. cruzi* respiration[11,12], it is assumed that it prevented the flow of electrons escaping from the antimycin A inhibition and also from reduced c_{558}. Difference between cyanide and antimycin A levels of reduced 'b' (Figure 2) indicated about 30% interference by non-b cytochromes at 562 nm.

DCCD, an inhibitor of oxidative phosphorylation, which mimics olygomycin action, was used as suggested by the experiments of KOVAC et al.[15] with yeast. Figure 3

Table I. Effect of inhibitors on intracellular ATP[a]

Inhibitor (μM)	ATP concentration (mM)
None	3.8
CCP (10)	1.5
DCCD (600)	2.3
Cyanide (100)	1.9

[a] Epimastigotes (66 mg) were suspended in 6 ml of standard saline medium plus the additions stated above. After 5 min incubation, 2.0 ml duplicate samples were taken with an automatic pipette and thoroughly mixed with 1.0 ml ice-cold perchloric acid (50% w/v). After centrifugation, the supernatant was analysed for ATP. ATP concentration was calculated by assuming that the water content of fresh epimastigotes was 85% of the total weight[9].

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shows the effects on the redox state of 'b' and respiration. The sequence of events may be described as follows: 1. addition of DCCD increasingly inhibited respiration and shifted 'b' towards the reduced state; 2. addition of 14 μM PCP stimulated respiration by 50% (with respect to the inhibited rate) and slightly reverted the reduction of 'b'; 3. further addition of 72 μM PCP stimulated respiration by 100% (with respect to the inhibited rate) and caused an extensive oxidation of 'b'; 4. final addition of cyanide plus antimycin A completely inhibited respiration and restablished 'b' in the reduced state, demonstrating the reversibility of PCP effect.

T. cruzi epimastigotes contained ATP in a concentration not very different from those reported for animal tissues such as skeletal and heart muscle[16]. Treatment of epimastigotes with cyanide, CCP and DCCD determined significant diminutions of the intracellular concentrationof ATP (Table I). In accordance with those results, antimycin A inhibited phosphorylation (Table II), as shown by the decrease of ^{32}P incorporation into P_7 and P_0, and by the increase of the intracellular concentration of P_i. The effect on the labelling of P_0 (that represents glucose-6-P and derivates[17]) is explained by considering that part of the ATP required to phosphorylate glucose originated in oxidative phosphorylation.

Table II. Effect of antimycin A on a) $^{32}P_i$ uptake in high-energy phosphate and b) the intracellular concentration of P_i

Experiment	Antimycin A (μg/mg of cells)	Uptake of ^{32}P-phosphate in fractions (μg atom ^{32}P) (g of cells [c]) P_7	P_0	Inhibition of respiration (%)
a)	None	0.8	0.4	—
	0.19	0.3	0.2	80
		Diminution of intracellular P_i (μg atom P) (g of cells [c])		
b)	None	6.0		—
	0.11	−1.0		78

Experiment a). Epimastigotes (16 mg phosphate deficient PD-1 (ref. [7]); 3.9 mM ^{31}P-phosphate (6.0×10^8 cpm/mg atom ^{32}P); 5.0 mM glucose; standard saline medium to 3.0 ml. Incubation in Warburg manometers for 2 h at 30 °C. After incubation the cells were analyzed for the incorporation of ^{32}P in P-fractions. Control Q_{O_2}, 5.0 Experiment b). Epimastigotes, 8.9 mg; glucose, 5.0 mM; incubation for 3 h. Initial P_i concentration, 27 μg atom P per g of cells. Other conditions as in experiment a). Control Q_{O_2}, 9.0. After incubation the cells were analyzed for P_i concentration. [c] Fresh weight.

In conclusion, occurence of oxidative phosphorylation in *T. cruzi* is supported by: 1. the oxidation of reduced cytochrome 'b' after addition of uncouplers (Figures 2 and 3); 2. the consistent effects of antimycin A on the redox state of 'b', respiration and phosphate metabolism (Figure 2 and Table II), and 3. the effect of uncouplers and DCCD on respiration and phosphate metabolism (Figures 2, 3 and Table I). These effects are in good agreement with the presence of a Mg^{2+}-activated ATPase[18] and mitochondrial structures in *T. cruzi*[19–21].

Zusammenfassung. In Epimastigoten von *T. cruzi* wird die oxydative Phosphorylierung bewiesen durch: 1. Beseitigung der Atmungskontrolle durch Zugabe von entkoppelnden Substanzen (CCP und PCP); 2. Hemmung der Phosphorylierung durch Antimycin mit gleichzeitiger Reduktion von Cytochrom 'b'; 3. Atmungshemmung durch Dicyclohexylcarbodiimid (DCCD); 4. Verminderung des intrazellulären ATP durch CCP, DCCD und Cyanid.

A. O. M. STOPPANI[22] and J. F. DE BOISO[22]

Instituto de Química Biológica,
Facultad de Medicina,
Paraguay 2155, Buenos Aires (Argentina),
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[22] Career Investigator, CONICET.

Visual Cortical Cells: How Critical is Focus?

Although performance limits of the retina-central pathways can now be defined using techniques which essentially by-pass the optics of the eye[1–3], little, yet, is known of the specific effects of image blur on single cell performance at successive levels of these pathways.

Initial observations of cat retinal ganglion cell responses do indicate, however, that refractive errors of as little as 0.25 diopters can often be detected through use of a set of neurophysiological criteria[4,5]. A similar susceptibility to blur, but generally less acute, has been demonstrated for particular cells of the rabbit superior colliculus, as well[6].

But how critical is image focus to cortical cell performance? Is the efficiency of every cell equally degraded by blur? And, how is responsiveness to specific trigger-features in the environment, e.g. motion and direction, affected?

Material and method. 123 photically responding cortical cells of the rabbit were investigated here, using the same experimental methods and conditions as in the superior colliculus study reported earlier[6]. As previously, the receptive field of each cell was repeatedly plotted through a series of induced spherical refractive errors.

Results and discussion. The Figure illustrates 6 classes of cortical cell performance in the presence of induced retinal blur. The upper 3 profiles are in response to flashing $^1/_2{}^\circ$ spot where: (A) shows a cell with a very high susceptibility to induced blur (representative of about