The pH as Controlling Factor of Shape and Function of Mitochondria

Previous investigations[1] showed that the functional modifications of mitochondria are closely related to their morphological changes; in the swelling which, within certain limits, seems to occur in the cell according to a functional correlation, an increase in oxidations usually takes place; when swelling is considerable a loss of co-factors and uncoupling of oxidative phosphorylation may be observed.

Swelling appears to be under the control of hormonal and nervous factors[1,2] in physiological conditions, but nothing is known as yet of the mechanism controlling the morphological and functional regulation of mitochondria within the cell. As mitochondria have a semipermeable membrane, swelling was related to hypotonic variations of the medium[3,4]. Scarce data exist on the possible influence of the pH, and these are only incidentally mentioned in the course of other investigations[5–8], in spite of the importance of electric potential variations on membrana phenomena in general. We have also noticed that, although the pH within the cells is generally supposed to be acid[9,10], most recent investigations *in vitro* on mitochondria oxidative phosphorylation were carried out (with a few exceptions[11–13]) at a standard pH of 7.4. We therefore believed it would be interesting to carry out a systematic investigation on the influence of pH on the structure and function of mitochondria *in vivo* and *in vitro*. The most significant findings will now be described.

The difficulty of applying the electrometric method to the study of intracellular pH induced us to adopt the colorimetric method described by CLARK[14]. As indicators we used 0.04% aqueous solutions of Bromothymol blue (pH range 6.0–7.6), Bromophenol red (pH range 5.2–7), Neutral red (pH range 7–8). The indicators were injected into laboratory animals, tested on the fresh tissues by the squash technique or inoculated into the cells by the method of micro-injection, according to CHAMBERS and FELL[15]. Standard quantities of the intracellular components isolated by differential centrifugation were tested with the indicators in special, calibrated cells of 'Perpex' and the variations of the colour were compared with a colorimetric scale at various pH.

We were, in general, able to confirm that in most tissues intracellular pH are lower than that of blood plasma, as already observed by other authors[9,10]. Values from 6.4 to 7.3 were found by us in guinea-pig, rabbit, rat, and mouse hepatocytes; Kuppfer's cells clearly appeared to be more acid (pH of 6.2–6.5). Values from 6.7 to 6.9 were found for the brain, 6.6–6.8 for the kidney. Values of the single intracellular components (assumed only as indicative of difference between them) were as follows: nuclear fraction of liver, pH from 6.6 to 6.8; mitochondrial fraction, 6.9–7.3; lysosome fraction, 6.4–6.6; microsome fraction, 6.4–6.8; soluble phase, 6.8–6.9 (this was also potentiometrically calculated). On sections of liver we sometimes observed groups of cells with a different change in colour: the centrolobular part of mouse liver was almost constantly acid (pH 6.4–6.9), whereas the peripheral zone showed groups of cells with values of 6.9–7.3. It was interesting to note that mitochondria of centrolobular zones are mostly rod-like, whereas a swollen appearance is prevalent in peripheral areas.

We were able to observe in other experiments that in certain abnormal conditions in which there is a mitochondrial shrinkage (e.g. vacuolar degeneration of the liver due to hypoxia) the intracellular pH of hepatocytes shows more acid values: on the contrary, in conditions causing a marked swelling (fasting) the pH becomes more alkaline. During the muscular contraction of guinea-pig gastrocnemious after stimulation of the sciatic nerve, there is a mitochondrial swelling[2] which is accompanied by a prompt increase in alkalinity.

Isolated mitochondria suspended in 0.25 *M* sucrose, buffered at various pH between 6 and 8, appear at phase contrast examination to become more swollen the higher the pH; the swelling thus obtained is reversible if mitochondria are re-suspended in an acid solution. Spontaneous swelling of liver mitochondria *in vitro* was studied by observing the fall in optical density with a mod G 4700 Beckman DU spectrophotometer at 520 mμ according to CLELAND[5]. Determinations were carried out by suspending in different tonic solutions (0.44 *M*, 0.25 *M*, 0.10 *M*) sucrose and in distilled water and at various pH comprised between 6 and 8 in 0.02 *M* Tris-maleate buffer; these experiments showed a greater decrease in percentage optical density at alkaline pH than at acid pH (see Table).

Percentage decrease (Δ%) of optical density on spontaneous swelling of rat liver mitochondria (E 520 mμ, temp. 20°C, 35th min)

pH	Δ% H_2O distill.	Sucr. 0.10 *M*	Sucr. 0.25 *M*	Sucr. 0.44 *M*
6.0	42.25 ± 0.35	3.37 ± 0.17	3.54 ± 0.45	0.75 ± 0.25
6.6	45.16 ± 0.61	32.25 ± 0.15	20.84 ± 0.53	14.00 ± 1.22
7.0	46.25 ± 0.38	35.90 ± 0.41	28.65 ± 0.39	21.50 ± 1.17
7.4	47.12 ± 0.38	39.25 ± 0.92	35.15 ± 0.53	30.80 ± 1.57
8.0	50.46 ± 0.55	44.66 ± 1.45	46.66 ± 1.05	37.96 ± 1.42

It therefore seems that the progressive and gradual increase in spontaneous mitochondrial swelling between pH 6 and 8 is related to the hydrogen ion concentration of the medium as well as to its tonicity. A reversal of mitochondrial swelling can be obtained at the 35th min by addition of the acid and re-swelling by addition of the base. Because of the relationship between morphological appearance and mitochondrial enzyme activity, we at first considered the behaviour of the oxidative processes at various pH, between 6 and 8, in the presence of some

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substrate liable to oxidation, such as succinate, glutamate, alanine, cytochrome c.

The manometric determination of O_2 consumption[16] showed a progressive increase of the $QO_2(N)$ from acid to alkaline pH.

We then considered the problem of the utilization of the energy freed from oxidative processes. The behaviour of oxidative phosphorylation and of ATPase was studied at various pH comprised between 6 and 8 according to the technique described in a previous paper[17]. It was observed that an increase in oxidation runs parallel to an increase in phosphorylation only at the pH between 6.6 and 7.4, so that at these pH the P/O ratio tends to be constantly coupled. At the pH from 7.4 to 8, the P/O ratio was more and more uncoupled. On the other hand, at the extreme pH of 6 and 8 the ATPase activity was greater.

It therefore appears that pH variations simultaneously control both the shape and enzymic activities of mitochondria.

Riassunto. È stato osservato che quando il pH intracellulare è più basso di quello plasmatico la forma dei mitocondri è prevalentemente a bastoncino, aumentando il pH (in condizioni sperimentali e patologiche) i mitocondri tendono a rigonfiare. Il fenomeno è anche dimostrabile spettrofotometricamente sui mitocondri isolati il cui rigonfiamento spontaneo regredisce abbassando il pH del mezzo. Il $QO_2(N)$ e l'attività ATPasica aumentano a pH alcalini parallelamente al rigonfiamento mitocondriale. Il rapporto P/O si mantiene accoppiato fra pH compresi fra 6.6 e 7.4.

È possibile che attraverso il pH si verifichi nella cellula un meccanismo di autoregolazione dell'attività funzionale dei mitocondri.

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Action Potentials of the Guinea Pig Heart in Sodium Deficient Solutions[1]

According to the 'sodium hypothesis' of electrical activity, the upstroke of the action potential depends on a substantial increase of the membrane permeability to sodium ions, allowing a positive charge to enter the cell[2]. Evidence in favour of the 'sodium hypothesis' is available for a variety of cardiac preparations: frog ventricle[3], sheep and calf ventricle[4], and mammalian Purkinje fibres[5]. The ventricle of the guinea pig, however, does not seem to follow this general rule. According to reports by two different groups[4,6], the amplitude of its action potential remains unchanged even if the sodium content of the bathing solution is reduced to zero. The present report is concerned with the effect of sodium-poor solutions on the *auricle* of the guinea pig.

Strips of the thin-walled right auricle, 8–10 mm in length and 2-4 mm in width, were immersed in a Tyrode bath and driven by a transistorized stimulator[7] at a rate of 50–100 per min. Ling–Gerard electrodes[8] were used to record from the inside of single fibres. Atrial fibres are relatively thin (diameter 8 μ[9]); therefore, it was difficult to change solutions and keep the tip within the same fibre until a new steady state was reached. Results were discarded if the action potential did not return to its original value upon return to normal Tyrode's solution.

Figure 1 illustrates the effects of reducing the sodium concentration from 100% to 27% (isosmotic saccharose was substituted for NaCl): the resting potential remained unchanged while the amplitude and duration of the action potential decreased. The upstroke velocity of the action potential, as seen on high speed records, decreased to about 30%. Reduction of the sodium concentration to zero resulted in a total loss of excitability.

Figure 2 is a plot of resting and action potential values as recorded with various extracellular sodium concentrations. The dashed line would be expected if the fibre membrane at the height of activity were exclusively

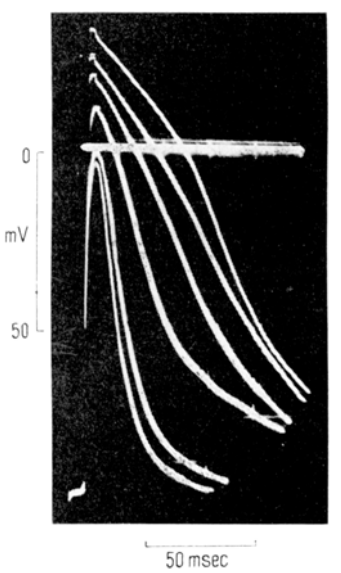

Fig. 1. Action potentials of the same auricular fibre, showing the effect of reducing the Na content of its Tyrode bath to 27%. Top trace: tissue in Tyrode's solution; lower traces: 4, 5, 10, 15 and 22 min after the change-over to Na poor solution. The rate of inflow was such that the bath was 50% exchanged at the end of 2 min.

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