Effect of pH Change Upon Influx of Anions and Cations into Mitochondria* (28369)

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Relatively fixed quantities of potassium and other ions are retained by mitochondria through washing procedures using isotonic sucrose(1-4). These ions appear to be contained in a compartment which occupies ⅓ of the volume of the centrifuged pellets and which may be the substance of the wall and the cristae(3). Experiments in which mitochondria were exposed to labeled K^+, Na^+, Cl^-, or Br^-(1,2,4) have shown that these ions penetrate into the above mentioned compartment at relatively deliberate and readily measurable rates. This penetration or influx may be due to a net inward movement with a gain in the total amount retained or to a process involving exchange of mitochondrial ions with the free ions in the surrounding fluids. The present report describes effects of change in the pH of the incubation medium. Alkalinization was found to accelerate influx of cations, and acidification was associated with increased influx of anions.

Methods. The methods and experimental procedures have been described(1,3). Sucrose, 0.25 M, buffered with Tris-HCl, pH 7.4, was used during the preparation of the mitochondria and for the final washing steps. Sucrose, 0.25 M, without added buffer, was used to suspend the mitochondria during the incubation period. The initial pH of the once-washed, freshly-prepared mitochondria in the unbuffered media usually was found to be between pH 6.8 and 7.0; and the individual aliquots were adjusted, upwards or downwards, with additions of 1-4 micromoles of KOH or NaOH or ½ to 2 micromoles of HCl. The final pH was determined by immersing the electrodes of a Beckman G pH meter directly into the media during incubation.

The standard procedure, as in previous studies(1-4), has been to incubate freshly prepared mitochondria in the presence of labeled anions or cations, to wash the particles to remove unbound electrolytes, and finally to determine the quantities of stable and radioactive materials remaining with the mitochondria. The mitochondria (1.5-2.3 mg nitrogen) were incubated for 10 minutes in sucrose (0.25 M) at 23°C. Influx was calculated by dividing the radioactivity of the washed mitochondria by the specific activity of the ions in the medium of incubation.

Results and discussion. The first figure summarizes results obtained from 3 separate experiments in which aliquots of rabbit liver mitochondria were exposed at different pH values to various concentrations of bromide, sodium or potassium. These ions were labeled with Br^{82}, Na^{24}, and K^{42}, respectively. The curves describe increased influx of the anion, bromide, with acidification, and increased influx of the cations, sodium and potassium, with alkalinization. There were large differences in the quantities incorporated. Raising the pH from 7 to 8 produced increments in influx of 50 millimicromoles of potassium and 3 to 5 of sodium; while the decrease with bromide over this range amounted only to ¼ millimicromole. The small change with bromide could be detected only in dilute solutions, less than 0.002 M. These data alone, based on changes in radioactivity, do not serve to distinguish between two possibilities: increased influx due to a net inward movement with increase in the amount retained and increased influx due to a more rapid exchange between the free and the retained ions without change in the total content of mitochondrial electrolyte.

Measurements of changes in the total amount of bromide were not undertaken, but additional observations have demonstrated increase in the content of chemically determined chloride. In one experiment, lowering the pH from 7.0 to 5.8 resulted in increase

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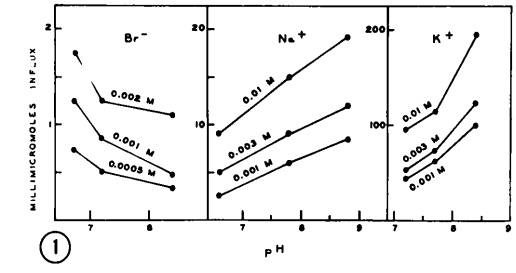

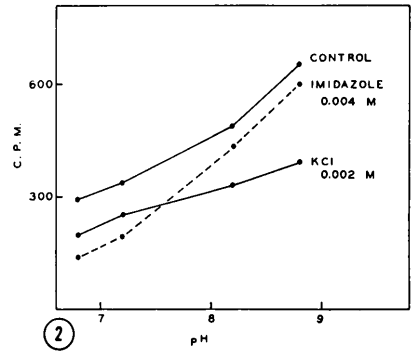

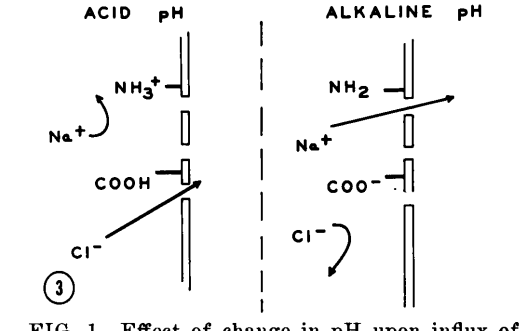

FIG. 1. Effect of change in pH upon influx of labeled bromide, sodium and potassium into mitochondrial substance.

FIG. 2. Effect of adding imidazole upon influx of radioactive sodium into kidney mitochondria at different pH values.

FIG. 3. Scheme to explain change in membrane permeability to anions and cations with change in pH.

in mitochondrial chloride from 0.04 to 0.15 μm per mg nitrogen. It is of interest, in this case, that the net inward movement was associated with release of approximately equimolar amounts of phosphate. Apparent exchange of phosphate for chloride has been described also as an effect of exposure to dinitrophenol(4).

In the sodium study (Fig. 1) the mitochondria were found to contain 50 to 61 millimicromoles of total sodium, and the values averaged 5 millimicromoles higher at pH 8.8 than at 6.6. The data lacked sufficient precision to distinguish net uptake from increased exchange activity.

The increments in potassium influx with elevation of the pH from 7.2 to 8.8 (Fig. 1) were achieved with little change in total content. Mitochondrial potassium remained between 1.06 and 1.18 μm; the average values being 0.02 μm larger at the higher pH. An additional study was carried out in 0.04 M KCl, and a greater influx, increasing from 28 to 72% of the total (1.6 to 1.8 μm per mg nitrogen), was obtained. In this case, the increased incorporation must have been due to a more rapid exchange between mitochondrial potassium and free ions in solution(1). It has been found that these steady state conditions are lost with lowering of the pH from 7.0 to 6.0. In this range the exchange rate actually increases, but as an associated response there is a sharp reduction in the total amount retained, and there is evidence of mitochondrial swelling. These changes are seen also with aging and with exposure to the inorganic mercurials(5), and they may represent a relatively non-specific reaction to partial disorganization.

In the presence of the buffer ion imidazole the effect of change in pH upon influx of sodium is exaggerated (Fig. 2). The slope of the curve becomes steeper due to an apparent inhibition at pH 7.0. Reduced influx can be correlated with increase in the concentration of imidazolium cations since, at pH 7.0, 50% of the molecules carry a positive charge compared to 1% at pH 8.8. The inhibition was anticipated on the basis of previous work showing inhibition of sodium or potassium influx with addition of other cations to the medium(4). These observations had suggested some sort of competitive interference between cations, presumably at sites of entry. Substitution or replacement of one ion for the other in the mitochondrial substance was not observed. The concentration of sodium in the present experiment (Fig. 1) was 0.002 M. Addition of KCl (0.002 M) produced a 30-40% inhibition at all pH values. These experiments were carried out with kidney mitochondria, and comparable results

have been obtained with mitochondria from liver.

As proposed in the drawing of Fig. 3, an increased rate of influx of labeled anions with acidification, or of cations with alkalinization, might be anticipated on the basis of change in permeability due to change in surface charge. Alteration in permeability would affect bidirectional flux and would serve to explain the changes with potassium, at least in the range between pH 7.2 and 8.5. On the other hand, if a net increase in content is involved, such as is the case with chloride below pH 7, permeability would be critical only if it were the rate limiting step. One may presume that cell membranes do have end groups with dissociation constants in the physiological range, and hence that the reaction pattern of Fig. 3 does have some importance generally. There is, however, little to go on with respect to quantitative significance. The results obtained with imidazole emphasize a synergistic effect due to change in charge of the free ions in solution.

Summary. Change in the pH of the incubation medium has been shown to have opposite effects upon influx rates of cations and of anions into mitochondrial substance. Alkalinization was associated with increase in influx of Na^+ and K^+; and conversely, acidification resulted in more rapid influx of Br^- and Cl^-. Increased sensitivity to pH change was observed in the presence of the buffer ion imidazole. At pH 7 imidazole carries a positive charge and inhibition to influx of Na^+ was observed. The inhibition was absent at pH 8.5 with conversion to the uncharged free base.

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Decreased Resistance to Tumor Cells after Stress, Followed by Increased Resistance.* (28370)

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Numerous workers have shown that various types of stress (celiotomy, liver damage, cold, etc.) will reduce the resistance of animals to inoculated cancer cells(1,2,4,5,6,7). Slawikowski(7) has shown that this reduced resistance lasts between 48 and 96 hours, and that the stress involved is different than the Selye stress. However, it is not known whether the animals develop an increased resistance to cancer cells after this period of reduced resistance terminates. Such a reversal or rebound effect is well known in many reactions involving immunity(8).

The reduced resistance to cancer after stress, referred to in most of the articles listed above, was demonstrated by an increased percentage take of cancer cells (Walker 256 carcinosarcoma) which were injected immediately before or after the stress. This increased take was noted in rats undergoing a stress, such as celiotomy or liver trauma, over the percentage take in control rats having an anesthetic, but no surgical stress. In general, the takes were about twice as high in those rats having the stress at time of cell injection as they were in the control rats not having stress at time of cell injection.

Materials and methods. In this experiment white female Sprague-Dawley rats (180-200 g) were used as the experimental animals. The stress induced was a celiotomy, performed under Nembutal anesthesia, similar

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