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Meeting Report

THE BIOCHEMISTRY AND BIOPHYSICS OF MITOCHONDRIAL MEMBRANES.

A report on the International Symposium held in Bressanone, Italy, June 1971

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The International Symposium on the "Biochemistry and Biophysics of Mitochondrial Membranes" which took place in Bressanone, Italy, from June 26–29, 1971, can be considered as a continuation of similar meetings arranged in the previous 6 years in the Bari area. From the beginning, in these meetings the importance of membranes in mitochondrial metabolism has been stressed, an aspect which had been largely neglected in the earlier research on bioenergetics. The following brief report does not attempt to cover all the material that was presented but rather concentrates on the main streams of research.

In the first session, devoted to electron transport, the interaction between ubiquinone and dehydrogenases was the main focus. In part based on the extraction-reactivation studies of ubiquinone (UQ), the groups of Ernster and Singer discussed the significance of their results that ubiquinone can influence the activity of succinate dehydrogenase. Reduced ubiquinone appears to activate succinate dehydrogenase when it is reduced from NADH. This should be interpreted to indicate a common pool of UQ for succinate and NADH oxidation. Reconstitution by UQ of extracted respiratory chain preparations shows that succinate dehydrogenase is fully reactivated at much lower concentration of UQ than NADH dehydrogenase. This has been interpreted to indicate a compartmentation of UQ, whereas Kröger argues that UQ has different K_m values for succinate and NADH dehydrogenases. Other studies (Nelson et al.) discussed the interaction of succinate dehydrogenase with cytochrome *b* in UQ extracted preparations in the presence of antimycin A. The reduction of cytochrome *b* is not abolished by malonate but rather by TTFA. This inhibition is competitive with ATP, GTP etc., indicating a common site for competitive interaction of TTFA and ATP. It was pointed out (Klingenberg), however, that the residual UQ (about 2% of the original) may still be sufficient to catalyze electron transfer from succinate dehydrogenase to cytochrome *b*.

A considerable portion of the contributions and discussions was devoted to the problem of *b*-type cytochromes. The non-linearity of the interaction of antimycin A with respiration and of its binding, possibly indicative of regulatory (allosteric) interaction between two or more cytochrome *b* molecules, was discussed by Slater. Studies with antimycin A based on the fluorescence quenching (as reported by Slater) are non-linear in energized and linear in non-energized membranes. The degree of cooperativity, as calcalulated from Hill-plots or from the equations for allosteric interactions of the target compound with antimycin A, is very high. Indicative of a different interpretation is the non-linear antimycin titration curve of the respiration which was shown by Kröger to depend on the participation of mitochondrial UQ. As a result of the pool function of UQ and from kinetic considerations, three quinones with different redox potentials were used as hydrogen donors to the cytochrome *b* region. With quinones of sufficiently negative potential (aurantiogliacladin) UQ also becomes reduced resulting in non-linear dependence on antimycin A, whereas with high redox potential quinones (duroquinone, menaquinone) titration with antimycin A is linear and UQ remains oxidized.

The contributions of Slater, Chance, Chappel, Dutton and Wikström were concerned with the identification of various cytochrome *b* components with high or low redox potential, energized and non-energized forms and with the antimycin A target. There was an essential agreement that there are at least two cytochrome *b* species, *b*-558 and *b*-565, with diverging nomenclature according to the group presenting the report. There was disagreement concerning the existance of a third separate *b* cytochrome (*b*-562) which, in particular according to Chance and Dutton, belongs to a species with a double peak together with *b*-558, called cytochrome b_T. Wikström argued against energy-rich forms of the cytochrome *b* since low potential cytochrome *b* can be reduced also in the presence of uncouplers. He suggested that cytochrome *b* may actually serve as a H^+-carrier in the membrane and the varying redox potential reflects different localized H^+-concentrations. Of interest was the introduction by Chappel of an antibiotic which possibly inhibits between the two cytochrome *b*'s.

A considerable part of the meeting was devoted to fluorescent probes in the study of mitochondrial membranes. In the last year the sophistication in the use of these probes has increased and consequently also in "mitochondriology" a more substantial appraisal of the use of fluorescent probes becomes evident. Various parameters have been assumed to be measured by the fluorescent probes in the different studies. The central issue is still the energization of the mitochondrial membrane. The influence of membrane charge on positively or negatively charged probes was pursued by Azzi. Accordingly the charge distribution in the mitochondrial membrane may be complex. Fluororescent probes were applied by van Dam for assaying energy conservation parallel to cytochrome *b* absorption changes. The intramitochondrial pH was claimed to be measured by Lee with a positively charged hydrophilic fluorophore. The use of fluorophores for the structure and phase transition of membrane lipids was shown and discussed by Luzatti and Shechter. New fluorophores have been introduced with structural features which permit more stringent predictions on the localisation in the membrane. This has been discussed in a lucid survey by Radda. A particularly lipophilic fluorophore has been used by Chance to indicate redox changes of ubiquinone. Perturbation of the fluorescence by ubiquinone was more pronounced with the reduced than with the oxidized form.

A particularly notable development reported on during this meeting was the identification of a calcium binding factor from mitochondria by the groups of Sottocasa and Carafoli. It was reported by Sottocasa that this factor contains sialic acid and accounts for the high affinity binding of mitochondria believed to be involved in the calcium transport. Its separation by hypotonic shock suggested that the component may be equivalent to the binding factors isolated from bacteria.

A new type of membrane probe has been described by Azzone. These are organic dye ions showing metachromatic shifts on binding to the mitochondrial membranes. With neutral red a pronounced shift of its metachromasy and of its p*K* is related to the structure of the energized membrane.

Another issue at the meeting was the transport of metabolites through the mitochondrial membrane as catalysed by specific carriers. This aspect has been a major issue at the earlier meetings. Kinetic measurements of the uptake of di- and tricarboxylates and phosphate had been used by Palmieri et al. to elucidate the specificity of the carrier sites. Whereas phosphate and dicarboxylate use different sites at the carrier, di- and tricarboxylate have identical sites, being competitive. Also competitive is the interaction of metal chelating agents indicating the presence of metal at the binding site. The fact that the metabolite exchanges are electroneutral, with the exception of the ADP/ATP-exchange, has been discussed by Papa using pH measurements. The elucidation of the ADN carrier was pursued both by Vignais and Klingenberg using ^{35}S-atractyloside (ATR). A similar inhibitor, ^{35}S-gummiferin (Vignais), has a strongly non-linear binding curve to the membranes. By combined measuring (Klingenberg) of the interaction between ADP, ^{35}S-ATR and BA* binding at the carrier the mechanism of the carrier was investigated. A possible conformation change of the carrier induced by BA was shown to fix ADP and to released all ^{35}S-ATR. The nature of this change as a transport transition state was discussed. The transport of di- and tricarboxylates through bacterial membranes was reported by Williams to be different from the mitochondrial counterpart in various respects. This bacterial transport seems to be less specific and has no counterexchange. Another metabolite transport system in liver mitochondria was described for ornithine and citrulline, which are both members of the urea cycle (Lehninger et al.). These

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* BA:bongktetic acid.

are organic cations and their uptake is influenced by membrane potentials opposite to that of anionic metabolites.

A number of contributions dealt with the mechanism of energy transfer leading to ATP synthesis. Thus an attempt to detect ultraviolet absorbing energy-rich bonds was reported by Storey. Further contributions by Bäuerlein dealt with model systems of oxidative phosphorylation. Boyer discussed the possibility of carboxyl activation linked to energy transfer and the preliminary demonstration of ^{32}P incorporated to protein with an acidic linkage. The present state of the isolation of factors involving ATP synthesis was discussed by Beechey and attempts by Griffith and Linnane on the genetic elucidation of the energy transfer mechanisms in mutants were presented.

It seems to be remarkable that research now has progressed to elucidate mechanisms of oxidative phosphorylation in terms of its molecular parameters, thus leaving the stage of an overall chemi-osmotic hypothesis. Whereas the existence of membrane potential etc. is broadly accepted, its utilization was discussed mainly in terms of ion transport and probe interactions. Only a brief discussion turned to the thermodynamic problems involved in the chemi-osmotic hypothesis, as criticised by Massari and by Pressman, with varying views, on the basis of the stoichiometric problems involved in the K^+-transport and the ATP/K^+-ratio.

There were other interesting and valuable contributions on various facets of mitochondrial bioenergetics and enzymology which gave the meeting a rather encyclopedic survey of the present state of the field. The high level of the contributions and the high intensity of the discussion contributed to the success of the meeting which certainly was of great profit to all its attendants.