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Defective mitochondrial cation transport during dietary protein deficiency in rats

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Summary

Mitochondria were isolated from the livers of male weanling and adult *Wistar* strain albino rats fed low-protein diet *ad libitum* for 30 and 84 days, respectively. Control animals consisted of adult and weanling rats fed purina chow for the same periods. Spectrophotometric estimations of mitochondrial passive transport of protons by following changes in matrix volume at 520nm revealed that the mitochondria of protein-deficient rats are more permeable to protons than those of control rats; the mitochondria of protein-deficient weanling rats being more permeable than mitochondria of protein of malnourished adult rats. Similar results were obtained when a sensitive pH-glass electrode was used to monitor mitochondrial proton translocation. Although protons were slowly ejected from the mitochondrial matrix of protein-deficient rats, there was a significantly high rate of influx of the ion into the matrix of these mitochondria when compared with controls. Furthermore, FCCP, a classical protonophore carried protons less rapidly across the mitochondrial membrane of malnourished animals thus indicating that a small pH difference probably exists across the mitochondrial energy-coupling membrane of protein-deficient rats. Consequently, the rates of mitochondrial Ca^{2+}- translocation were lower in protein-deficient animals than in normal rats.

These defects are probably due to diet-induced looseness of mitochondrial bi-layer structure.

Résumé

Apré avoir suivi un regime insuffisant en protéine pendant 30 et 84 jours respectivement, on a isolé des mitochondres de la foie des nourrissons males et des advttes de *l'espéce Wistar* des rat albinos. Les animaux de controle comprenasent des rats, nourrissuns et aduttes alimentés de purina chow pendant la même periode. En suivant les changements de volume de matrice à 520nm les estimations spéctrophotométriques des transports passifs des protons par les mitochondries unt révélé que les mitochondries des rats ayant des carences en protéines étaunt plus perméables aux protons que celles des rats adultes sous-alimentés, tandis que les mitochondries des nourrissons ayant des carences en protéins etacest plus perméables celles des rats adultes sours-alimentés. On a obtenu des résultats similaires utilisant une électrode en verre sensible au pH à suivre la translocation mitochondriale des protons. Bien que les protons aient été explusés lentement de la matrice mitochondriale des rats avec des carences en protéines, le taux d'influx des ions dans la matrice des mitochondries était trés élevé comparé aux meme taux dans les animaux de contróle. Qui plus est, le FCCP une protonophore classique a transporté les protons de fac, on moins rapid à travers la membrane mitochondriale des animaux sour-alimentés ainsi indiquant l'existence probable d'une petite différence de pH à travers la membrance mitochondriale couplant l'énergie des rats avec des carences en protéines. Par conséquent, les taux de translocation mitochondriale de Ca^{2+} -étacent plus bas dans les animaux ayent des carences en proteins que dans les animux normaux.

Ces déficiences sont peut-être dues à un relachement de la structure des couches doubles mitochondriales rausées par le régime.

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Introduction

The physiological significance of mitochondrial calcium accumulation lies in the ability of this organelle to regulate intracellular free calcium ion concentration[1,2]. This regulatory mechanism is complemented in non-muscle cells by the plasma membrane Mg^{2+}-dependent, Ca^{2+}-pumping ATPase and the Na^{+}/Ca^{2+} exchanger; and in addition to these mechanisms, the sarcoplasmic reticulum Ca^{2+}translocating ATPase in the case of skeletal muscle[3-8]. To perform its functions, therefore, the mitochondrion must regulate its own ionic composition while at the same time provide a high proton electrochemical potential gradient required for ATP synthesis [9-11].

There is evidence that the structural and functional features of this organelle are altered in several pathological conditions such as cancer [12], Luft's disease [13], mitochondrial myopathies [14,15] as well as in a class of genetic disorders recently termed 'Cytochrome oxidase (COX) deficiency diseases'[16]. The unique properties of the protein components of the mitochondrial energy-transducing membrane seem to make the functions of these membranes highly susceptible to genetic and diet-induced manipulations. Specifically, we have demonstrated that the mitochondrial energy-synthesizing assembly is defective in a rat model of protein-energy malnutrition[17,18]. Our findings, together with reports that the lipid composition of subcellular membranes are modified by diet and nutrition [19], suggest that the molecular architecture of biologically active membranes could be sensitive to dietary protein deficiency. In addition, the increased lecithin content of the erythrocyte plasma membrane of kwashiorkor children[20] further supports the notion that biomembranes could suffer structural changes during dietary protein deprivation. The capacity of mitochondria of protein-deficient weanling and adult rats to transport protons and Ca^{2+}ions was investigated in this study in order to determine the influence of protein-energy malnutrition on the structural and functional integrity of mitochondrial energy-coupling membrane.

Evidence is presented in this paper to show that the mitochondrial energy-coupling membrane of the liver of malnourished rats has a significantly high permeability to protons; the membrane of severely malnourished weanling rats being more permeable to protons than that of adult rats.

Materials and methods

Materials

Carbonylcyanide-p-trifluoro-methyoxyphenylene hydrazone (FCCP), oligomycin, rotenone, valinomycin, 4-(20 hydroxyethyl)-1-piperazine ethane sulphonic acid (HEPES), potassium succinate, potassium pyruvate, potassium malate, and the sodium salts of adenosine 5^1-diphosphate (ADP)and adenosine 5^1-triphosphate (ATP) were purchased from Sigma Chemical Co. London, U.K. All other chemicals were of the highest purity grade and were purchased from Fluka AG (CH-9470, Buchs), Switzerland and British Drug Houses, London.

Experimental animals

Two groups of experimental animals were used in this study. In the first group, litter mate weanling rats approximately 20 days old were fed low-protein diet (3.4 per cent (w/w) protein) for 30 days according to a slight modification of the regimen of Boyd and Carsky [21]. The second group of experimental animals consisted of sexually mature male rats weighing approximately 125g. The animals were similarly fed the low protein diet *ad lib* until they were severely malnourished i.e. approximately 12 weeks. The control animals used were weanling and sexually mature rats fed the normal *purina chow* for 30 days and 12 weeks, respectively. Mitochondria were isolated by differential centrifugation from the livers of these animals following homogenization in ice cold 0.25M sucrose as previously described [22]. The mitochondrial fractions were washed two or three times with cold 0.25M sucrose and stored as stock suspensions in 0.25M sucrose at 0°C, at a concentration of 50mg of mitochondrial protein/ml. the mitochondrial preparations were usually used within 3-4 hours of isolation. Mitochondrial protein was determined by a modification of the biuret method[23].

Polarographic measurement of oxygen uptake

The rate of mitochondrial oxygen utilization was determined at 37°C in a closed 1.2ml vessel equipped with a stirring bar and a thermostatically controlled water bath (Thermomix 1420, B., Braum Bender and Hobein, Zurich, Switzerland) by use of the conventional Clark-type oxygen electrode (Yellow Springs Instrument, Ohio, U.S.A.). The reaction vessel contained 1.1ml reaction medium (600mM dialysed mannitol, 20mM Tris HCl (pH 7.4) 5mM KH_2PO_4 and 5µg bovine serum albumin). Oxidizable substrate for individual experiments consisted of any of potassium pyruvate/malate (3.98mM/1.98mM) or potassium succinate (3.98mM). Where potassium succinate was used as electron donor, 10µM rotenone was included in the reaction medium in order to prevent the transfer of electrons from endogenous substrates. An aliquot of the mitochondrial fraction (final concentration: 3mg protein/ml) was introduced into the reaction vessel on achieving a steady recorder tracing. After the stabilization of the recorder pen, an aliquot of either pyruvate/malate or succinate was carefully introduced by use of a Hamilton syringe inserted through the opening in the glass stopper of the reaction vessel. Aliquots

of ADP (final concentration 50nmol ADP/mg mitochondrial protein) or FCCP were added in some experiments. Respiratory rates and control ratios were calculated as described by Estabrook[24].

Measurement of mitochondrial passive transport of protons

Energy-independent fluxes of protons across mitochondrial energy- coupling membrane were followed by measuring light-scattering changes of the mitochondrial suspension according to Cunarro and Weiner[25]. These absorbance changes have been shown to reflect passive changes in mitochondrial ion and water content secondary to electrochemical gradients because the mitochondrial volume changes are not energy-linked, respiration being inhibited by the addition of rotenone and antimycin A. The medium used in this study was O.15N NH_4Cl because mitochondria swell least in this medium compared to other isotonic solutions. Absorbance changes in a Beckman DU-8 spectrophotometer at 520nm on addition of mitochondria were followed at 30°C in a 3ml, 1-cm light-path cuvette containing 0.15N NH_4Cl, 10µm rotenone 0.05µg antimycin and 10mM Tris-HCl (pH 7.4).

Measurement of Ca^{2+} flux across mitochondrial membrane

Using a calibrated Ca^{2+} selective electrode (F2112 Ca^{2+} -selectrode radiometer) as described by Lehninger *et al.*, [26], the changes in the extramitochondrial concentrations of free Ca^{2+} were monitored by a Phillips pH meter linked through a bucking voltage box to a Perkin-Elmer recorder Model 56. Since the response of the electrode to changes in free Ca^{2+} concentration is logarithmic, the electrode was first calibrated with several additions of aliquots of pure $CaCl_2$ solution. Mitochondria (2mg per ml) were incubated at 37°C in a 2ml incubation vessel which contained, in final concentrations, 120mM KCl, 20mM Tris-HCl (pH7.4) and 1µM rotenone. Different amounts of Ca^{2+} ions were introduced into the reaction vessel by a Hamilton syringe on obtaining a steady recorder tracing. Ca^{2+} influx was initiated by the addition of 1nM succinate. The experiment was repeated five to six times for each mitochondrial preparation. The rates of Ca^{2+} accumulation were calculated from the tracings according to Lehninger *et al.*, [26].

Assay of F_oF_1 ATPase

Mitochodrial basal F_oF_1 - ATPase activity was estimated by the method described by Chefurka [27]. The basic reaction medium contained 50mM KCl, 50mM sucrose, 55mM Tris-HCl pH 7.4, 1mM EDTA and 6mM ATP. The reaction was allowed to proceed at 37°C and was initiated by the addition of 2mg protein per ml. At exactly 15 minutes, the reaction was quenched by adding ice-cold 10% trichloroacetic acid. The precipitated protein was separated by differential centrifugation and the phosphate content of the supernatant was immediately estimated by the method of Goldenberg and Fernadex [28]. A solution which contained all components of the basic reaction medium including ATP but devoid of mitochondria was used as blank. ATPase activities are reported as nanomoles of phosphate liberated per minute per mg of mitochondrial protein.

Measurement of proton translocation

Proton translocation was measured according to the procedure described by Reynarfarje *et al* [29]. Phosphate-depleted mitochondria were prepared by pre-incubating washed mitochondria with 400 mM sucrose,, 10mM KCl, and 30mM HEPES, pH 7.1 for 10mins, and in the cold, after which the suspension was diluted with more of the incubation medium and the mitochondria re-isolated and finally suspended in a small volume of the incubation medium. The all-glass thermostated reaction vessel contained 120mM LiCl, 10mM KCl, 3mM HEPES, 4µM rotenone, and 100ng valinomycin per mg mitochondrial protein (pH 7.2). changes in the pH of the reaction medium were followed by use of a sensitive Phillips pH-glass electrode linked through a bucking voltage box to a Perkin-Elmer recorder Model 56. After 2 min of incubation, in order to ensure that endogenous substrates and ATP were depleted, 1mM potassium succinate was added to induce proton translocation. The rate of proton flux was computed as ng-ion H^+ per mg mitochondrial protein per minute. FCCP was used as the standard proton mitochondrial translocator.

Sodium dodecylsuphate (SDS) polyacrylamide gel electrophoresis

Mitochondrial membrane proteins were electrophoretically separated on a 5% - 20% acrylamide gradient gel using the procedure of Weber and Osborn [30]. Aliquots of the mitochondrial preparations which have been previously dissolved in 2.5% SDS, 0.05% mercaptoethanol, and 0.1M so-

dium phosphate buffer pH 7.0 were warmed at 95°C for 3 min. By use of a Hamilton syringe, the aliquots were carefully introduced into the bottom of the sample wells of the gel which was run at a constant voltage (50V) overnight at room temperature in 0.1M Tris-glycine and 0.1% SDS buffer, pH 8.3. The gel was stained with 0.25% Coomassie Brilliant Blue in 50% methanol/7.5% acetic acid (v/v) after fixing in 10% trichloroacetic acid in 5% methanol. In order to destain, the gel was rinsed with distilled water and rinsed several times in 7.5% acetic acid/20% methanol (v/v).

Results

In this study, mitochondrial volume changes were measured in the presence of inhibitors of oxidative phosphorylation, such that, the uptake of NH_4Cl by tightly coupled mitochondria was limited only by the rate of electrogenic proton entry into the matrix. Fig. 1 shows the pattern of passive swelling of mitochondria from adult and weanling rats fed low protein for the periods described under 'Materials and methods'. As seen from the figure, mitochondria isolated from the livers of animals fed high protein (normal) diet did not swell appreciably after 4 mins in 0.15N NH_4Cl and in the presence of inhibitors of oxidative phosphorylation, whereas, mitochondria of weanling and adult animals fed low protein diet swelled somewhat steadily in 0.15N NH_4Cl for about 4 mins after which there was no longer any increase in matrix volume.

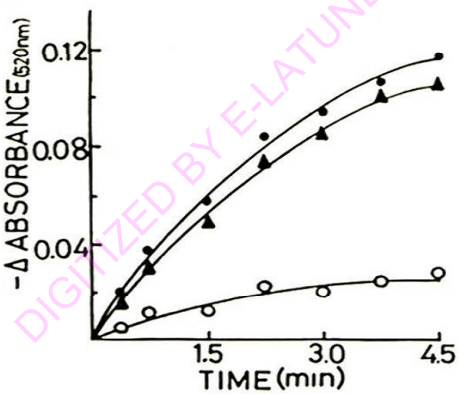

Fig 1. Changes in light scattering by mitochondria isolated from the livers of normal rats (○), adult (▲) and weanling (●) rats fed on low-protein diet. Reaction medium contained 0.15N NH_4Cl, 0.05µg antimycin, 10µM rotenone, and 10mM Tris-HCl, pH 7.4

Specifically, mitochondria of malnourished adult and weanling rats swelled after 3 mins by 4.5 and 4.8 times the degree of swelling of mitochondria of control rats. Finally, the total increases in mitochondria volume changes over a period of 4.5 minutes in 0.15N NH_4Cl are; $-\Delta A = 0.022 \pm 0.001$, $-\Delta A = 0.122 \pm 0.009$, $-\Delta A = 0.114 \pm 0.006$ for control, malnourished weanling and malnourished adult rats, respectively.

The results obtained from several determinations of the rate of proton flux across mitochondrial membranes of normal and malnourished rats are summarised in Table 1. As seen from the data, the rates of succinate-induced transient ejection of protons across the mitochondrial energy-coupling membrane are comparable in normal adult and weanling rats. The data show furthermore, that the rates of passive influx of protons into the matrix of normal mitochondria are only 2.5 per cent the rates of proton ejection during succinate oxidation. This shows that mitochondrial coupling membrane of normal mitochondria has a very low permeability to protons. However, these low rates of influx of protons were increased by at least 60 times on addition of FCCP indicating that this protonophore carries protons very rapidly across the mitochondrial membrane of rats fed normal or high protein diet. In contrast, the rates of efflux of protons from mitochondria of malnourished adult and weanling rats on addition of succinate were at least 30 and 45 per cent respectively, lower than the rates obtained for mitochondria isolated from the livers of normal rats. In addition, the rates of passive influx of protons into the matrix of mitochondria isolated from the livers of malnourished adult and weanling rats were 65 and 48 per cent lower than the rates of succinate-linked ejection of protons from these mitochondria. These results show that mitochondria of severely malnourished rats are permeable to protons when compared to normal mitochondria in which the rate of passive influx of protons was only about 3 per cent the rate of its ejection.

Consequently, the rates of FCCP-induced proton translocation by mitochondria of malnourished adult and weanling rats were 3.2 and 2.4 times, respectively, higher than the rate of passive influx of protons in the absence of FCCP.

Table 1 Cation translocation across mitochondrial membrane of protein-deficient rats

Mitochondria	rate of proton flux (ng-ions H min^{-1} mg $protein^{-1}$)			rate of Ca^{2+} ion accumulation ($ng\text{-}ion Ca^{2+} min^{-1}$ mg $protein^{-1}$)	
	succinate-linked ejection	influx	FCCP-induced influx	succinate	ATP
normal[1]	319.6±6.3	8.2±0.9	493.6±1.3	109.3±3.1	126.3±44.2
normal[2]	293.8±7.6	7.6±0.7	489.3+11.6	111.6±2.7	121.7±3.6
protein-deficient adult rats	[a]216.7±4.3	[a]75.9±3.4	[a]245.6±3.2	[a]63.6±1.6	[a]45.6±1.6
protein-deficient adult weanling rats	[a,b]163.8±4.9	[a,b]85.3±2.8	[a,b]205.7±3.4	[a,b]56.3±1.7	[a,b]41.2±1.3

Each value is a mean of at least six determinations ± standard deviation.
Significance was estimated by student's *t*-test
[a]Values are significantly ($P \leq 0.01$) different from those of normal mitochondria. [b]These values are significantly ($P \leq 0.02$) different from those of protein-deficient adult rats.
[1]adult rats fed high-protein diet
[2]weanling rats fed high-protein diet

Table 2 Mitochondrial proton translocating ATPase of malnourished rats

Mitochondria	ATPase activity (nanomoles Pi mg $protein^{-1}$ min^{-1})		
	basal	FCCP-induced	Oligomycin-inhibited
normal	156.6±7.4	800.7±18.3	30.3±2.6
malnorished adult rats	75.8±7.5	150.7±6.8	18.7±1.7
malnourished weanling rats	66.3±4.1	137.5±6.3	16.4±1.5

Each value is a mean of at least 5 different determinations ± standard deviation

Table 3 Mitochondrial bioenergetic parameters during protein deficiency in rats

Mitochondria and Substrate	Respiratory rates (ng atoms 0/min/mg protein)				
	State 3	State 4	[a]FCCP	ADP/0 ratio	[b]RCR
normal rats					
Pyruvate/malate	62.61±2.32	15.21±0.61	71.63±5.21	2.85±0.17	4.38±0.21
β-hydroxybutyrate	54.62±1.41	13.96±0.78	59.36±4.61	2.73±0.18	4.15±0.18
Succinate	156.26±6.76	37.30±1.31	171.48±0.11	1.85±0.11	4.10±0.13
protein-deficiency-adult rats					
Pyruvate/malate	38.86±1.08	15.23±0.91	39.20±0.96	2.03±0.16	2.21±0.08
β-hydroxybutyrate	33.11±1.21	13.29±0.55	36.20±1.61	2.05±0.13	2.28±0.06
Succinate	54.26±1.21	25.17±1.21	67.63±2.21	1.40±0.09	2.11±0.07
protein-deficiency weanling rats					
Pyruvate/malate	33.61±1.66	14.26±1.16	33.61±0.93	1.85±0.10	2.11±0.13
β-hydroxybutyrate	27.54±1.35	13.16±0.91	29.37±0.63	1.96±0.13	1.95±0.11
Succinate	50.26±1.78	24.61±0.97	54.63±3.76	1.27±0.17	1.99±0.16

Each value is a mean of at least five different determinations ± standard deviation [a]FCCP-stimulated state 4 respiratory rate. [b]RCR is ratio of state 3 to state 4.

The data obtained from several determination of the rates of respiration and ATP-supported Ca^{2+} accumulation by mitochondria from normal and malnourished animals are shown in Table 1. The results show that mitochondria isolated from the livers of adult and weanling rats placed on a high protein diet accumulated Ca^{2+} at identical rates (109.3 ± 3.1, and 111.6 ± 2.7 ng-ions Ca^{2+} min^{-1} mg $protein^{-1}$, respectively). However, mitochondria from the livers of severely malnourished adult and weanling rats, respectively accumulated Ca^{2+} ions at rates that were 41.8 and 49.6 per cents lower than those of mitochondria isolated from animals fed adequate protein. Similar results were obtained when the membrane potential generated by ATP hydrolysis was used in palace of succinate oxidation. In this instance, the rates of ATP driven Ca^{2+}- uptake by mitochondria from malnourished animals were over 60 per cent less than those of mitochondria of rats fed high protein.

Results obtained by determining the ATPase action of mitochondrial preparations obtained from normal and severely malnourished adult and weanling rats are summarized in Table 3. The basal activity (156.6 ± 7.4 nanomoles Pi released mg $protein^{-1}$ min^{-1}) of the control enzyme was 2.1 and 2.4 times the activities seen in the mitochondria of malnourished adult and weanling rats, respectively. Inclusion of FCCP in the assay cocktail resulted in a five- and a two-fold enhancement, respectively, of the ATPase of the mitochondria of rats fed high protein and low protein diet. Oligomycin reduced the ATPase activity to 40.3 ± 2.6, 18.7 ± 1.7 and 16.4 ± 1.5 nanomoles Pi released mg $protein^{-1}$ min^{-1}, respectively for rats fed high protein diet, and malnourished adult and weanling rats. These values represent at least a 75 per cent reduction in the ATPase activity of the various mitochondrial preparations by oligomycin.

Discussion

In this study, spectrophotometric estimations of the degree of permeability of protons across the mitochondrial energy-coupling membrane under conditions of limiting electrogenic proton re-entry into the matrix revealed that the energy-coupling membranes of protein-deficient adult and weanling rats exhibited a significantly high proton permeability; the membrane of malnourished weanling rats being more permeable to protons than that of malnourished adult rats (Fig. 1). This means that the energy-coupling membranes of malnourished animals do not possess an extremely low proton conductance as required by the chemiosmotic theory. Clearly, a fairly high proton permeability of the inner mitochondrial membrane could result in an inability of the membrane to generate a sufficiently high pH for ATP synthesis. To confirm this inference, the ability of the energy-coupling membranes of malnourished rats to translocate protons during succinate oxidation was assessed using a sensitive method of measurement of proton flux. The results obtained show that although protons were translocated out of the matrix rather slowly during oxygen utilization by mitochondria of malnourished animals, there was an appreciable leakage of the ion into the matrix of these mitochondria when compared to normal mitochondria (Table 1).

It seems likely that an increased permeability of the membrane to protons could be due to a less tightly -packed structure of the coupling membrane during dietary protein-deprivation, since phospholipid metabolism is grossly impaired in protein-energy malnutrition [20]. Whether such a less tightly-packed bilayer structure results from a reduced rate of phospholipid synthesis *per se* or from reduced rates of synthesis/turnover of membrane proteins remains an open question. It seems probable however, that where a multimeric protein is immersed in the bilayer structure, the absence of one or more subunits of the complex could bring about a looseness of the bilayer, even if the composition of the annular lipid domain of the protein assembly is not altered. Furthermore, the observation that FCCP, a very potent protonophore, did not rapidly shuttle protons across the coupling membrane of the mitochondrial membranes of malnourished animals (Table 1) suggests that a low gradient of protons probably occurred across the membrane. Consequently, the large negative inside membrane potential required, in addition to the pH difference, for the generation of a proton electrochemical potential gradient, becomes so small during severe protein malnutrition. Evidence for this is seen in the inability of mitochondria isolated from severely malnourished animals to actively accumulate Ca^{2+} during succinate oxidation (Table 1). However, because the hydrolysis of ATP by the F_oF_1-ATPase in intact mitochondria could result in the formation of the membrane potential needed for driving Ca^{2+}-uptake [5], ATP was used to power Ca^{2+} uptake by the mitochondria of normal and malnourished animals. The results show conclusively, that the rates of respiration and ATP-supported Ca^{2+} accumulation of

mitochondria were significantly reduced during protein deficiency (Table 1).

It seems therefore, logical from the foregoing to imagine that mitochondria of malnourished animals were uncoupled, at least to a first approximation, since the proton conductance of the membrane was increased. The pre-chemiosmotic criteria for an uncoupled mitochondria include

(1) an enhanced rate of resting state or state 4 respiration,
(2) a reduced state 3 respiration,
(3) a reduced respiratory rate of ADP-control ratio, and
(4) an enhanced basal ATPase action[5].

Results obtained by polarographic determinations of the respiratory rates of mitochondria in their various metabolic states, using succinate and pyruvate/malate as electron donors show that, although, the respiratory control and ADP:0 ratios of mitochondria of malnourished animals were lower than those values obtained for normal mitochondria, as if in an uncoupled state, the rates of resting state respiration of mitochondria of malnourished rats, were not significantly different from the rates seen with normal and tightly coupled mitochondria, especially during the oxidation of β-hydroxybutyrate or pyruvate/malate (Table 3). Surprisingly, the rates of state 4 respiration were even lower during succinate oxidation in malnourished rats when compared to the values obtained for normal mitochondria (Table 3). Moreover, the rates of FCCP-stimulated respiration of mitochondria isolated from the livers of protein-deficient adult rats, when compared to the values obtained for normal mitochondria, were reduced by at least 46, 40 and 60 per cent, respectively, during the oxidation of pyruvate/malate, β-hydroxybutyrate, and succinate. Similarly, the rates of FCCP-stimulated respiration of mitochondria of severely malnourished weanling rats, were reduced by at least 54, 51 and 68 per cent, respectively, for pyruvate/malate, β-hydroxybutyrate, and succinate oxidation (Table 3). Consequently, the ratio of FCCP-stimulated respiration to state 4 respiration was at least 4.2 for normal, 2.6 for adult malnourished, and 2.2 for weanling malnourished rats. Altogether these results clearly demonstrate that the mitochondria of protein-deficient rats were not truly uncoupled.

Additional evidence for the non-uncoupling of mitochondrial respiration during dietary protein deficiency, is the finding that basal ATPase activity was markedly reduced in mitochondria of malnourished rats which, incidentally, showed a lower sensitivity to both oligomycin and FCCP (Table 2). These findings suggest that the F_oF_1-ATPase of the mitochondria of protein-deficient rats is probably structurally defective. It is not clear whether the reduced activity and the lowered sensitivities of the enzyme to FCCP and oligomycin have any relatedness to the composition and/or the biogenesis of this multisubunit protein during malnutrition in the rat. However, because this protein also catalyzes the proton motive force-dependent synthesis of ATP when the mass action ratio of the reaction favours the synthesis rather than the breakdown of ATP, it seems probable that, the reduction of the rate of state 3 respiration, the loss of respiratory control and the extremely low ADP:O ratios seen during dietary protein-deficiency could be due to the inability of this enzyme to act as a synthetase.

In orther to have an insight into the structural intactness of the proteins of the inner mitochondrial membrane during dietary protein deficiency, the mitochondrial membranes obtained from malnourished and normal animals were electrophoresed on polyacrylamide gel in the presence of sodium dodecylsulphate. Because there are fewer polypeptides (Fig. 2) in the mitochondrial membranes of malnourished rats, it seems reasonable to surmise that some of the missing protein bands were lost during membrane synthesis in the liver of protein-depleted animals. This may be true especially for cytochrome C oxidase whose activity was reduced by over 70 per cent during protein depletion in weanling and adult rats[17,18]. Although, it has been shown that a reduction of activity is a common property of all multimetric protein complexes of the energy-coupling membrane during dietary protein-depletion [17], it is not clear if this feature is related to the mode of biogenesis of these proteins during malnutrition.

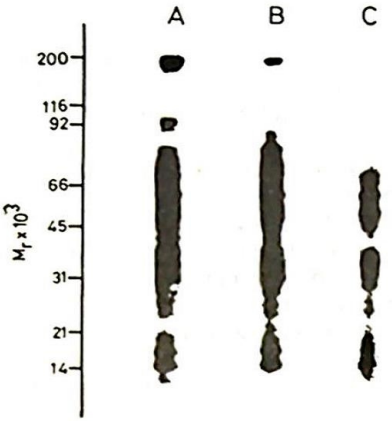

Fig. 2 SDS-polycrylamide gradient (5%-20%) gel electrophoresis of mitochondrial membrane proteins. A, normal rats; B, protein-deficient adult rats; and c, protein deficient weanling rats.

In essence, therefore, the defect in the transport of cations across the mitochondrial energy-coupling membrane as well as the loss of control of respiration by ADP may have been caused by a mere looseness of the bilayer structure of the energy-coupling membrane during malnutrition. In order to have a clearer picture of the molecular basis of these defects, it is pertinent to elucidate during protein deficiency, the structural integrity of those proteins and the composition and assymmetry of the various phospholipids that make up the inner mitochondrial membrane. This information will certainly enhance our understanding of the molecular basis of the pathophysiology of human protein-energy malnutrition, especially now that kwashiorkor has been suspected to play a key role in the development of aflatoxin-induced liver cancers in developing and poverty stricken countries of the world.

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