Journal of Bioenergetics and Biomembranes (1977) **9**, 289–301

Electrical Properties and Structural Transitions in the Mitochondrion

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Received 1 September 1977

Abstract

Charge carrier generation and transport in the mitochondrial lipoprotein system has been investigated by electrical conductivity, low frequency dielectric relaxation, and thermoelectric power. A parallel study was conducted on morphological/structural changes by DTA and NMR. The results obtained confirm the need to consider concurrently free charge carrier processes and polarization phenomena. All techniques show a "transition" at the same temperature. The steady state conductivity is correlated with main chain segmental reorientations of the phospholipid moiety below the transition and with an interfacial polarization process above it. The Seebeck coefficient provides a useful new aid to characterizing the charge carriers, confirming that they are electronic. The terminal cytochrome oxidase component was investigated separately but it largely reflected ionic impurities characteristic of the isolation process, so that the results were of no intrinsic value.

Introduction

The possibility of solid state semiconductivity in biological materials and its ramifications for biological processes and biomedical problems [1–3] have long been the focus of theoretical and experimental attack. Even superconductivity is being claimed [4–6], sometimes with minimal justification. The steady state conductivity, in the hydrated state at least, is undoubtedly appreciable, but the nature, origin, and transport

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mechanisms of the carriers involved will remain uncertain until reliable supporting data from related techniques become available.

The importance of electric *polarization* phenomena (as distinct from conductance) is well established for synthetic insulating polymers—for example, the electret state, the piezo-, pyro-, and ferroelectric effects, and triboelectricity, to name a few. Similar phenomena are being recognized in biological systems and living processes [7], particularly in the hydrated state, and their significance is being probed. Conduction and polarization viewpoints have developed independently despite the likelihood that they can represent transport and storage, respectively, of the same charge species. For instance, dielectric experiments on polymers interpret polarization in terms of dipole orientation or accumulation of electronic or ionic "space" charges (perhaps injected) at interfacial boundaries. Semiconduction experiments interpret it in terms of charge carriers captured in traps associated with a definite activation energy for release to a "conduction band." Thermally stimulated depolarization studies could equally well reflect either this trap depth distribution or release of the dielectric saturation through successive stages of molecular reorientation processes. Similarly, "conduction" and "dielectric loss" in an ac field are basically indistinguishable, especially in relation to a "hopping" mechanism of conductivity over potential barriers whose physical nature is unspecified, but which could well involve these same activated molecular motions.

Mixed conductivity in dielectric materials presents another problem, which in principle is insoluble. The true electronic conductivity refers to times much longer than the slowest dielectric polarization process. The true ionic conductivity refers to very short times prior to the buildup of electrolysis products unless totally nonblocking "ionic" reversible electrodes are involved. This is why ac techniques are essential for ionic solutions. Such a conflicting requirement is the principal cause of controversy surrounding the effect of hydration on the conductivity of biopolymers. Nor is there any *prima facie* distinction between the macroscopic laws of ionic and electronic conduction—both may show exponentially activated intrinsic and extrinsic regions, and theories [8] relating current to voltage, thickness, etc., are not sufficiently discriminating when applied to polymers. It is particularly hard to categorize ionic conductivity at low concentrations and in the absence of electrolysis [9]; even dissolved gases in polymers may accept electrons and diffuse as ions in a pseudoelectronic mechanism [10].

Thus it remains surprisingly difficult to determine unambiguously the details of electrical conduction even for synthetic polymers of less heterogeneity and morphological complexity than the biopolymers. Our

personal appraisal of the conflicting literature of electrical conductivity in biopolymers is that globular proteins and probably phospholipids are electronic when dry with the possibility of an increasing protonic contribution at higher hydrations. There remains an urgent need for appropriate techniques to complement established conductivity results. The highly promising contactless microwave Faraday rotation method [11] is less restricted by the fundamental physical nature of biological samples than conventional ancillary techniques [12] such as the dc Hall effect, the drift mobility, or the various photoexcited phenomena. However, the Hall mobility values previously reported for biological materials [11, 13] now have to be reassessed [14] following full understanding and elimination of technical problems [15].

Accordingly we have chosen to apply to the problem two techniques which provide a link between conductivity and polarization phenomena, namely the thermoelectric effect and low frequency time domain dielectric measurements.

A thermal gradient within a solid predicates majority carrier diffusion $D\nabla n$ from hot to cold end until a balancing electric potential difference $\mu\nabla V$ is achieved, where D is the diffusion coefficient, n the carrier concentration, μ its mobility (the parameter of most interest), and V the electric potential. The Seebeck coefficient, denoted by Q, is one of three interrelated thermoelectric effects, and is the voltage produced per unit temperature difference applied. Such thermoelectric voltages arising from free carrier migration are, in insulating materials, associated with an ubiquitous static polarization (cf. electric behavior) which may be complicated further by the influence of the highly polarizable water of hydration in the case of biopolymers [16].

Step function time domain dielectric spectroscopy also provides for integration of free carrier transport with dielectric polarization, since dc conductance is the low frequency limit of the general Fourier transform of transient currents into the frequency domain. Many reported apparent "conductivities" of insulators represent predominantly a very low frequency loss phenomenon [17] or other artifact [18] rather than steady state charge transport through the bulk. Insofar as the distinction is meaningful, step response charge/discharge cycles permit separation of conductivity and dielectric contributions, unlike continuous wave dynamic measurements. If the conductivity is taken independent of frequency, the resulting dielectric spectrum, in common with mechanical relaxations, is interpretable in terms of structural dynamics of molecules. At the other extreme, if the dielectric contribution is frequency independent (as in simple inorganic materials), the conductivity spectrum is interpretable by amorphous semiconductor theory.

Results from these two techniques applied to representative homogeneous biopolymers are reported in an associated paper [16]. However, several more heterogeneous biological organelles (chloroplasts, melanosomes, mitochondria, nerve systems, retinals) are better candidates for the involvement of semiconductivity *in vivo*. In particular, there could be a significant difference between their electrical properties, even when "dry," and those of synthetic insulating polymers. The present paper is concerned with the mitochondrion, the power plant of cells wherein the chemical energy of foodstuff molecules is utilized in respiration, the process of enzymatic oxidation. The ultimate goal in this field is to establish the molecular and electronic basis of the biological activity of this assembly of proteins and lipids, and to correlate it with the physical and chemical structure. On the basis of the microwave Hall effect in the complete and partial respiratory chain we speculated in some detail on electron transport via semiconducting pathways and tunneling [13]. The present paper provides data with which to modify and refine these speculations. The electrical measurements are supplemented by differential thermal analysis (DTA) and nuclear magnetic resonance (NMR) to help the structural dynamic aspects of this lipoprotein assembly.

Experimental

The apparatus developed for Seebeck coefficient measurements on high resistance, low mobility materials is described elsewhere [19]. The stainless steel conductivity/dielectric cell with pressure electrodes was a more compact version of a previous design[20], but was enclosed in a glass jacket for temperature control with electrical isolation as for the Seebeck cell. Voltage of up to 120 V from a battery generated currents detected on a Vibron 62A high impedance electrometer, and displayed as a function of time on a chart recorder. The Vibron head resistor was at least 10 times smaller than the sample resistance for the current mode measurements; Seebeck results via the voltage mode were made without head resistor. Battery, leads, and cell were all screened and final charging voltages were selected from the linear current–voltage region. Attainment of equilibrium on charging or discharging required times significantly longer than the median relaxation time expected for the lowest frequency polarization process; in practice voltage application for 5 hr or more was necessary for discharge current transformation to 10^{-5} Hz at low temperatures. In cases in which residual polarization of samples produced variable "zero" settings on the Vibron different from the earth level, the reverse polarity discharge was required to obtain the correct reference levels and so eliminate spurious "currents".

The steady conductivity was evaluated from the final charging current, and the time-dependent discharging current $i(t) \alpha t^{-n}$ was transformed by the Hamon [21] approximation to give the loss factor ϵ'' in the frequency range 10^{-1} to 10^{-5} Hz. The validity of the transformation is adequately discussed elsewhere [22].

Heavy beef heart mitochondrion samples were prepared by the method of Smith [23]; electron microscope examination [24] showed some 30–50% to be intact mitochondria and the rest submitochondrial particles in vesicles. Samples were prepared for electrical measurements in the form of discs either 5×10^{-3} or 1.3×10^{-2} m in diameter, pressed at 4×10^{6} to 5×10^{8} Pa under a vacuum of 10^{2} Pa. The samples were equilibrated at this vacuum in the measuring cell for some time and the measurements then proceeded in the presence of a small pressure of dry nitrogen as heat transfer agent. Unlike the associated work on hemoglobin and DNA [16] the most significant variable was not hydration, but temperature, and the experiments were carried out accordingly.

The DTA runs involved milligram quantities of mitochondria in an atmosphere of dry nitrogen at a heating rate of 10 K/min. A Varian A60 wide line NMR spectrometer with variable temperature probe was used for the NMR experiments. These techniques were purely supplementary; neither was investigated in detail.

Results

The DTA results (not reproduced) show a broad endothermic transition with two small shoulders, centered about 342 K, with noticeable baseline shift that possibly reflects loss of water from the sample. The NMR line (not shown) reveals accentuated motional narrowing from a width at half-maximum height of about 1500 Hz to about 1000 Hz in the vicinity of 340 K.

The thermoelectric data in the form of representative voltage versus temperature gradient plots are shown in Fig. 1. This thermoemf sometimes had two components, one reaching equilibrium concurrently with the thermal gradient, and the other a slow drift over an hour or so, which probably reflects a change in the background polarization corresponding to $\Delta T = 0$. Despite this generally variable and often substantial residual polarization, the plots are linear and their slopes, giving the thermoelectric coefficient Q in volts per degree Kelvin, are constant and reproducible. Identical behavior at positive and negative ΔT is particularly encouraging. Figure 2 shows this Q value as a function of average temperature for two samples. The errors do not obscure the trend from a negative to a small

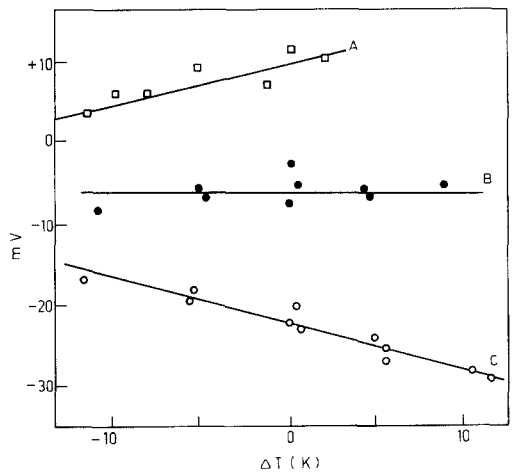

Figure 1. Thermoelectric voltage (mV) versus temperature gradient ΔT (K). The plots are displaced vertically relative to each other for clarity of presentation. Average temperatures (K): A, 347; B, 343; C, 333.

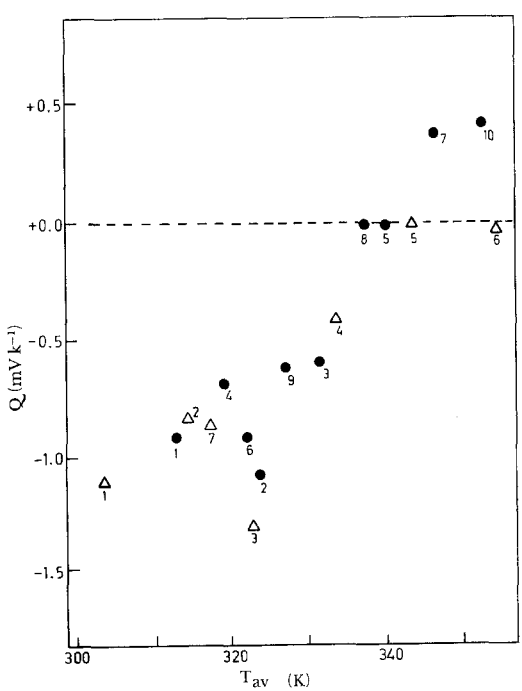

Figure 2. Thermoelectric coefficient Q (mV K^{-1}) versus average temperature T_{av} (K). The sequence of readings is important and is indicated by numbers on the points.

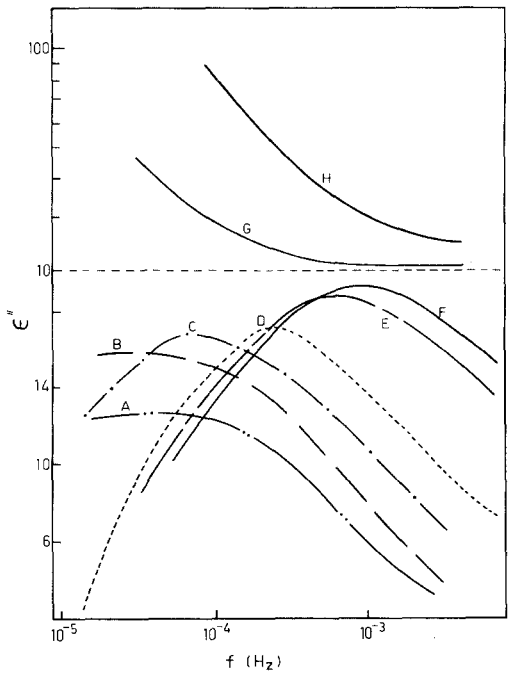

Figure 3. Dielectric loss ϵ'' versus frequency (Hz) at various temperatures (K): A, 306; B, 311; C, 316; D, 321; E, 330; F, 336; G, 346; H, 351.

positive Seebeck coefficient and the changeover that occurs around 340–345 K.

The low frequency dielectric loss factor ϵ'' from the Hamon treatment of $i\alpha t^{-n}$ is displayed in Fig. 3 as a function of frequency and temperature. There are two relaxations that are designated Ω and α for the higher and lower temperature processes, respectively, at any one frequency. The Ω relaxation is dominant above a transition temperature around 340 K in the initial run while the α relaxation dominates below this temperature. Thermal cycling to 383 K shifts the α peaks to lower frequencies for the same temperature without affecting the peak magnitude ($\epsilon''_{max} \sim 10$–25) and breadth (Cole–Cole parameter n remains ~ 0.6); it is also found that the frequency at which ϵ''_{max} occurs is the same for both old and new transition temperatures. The Ω relaxation is harder to characterize because the increase in amplitude (to as high as $\epsilon'' = 10^3$) and frequency with temperature fails to yield an observable maximum even at the lowest frequency experimentally accessible. We have, however, no reason to

suspect any artifact here. Further points to note are the nondependence of ϵ'' of the α relaxation on charging voltage, in contrast to ϵ'' of Ω which becomes inversely proportional to this voltage at lower frequencies. Also, ϵ' and ϵ'' measured above the transition at 50 Hz on an ac bridge are independent of dc bias voltage applied.

The steady conductivity evaluated from the final charging current is approximately ohmic with respect to electric field and sample thickness; the time to final equilibrium varies from several hours at low temperature to a few minutes at higher temperature. Above 383 K current decay continues well past the equilibrium time expected. There are two conductivity regimes, with slopes that remain constant after the thermal cycles that decrease the conductivity and raise the transition temperature. The initial value of the latter is again about 340 K.

Figure 4 summarizes the conductivity and polarization data for the third heating run as logarithmic plots against reciprocal temperature, of the conductivity, $\sigma = \sigma_0 \exp(-E_\sigma/RT)$ where $\sigma_0 \sim 10^8$ or $10^{26}\,\Omega^{-1}\,m^{-1}$ (below or above the kink), the frequency of the α peak to which rate theory applies [25], and the ϵ'' value at 10^{-4} Hz for the Ω peak. The initial runs give for

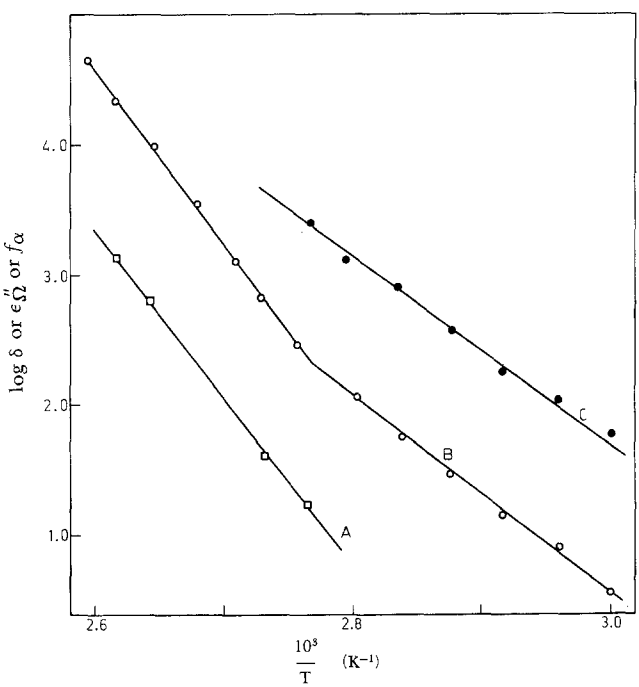

Figure 4. Conductivity σ, loss factor at 10^{-4} Hz for Ω relaxation ϵ''_Ω, and frequency at a α peak (K^{-1}). f_α, versus reciprocal temperature. A = log ϵ''_Ω ; B = 13 + log ; C = 6 + log

$T < 345$ K, $E_\sigma = 115$–135, $E_\alpha = 85$, and for $T < 345$ K, $E_\sigma = 230$–245, $E_\Omega = 200$–210. Subsequent runs, after thermal cycling has shifted the transition, give for $T < 360$ K, $E_\sigma = 145$–155, $E_\alpha = 130$, and for $T > 360$, $E_\sigma = 240$, $E_\Omega = 230$–240. All E units are in kilojoules per mole.

Extensive results were also obtained for the cytochrome oxidase fraction representing the terminal component of the respiratory chain, but analysis showed the dominance of extrinsic effects for ionic impurities used in the isolation process. These results have therefore been omitted, and we are unable to confirm the interesting data of Cope and Straub [26].

Discussion

The DTA indicates a reversible thermodynamic transition close to 342 K in the mitochondrion that, from the NMR results, reflects increased molecular mobility, most likely in the structural phospholipid in view of the known liquid crystalline behavior of this moiety [27]. The mitochondrion samples are estimated to contain 60% protein, 30% lipid, and 10% other species [28], and whether the transition is internal to the phospholipid or related to phospholipid/protein interaction is uncertain. The breadth of the transition favors the latter. This transition is also unequivocally revealed by all three electrical techniques, showing that charge generation, transport, and storage all depend on the physical structure and mobility of these molecular entities constituting the mitochondrion. Since this is the basis of the biological function, it is probable that these electrical processes have a role to play. At high temperatures, above about 383 K, there occurs either a loss of residual water or an irreversible (degradation or morphological) transition in the integrated lipoprotein structure–either event could stimulate ionic processes. This proposal is based on the evidence in the literature from a variety of techniques for desorption of water from many biopolymers at temperatures on the order of 373 K, even in apparently dry samples [29]. There is also electron microscopic evidence of certain structural changes in the mitochondrion around this temperature [30].

As far as the thermopower results are concerned, it is difficult to conceive any explanation other than free charge carrier migration, whether intrinsic or injected, since unlike the study on hydrated biopolymers [16], there is no likelihood of a gradient of chemical potential interfering. Below the transition temperature the cold junction polarity portends a negative majority carrier, i.e., electrons, while the relatively quick changeover to a positive coefficient above the transition favors an increasing contribution from less mobile electrons or from holes—the

coefficient reflects the mobility ratio of positive and negative carriers—as temperature rises. It is possible that protons contribute to the change; however, even if they were not blocked at the electrodes, their Seebeck effect should be small. It is just possible that conduction is intrinsic near room temperature, and extrinsic above the transition at 342 K but the formulas relating the Seebeck coefficient to carrier concentrations and mobilities for band (both intrinsic and extrinsic) and hopping mechanisms [31, 32] are not sufficiently unique to permit a distinction. The conductivity/dielectric results indicate that both the carrier generation and mobility activation energies are involved, so that the significance of the Seebeck results is more in relative terms than absolute ones.

In considering the low frequency dielectric spectrum the value of n in $i\alpha t^{-n}$ ($\simeq$0.6 for the α peak here) is commonly taken to represent the Cole–Cole parameter associated with a relaxation time distribution, and Algie and Stuart [33] have interpreted their results for keratin in terms of at least six overlapping Cole–Cole type relaxations. However, Jonscher [34] points out that many features of polymer relaxation peaks are inconsistent with the concept of a relaxation time distribution.

The polarization peak designated Ω, appearing above the transition temperature, has a large magnitude that undoubtedly reflects an interfacial polarization process. It appears similar to the purely electrical process observed in a few synthetic polymers that has no counterpart in mechanical experiments [25, 35]. The Ω enhancement by high temperature cycling links the physical nature of these boundaries with the loss of water and/or irreversible change in the protein/lipid structure already proposed. On account of the heterogeneous nature of the material, the α relaxation too might reflect another process of charge accumulation at interfaces. However, in comparison with hemoglobin [16], and in the light of observations on certain synthetic polymers [36] it is possible to assign it segmental motions of the protein and/or lipid main chains. The magnitude of ϵ'' for the α peak, being larger than for hemoglobin, supports the idea that the phospholipid moiety is responsible, in line with the conclusions of higher frequency ac bridge studies [24] on the mitochondrion. The shift of the α peak on thermal cycling is consistent with possible loss of water that had been plasticizing the segmental mobilities—in fact, loss of water from phospholipids is known to raise the transition temperature [27] for hydrocarbon chain melting. However, similar effects could also well arise from the alternative possibility that morphological changes reduce protein/lipid electrostatic interactions, which would also raise the chain melting temperature. The rather large shift in the α peak activation energy tends to support the latter explanation. Probably it is some combination of both.

The steady conductivity in both regimes is also probably due to the phospholipid. Although high activation energies and high σ_0 values ($>10^{20}$ here) are known for phospholipids, normally higher temperatures would result in a lower conductivity energy consequent to increased chain mobility. This is not borne out in the present case—the high E_σ above the transition is more characteristic of electronic excitations (perhaps injection) than polymer motions. Therefore, while it is quite likely that the phospholipid dominates the conductivity (and polarization processes), the detailed mechanism is still uncertain. As a point of interest, the mitochondrion response is quite different from cytochrome oxidase fraction, which has no steady conductivity even after long charging times. It is therefore fairly certain that the charge carriers in the mitochondrion are electronic.

Further observations may now be made from the comparison of conductivity and polarization parameters. The E_Ω constitutes about 90% or more of the total conductivity energy E_σ, and the E_α abour 70%, rising to over 80% after a high temperature cycle. That E_α and E_Ω both exhibit large increases after this cycle is solid evidence that they are closely related. In hemoglobin the E_α was equal to E_σ [16], whereas for keratin [37] E_Ω was equal to E_σ. It is reasonable to propose that the major part of the conduction energy in the mitochondrion involves the transport step, which might be a hopping process from one phospholipid chain to another, and hence dependent on the segmental reorientation below the transition generating a favorable configuration. Above the transition the structure may no longer be favorable to hopping, and the carriers might accumulate at lipid/protein or other heterogeneous boundaries between areas of differing conductivity. The resultant polarization may be enhanced by injected carriers, possibly even by protons subsequent to thermal cycling; however, these should change the ac capacitance under dc bias. An electrode/sample barrier could be created rather than any internal sample boundary effect, and this accords with the space charge type dependence of ϵ''_Ω on the inverse charging voltage. In polyproline the contactless [38] method gives the same results as the conventional method, proving that the dielectric behavior originates in the bulk sample and not at the electrode interface.

At any rate release of the large Ω polarization with a high energy barrier parallels the slow dissipation of charges toward or at the electrodes that constitute the conductivity. In $E_\sigma = E_n + E_\mu$, E_n is apparently small compared to E_μ, being about the same order of magnitude as the drift mobility parameter [39]. It need not necessarily be a generation term—it could represent a tunneling process [13, 40] or some other transfer potential barrier not directly connected with the α or Ω process. Physically

this could involve transfer across protein/lipid boundaries or between separate portions of the intergrated lipoprotein structure, but such interpretation is purely speculative.

In conclusion there is no question that the low frequency dielectric polarization and thermoelectric effect can provide a valuable insight to charge carrier processes in biological materials that is not available from conductivity measurements alone.

Acknowledgments

The authors acknowledge the contributions of Dr. M. Landon and Dr. R. J. Mayer (Medical School)—mitochondrion samples; Dr. A. J. I. Ward (Chemistry Department)—NMR data; and Mr. E. L. Charsley (Stanton Redcroft Ltd.)—DTA data.

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