Evidence for a New Type of Iron Containing Electron Carrier in Mitochondria*

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Sands and Beinert (1960) and Beinert and Sands (1960) reported on an unidentified component of mitochondria and derived fragments, which showed a characteristic EPR signal in the reduced state. They attributed this signal tentatively to Fe^{++}. We would now like to present additional evidence that this type of signal is due to at least one new iron containing electron carrier (or possibly a small group of related ones) which function(s) near the flavin region of the electron transport sequence. We would like to emphasize that this material, which is present in amounts approximately stoichiometric to the other electron carriers, accounts for only a small portion of the tightly bound "non-heme iron" present in mitochondria and derived preparations. So far our EPR data have given no information on the role of the bulk of the non-heme iron.*

It would appear that the observed EPR signal is very specific for the particular structure of the new component, as we have been unable to reproduce the signal under the same conditions using model Fe^{++} systems. The signal, however, is very sensitive to temperature as would be expected for a signal due to Fe^{++}. Of other metals, which could conceivably produce a signal in this area of the EPR spectrum, Mo could not be detected by chemical analysis, nor could the

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** The previously reported Fe^{+++} signal at g =4.3 (Beinert and Sands, 1959, 1960) is of doubtful significance, since such signals have only been found in preparations exposed to certain chelating agents.

typical EPR signal of Mn^{++} be found after ashing. Added Mn was readily detected in this way. Cu and Fe appear to be the only heavy metals present in sufficient quantity to account for the signal, but copper does not have to be considered, because the reduced form, Cu^{+}, is not paramagnetic. The EPR signal is no longer observed after treatment with heat, acid, urea or proteolytic enzymes, which indicates that the specific Fe^{++} structure is sensitive to agents which denature or degrade proteins. The signal is not found on reduction of purified preparations of cytochromes $\underline{a} + \underline{a}_3$, $\underline{b}$, $\underline{c}$ and $\underline{c}_1$ but is readily observed after reduction of preparations of succinic dehydrogenase (Singer et al., 1956) and DPNH CoQ*** reductase (Haavik and Hatefi, 1961) which do not contain heme components. It is unlikely, therefore, that the signal is due to a hemoprotein. Flavin, however, has always been found to be associated with materials, which showed the Fe^{++} signal. Whether this association is of any specific significance is not clear, but it might at least be taken as an indication that flavin and the iron compound are adjacent electron carriers. Artifacts appear to be excluded, since the characteristic asymetric signal ($g_{\parallel}$=2.00, $g_{\perp}$=1.94) can be clearly seen in the EPR spectrum of a piece of freshly frozen untreated rat heart or beef heart as shown in fig. 1. Spectra of mitochondria and submitochondrial particles are also included for comparison. DPNH was added to the latter to achieve the same state of reduction as that due to endogenous substrates in the other three samples. The predominant Fe^{++} signal can be readily distinguished from the overlapping Cu^{++} and free radical signals by its temperature sensitivity.****

Fig. 2 depicts the progressive reduction of electron carriers in submitochondrial particles of the phosphorylating type by DPNH and succinate, respectively. Initially only the Cu^{++} signal of cytochrome oxidase can be seen

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*** CoQ: coenzyme Q or ubiquinone, the subscript indicates the number of isoprenoid units of the side chain.

**** A temperature rise from -180° to -130° leads to a loss of signal height of 70-90%.

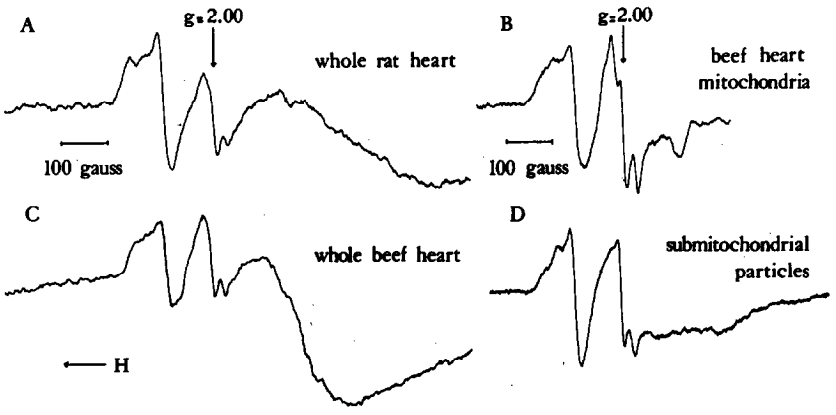

Figure 1 - EPR spectra (first derivative) of about 100 mg of fresh rat and beef heart, of beef heart mitochondria (8.6 mg. in 0.10 ml. of 0.25 M sucrose, pH 7.5) and of particles obtained by sonication of mitochondria (Linnane and Ziegler, 1958). 10 mg. of particles were suspended in 0.10 ml. of 0.25 M sucrose, pH 7.5, containing 0.015 M $MgCl_2$ and 0.001 M ATP and were frozen 70 seconds after addition of 0.45 µmole of enzymatically reduced DPNH at 0°. All spectra were recorded at a sample temperature of -178°.

(Sands and Beinert, 1959). On addition of substrate this signal rapidly disappears and a free radical signal (g=2.00, probably due to flavin semiquinones) immediately appears and declines as the reduction proceeds. It is clearly seen at the early stages that reduction of Cu^{++}, Fe^{+++} and flavin (evidenced by radical disappearance) occurs simultaneously. Spectrophotometric experiments, performed under the same conditions with the same concentrated preparation as was used for the EPR spectra, indicated that the iron compound was reduced at a rate comparable to that of the cytochromes. Reduction of the latter was followed by absorbancy readings at wave-length pairs characteristic of each component.

In addition to the predominant slope ($g_{\perp}$=1.94), the EPR spectra of reduced submitochondrial particles exhibit small peaks a, b and c (fig. 3) which vary according to whether DPNH or succinate is used as substrate. With DPNH peak 'a' is most pronounced whereas succinate accentuates peak 'b'. These differences in signal shape are evidently due to different structures which are

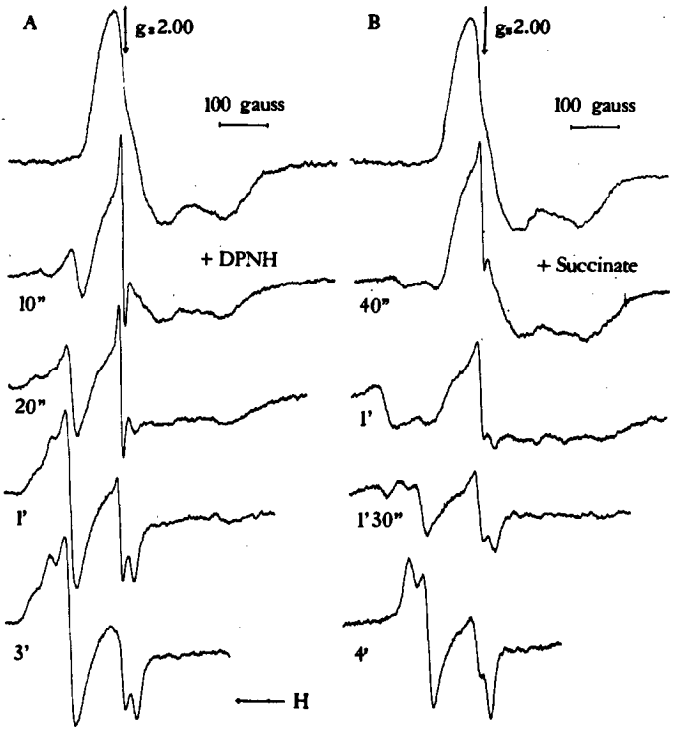

Figure 2 - EPR spectra of submitochondrial particles as in Fig. 1. Uppermost line: Untreated (Cu^{++} signal); lower lines: reduced by substrate (0.45 µmole of DPNH in A and 2 µmoles of succinate in B) and frozen as indicated after incubation at 0°. Spectra taken at -178°. The sharp signal at g=2.00, overlapping with the Cu^{++} signal, is due to free radicals. To the left at higher fields the Fe^{++} signal ($g_{\perp}$=1.94) shows by its characteristic deep slope. The broad trough to the right is due to Cu^{++} and is used to estimate the quantity of Cu^{++} present because it is less interfered with by other signals.

specifically reduced by the respective substrates. That the differences are not a result of interaction by the same structure with different substrates is evidenced by fig. 3 (B and D). EPR spectra of derived enzymes which are reduced either by DPNH only (3B) or by succinate (3D) give the typical DPNH type signal only (3B, peak a) or the succinate type (3D, peak b) whether reduced by substrates or by dithionite.*****

***** DPNH is able to reduce the iron compound of succinic CoQ reductase when a small amount of DPNH CoQ reductase is present. In this case also the succinate-type signal of the succinic CoQ reductase (Fig. 3D) is observed, although succinate is absent.

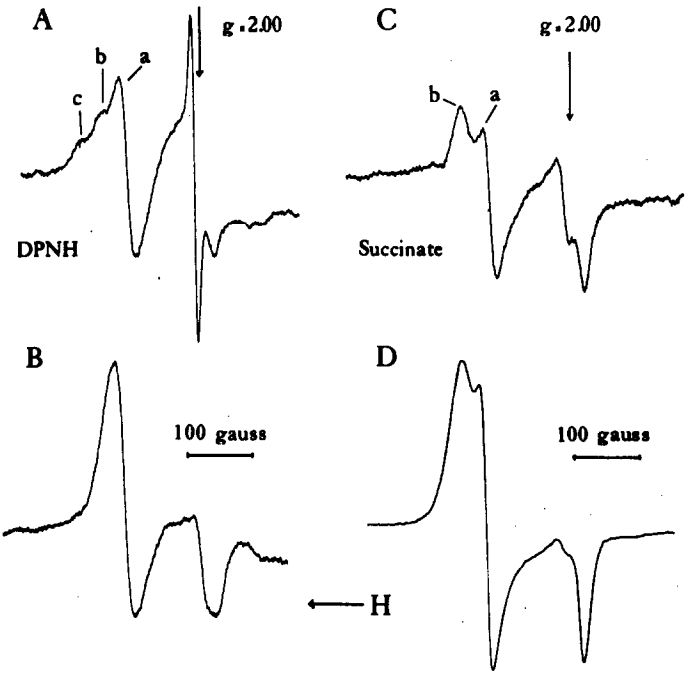

Figure 3 - A and C, EPR spectra of submitochondrial particles as in Fig. 1 and 2. A, reduced with 0.45 μmole of DPNH, frozen after 40 seconds at 0°; C, reduced with 2 μmoles of succinate, frozen after 2 minutes at 0°. B, 2.5 mg of DPNH CoQ reductase dissolved in 0.10 ml of 0.66 M sucrose containing 0.05 M Tris (pH 8), and D, 3 mg of succinic CoQ reductase, (Ziegler and Doeg, 1959) dissolved in 0.10 ml of 0.25 M sucrose, both reduced with dithionite. Spectra taken at -178°.

In DPNH and succinic CoQ reductases there is not more than one molecule of the specific iron compound per flavin and in mitochondria and derived particles the compound is also present in amounts not exceeding those of the flavin and individual cytochromes.****** If we assume that fresh heart has 15% protein and that one fifth of this is mitochondrial protein, the concentration of the compound in heart is accounted for by that present in mitochondria. Thus, after allowing for the iron content of the new respiratory catalyst, more than 95% of the "non-heme iron" in mitochondria and derived particles and 75-95% of that in various DPNH and succinic dehydrogenase

******Estimated by double integration and comparison to a Cu^{++} standard assuming four free spins per molecule of the Fe^{++} compound.

preparations still remains unaccounted for.

Study of the new component by chemical methods would obviously be very difficult in the presence of so large an excess of non-heme iron and results obtained by such methods would not necessarily apply to the iron component described in this note.

As exemplified in fig. 2, in all materials with an active electron transport system the iron compound is readily reduced and reoxidized. This is also true for DPNH and succinate CoQ reductases. In succinic dehydrogenase (Singer et al., 1956), however, which only links to phenazine dyes or ferricyanide, the iron compound is reduced less extensively by substrate if at all and is no longer reoxidized by compounds such as CoQ_2. It is of interest in this context that inhibitors such as cyanide (Tsou, 1951) or thenoyl trifluoroacetone (Doeg, 1961), which block interaction of succinic CoQ reductase with many acceptors, affect the iron compound in the same way, i.e. its reduction is less extensive and reoxidation is practically eliminated.

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