Swelling and Lysis of Rat Liver Mitochondria Induced by Ferrous Ions

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The possibility that ascorbate- and reduced glutathione-induced swelling and lysis of mitochondria (1, 2) were dependent on some metal, such as iron, which was contaminating reagents, or even the nonheme iron in mitochondria led us to investigate the action of added iron. The ferrous ion was of special interest as a result of its role in several reactions catalyzed by ascorbate or even the nonheme iron in mitochondria led us to investigate the action of added iron. The ferrous ion was of special interest because of its role in several reactions catalyzed by ascorbate and oxidized plus reduced glutathione (1). Whether or not such effects contribute to physiological control or pathological phenomena remains to be seen, but these studies with isolated mitochondria provide additional information about structure and permeability control in the mitochondrial membranes.

EXPERIMENTAL PROCEDURE

The swelling of rat liver mitochondria was measured as the turbidity decrease of dilute suspensions at 520 mm (D_{290}). Selected 8-ml test tubes gave a light path of approximately 11 mm. Changes in light scattering are correlated with mitochondrial swelling (10, 11). The Bausch and Lomb Spectronic 20 shutter control and entrance slit were modified so that volumes as low as 1 ml could be read.

The mitochondria were prepared as previously described (8), maintained at 0° in a concentrated stock suspension, and added in amounts giving an initial D_{290} of approximately 0.500 (protein = 100 to 150 μg per ml). In most experiments the basic medium was 0.175 M KCl-0.02 M Tris buffer, pH 7.4, equivalent in tonicity to our medium containing 0.33 M sucrose-0.02 M Tris (12). Earlier experiments had indicated that 0.25 M sucrose and its equivalents are probably hypotonic for rat liver mitochondria. Incubations were aerobic at 22-25°. Readings were taken or aliquots withdrawn at time intervals short enough to establish the character of the curves. This was every 2 or 3 minutes in the early time periods.

RESULTS

Comparison of Media—The swelling of mitochondria produced by Fe^{2+} is usually so slow in 0.33 M sucrose as to be negligible. It occurs more readily in mannitol medium, but KCl is the most satisfactory medium for studying this phenomenon (Fig. 1). For comparison it might be mentioned that with 0.3 mM ascorbate, the lag period before swelling was 30 minutes in sucrose and 20 minutes in KCl. Fe^{2+} has no effect at pH 8.0, possibly because of the low pH.

All common chemicals were of analytical reagent grade. Ferrous and ferric ammonium sulfate were used as the source of metal ions. Fe^{2+} ion stock solutions were prepared by dissolving the salt in cold unbuffered KCl solutions deoxygenated just before use. All concentrations of Fe^{2+} refer to the initial value in the experiment. Distilled water was redistilled in a two-step all-quartz still. Special chemicals were of the highest grade obtainable from Sigma Chemical Company, California Corporation for Biochemical Research, Nutritional Biochemical Corporation, and the Eastman Company. BSA was a crystalline product of the Armour Company. Antimycin A was obtained from the Wisconsin Alumni Research Foundation, and gramicidin from the Schering Corporation. The oligomycin was a gift from Dr. H. A. Lardy and Dr. F. M. Strong, part of the material prepared by the late Dr. W. H. Peterson. Octylguanidine was a gift of Dr. B. C. Pressman. The C-factor was a crude preparation made according to the method of Lehninger (13).

Lipid peroxide formation was followed by a modification of the thiorbarbituric acid method (14, 15). A 1-ml aliquot was withdrawn from the incubation mixture and pipetted into an 8-ml Pyrex tube. After the D_{290} was read, 0.25 ml of 40% trichloroacetic acid and 0.125 ml of 5% HCl were added. After mixing, 0.25 ml of 2% sodium 2-thiobarbiturate was added promptly. In many experiments the 3 reagents were combined before addition. The use of part HCl instead of all TCA essentially eliminates difficulties from adsorption of color on the protein precipitate. The tubes were topped with 18-cm air-cooled condensers and placed in boiling water for 10 minutes, cooled, centrifuged at 2500 r.p.m. for 10 minutes, and the color read at 532 mm. In plotting lipid peroxide formation in the graphs, the absorbancy reading has been graphed directly rather than by means of a fixed conversion to malonaldehyde equivalents.

The abbreviations used are: BSA, crystalline bovine serum albumin; HSA, human serum albumin; TBA, 2-thiobarbituric acid; DNP, 2,4-dinitrophenol.

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because of rapid conversion to Fe$^{3+}$ and precipitation of ferric hydroxide.

**Characteristics of Changes with Fe$^{2+}$**—The curves for swelling and lysis of mitochondria with Fe$^{2+}$ ion had a superficial resemblance to those with ascorbate, but detailed study revealed distinct differences. Between concentrations of 1 and 10 μM, Fe$^{2+}$ produces a swelling that shows essentially no lag period and a rate that is determined by the concentration of Fe$^{2+}$ (Fig. 2). With 10 μM Fe$^{2+}$ there may or may not be a brief lag period. As the concentration of Fe$^{2+}$ is raised, lag periods increase and the swelling after the lag period starts with a fairly sharp break and proceeds at a rapid rate which is nearly identical for all concentrations of Fe$^{2+}$ between 20 and 100 μM (Fig. 3). Provided sufficient time is allowed, all of these concentrations of Fe$^{2+}$ produce final plateau D$_{520}$ readings that are essentially the same. The final low D$_{520}$ reading (0.090 to 0.130) approaches that seen with ascorbate and GSSG + GSH (0.040 to 0.060), but it is distinctly and characteristically higher.

The very low D$_{520}$ values reached with Fe$^{2+}$ are suggestive of lysis, a term we have used in connection with the effects of ascorbate and GSSG + GSH. However, since the end point with Fe$^{2+}$ may be different from that with the other substances, we have conservatively retained the term swelling. As will be seen in later sections, both phenomena probably occur.

**Both Fe$^{2+}$ and Fe$^{3+}$ Ions Affect Swelling Process**—Experiments like that in Fig. 3 suggest that higher Fe$^{2+}$ concentrations have a definite inhibitory effect on the initiation of swelling. This lag-producing effect may disappear as the Fe$^{2+}$ concentration is lowered to optimal levels by conversion of Fe$^{2+}$ to Fe$^{3+}$. On the other hand, the shortening of the lag might be caused by the Fe$^{3+}$ ion formed. In a large number of experiments with 50 μM Fe$^{2+}$, the lag period ranged from 10 to 50 minutes. The longer lag periods were seen when Fe$^{2+}$ stock solutions were most carefully prepared to avoid oxidation.

When combinations of Fe$^{2+}$ and Fe$^{3+}$ ions are tested, the lag periods and swelling curves are often similar to those that would be seen with the same amount of Fe$^{2+}$ alone. Fig. 4 illustrates fairly typical effects of 1–40 μM Fe$^{2+}$ in the presence of a fourfold excess of Fe$^{3+}$. High Fe$^{2+}$ concentrations still inhibit initiation of swelling even in the presence of some Fe$^{3+}$ ion. In general the lower concentrations of Fe$^{3+}$ (0 to 40 μM) produce little or no effect, but at high concentrations, it does shorten the lag periods with Fe$^{2+}$ (Fig. 5). There may be an optimal Fe$^{2+}$ to Fe$^{3+}$ ratio for shortening lag periods, but its definition is difficult because some Fe$^{2+}$ is always oxidized...
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830 Fe++-Induced Xwelling of Mitochondria during the experiment and tests at higher concentrations of Fe+++ are limited by precipitation of Fe(OH)3. Fe+* alone (1 to 150 μM) causes little or no swelling over periods as long as 75 minutes.

The final D20s plateau or end point reached when ratios of Fe+++ to Fe++ are used is almost identical regardless of whether the lag period is 2 minutes or 40 minutes or the total concentration of iron is 5 or 150 μM. It is the same as that reached with Fe++ ion alone.

Formation of Lipid Peroxide (TBA Color Reaction)—During Fe++- or Fe+++ plus Fe++-induced swelling of mitochondria, there is formation of material which reacts with 2-thiobarbituric acid to give a colored product. The absorption spectrum coincides exactly with that for malonaldehyde (16). Lipid peroxides are believed to be the precursors of this material (15).

Formation of TBA color material shows remarkable correlation with the swelling and lytic changes as judged by the D20s reading (Figs. 2-5). During most of any lag period from 3 to 70 minutes, there is no color or a very small rise in color, but usually a minute or two before D20s changes start, the TBA color begins a rapid rise. However, in many experiments the increase in TBA color formation coincides with rather than precedes the beginning of swelling. The increase in lipid peroxides is much more rapid with Fe++ and Fe+++ plus Fe++ than with ascorbate.

The amount of TBA color material formed is determined by the amount of Fe++ added. Between 1 and 10 μM Fe++, the TBA color is roughly proportional to the amount of iron. The rise of TBA color occurs in a few minutes and then stops, as though the Fe++ were exhausted (10). It then remains unchanged for long periods instead of continuing to rise as with ascorbate. A second addition of Fe++ will produce a second rise (Fig. 6). With very low concentrations of Fe++, the amount of lipid peroxide is similar to that seen with 0.3 mM ascorbate lysis. As the concentration of Fe++ is increased, the amount of TBA color increases, even though a long lag period may precede the rise. With the highest concentrations of Fe++, the yield of TBA color is limited by the amount of unsaturated lipid of the mitochondria (Fig. 3). Fe+++ ion seems to have little or no effect on the amount of lipid peroxide formed.

The inhibitory or delaying effects of high Fe++ concentrations on swelling appear to be always associated with inhibition of lipid peroxide formation. Another type of experiment demonstrating such inhibitory effects is shown in Fig. 6; 10 to 20 μM Fe++ added late after 4 μM Fe+++ at zero time gives a proportional rise in lipid peroxide and an accelerated swelling. At 50-100 μM there is only a small rise in lipid peroxide. This is accompanied by a brief acceleration of swelling followed by some inhibition.

Morphological Change with Fe++-induced Swelling—Our earliest observations on Fe++-induced swelling raised the question whether or not complete lysis occurred, for the plateau of D20s was always distinctly higher than with ascorbate. This could be due to retention of structure, but it might also result from aggregation due to the Fe++. Phase microscope examination of mitochondria in samples withdrawn at various times during swelling experiments revealed two difficulties which prevented completely satisfactory results. In the KCl medium there was some tendency for mitochondria to aggregate in groups of 3 to 6. This tendency appeared to be increased somewhat by Fe++. The second difficulty was that when suspensions of mitochondria were placed between the coverslip and microscope slide, an appreciable number of the mitochondria became fixed on the surface of the glass and underwent very little further change once this occurred.

2 We are indebted to Miss Claire Hollingsworth for many phase microscope observations and for the tests on reversibility of swelling by ATP + Mg + BSA + C-factor.
However, despite the difficulties mentioned, some swelling of mitochondria under the influence of iron could be followed by observing both single mitochondria and some of the aggregates. The beginning and progression of swelling was well correlated with the D$_{590}$ change in the suspension. Swelling was followed by a variety of changes. Some mitochondria seemed to lyse quite clearly and just disappear. Others left distinct membrane outline ghosts or small clumps of membrane material.

**Reversal of Fe$^{++}$-induced Swelling** — ATP + Mg$^{++}$ + BSA, with which Lehninger (17) has demonstrated reversal of mitochondrial swelling induced by a variety of agents, was studied for possible reversal of Fe$^{++}$-induced swelling. When this combination is added after the D$_{590}$ plateau is reached in the usual (pH 7.4) Fe$^{++}$ swelling experiment, no reversal whatever is seen. The addition of a crude C-factor preparation either initially or after swelling made no difference. In two experiments where the ATP mixture (5 mM ATP + 2 mM Mg$^{++}$ + 1 mg per ml of BSA) was added at D$_{590}$ = 0.225, before the plateau was reached, a small contraction was seen. The partial reversal may represent the response of mitochondria that are not yet completely swollen. Because most contraction studies with C-factor addition were done at higher pH, we carried out experiments with swelling induced by Fe$^{++}$ at pH 7.4, followed by adjustment of the pH to 8.0. Still there was no reversal with the ATP mixture. The presence of added iron did not inhibit in any way the substantial contractions seen when ATP + Mg$^{++}$ + BSA was added after swelling produced by other agents.

**Acceleration of Lipid Peroxide Formation and Swelling Induced by Fe$^{++}$—Gulonolactone, $\alpha$-ketoglutarate, and GSSG were studied because they reduce or eliminate the lag period and accelerate lysis with ascorbate.** L-Gulonolactone, 5 and 10 mM, produced only a slight shortening of the lag period with 50 $\mu$M Fe$^{++}$, but 10 mM $\alpha$-ketoglutarate induced a sharp rise in TBA color and virtually eliminated the lag before swelling. GSSG at 1 mM, which greatly shortens lag periods with ascorbate, did not produce swelling or lipid peroxide with 50 $\mu$M Fe$^{++}$ within a 60-minute period. However, higher concentrations of GSSG reduce the lag period to 1 minute as the concentration is raised to 20 mM (Fig. 7). Such large amounts of GSSG alone result in some TBA color and a slow swelling. However, the primary determinant of the amount of TBA color is still the amount of Fe$^{++}$.

**Inhibitors of Lipid Peroxide Formation and Swelling Induced by Fe$^{++}$** — It was of interest to test several types of inhibitors for the following reasons: (a) Electron transport chain inhibitors block phosphate- and thyroxine-induced swelling. (b) Complete uncoupling of phosphorylation blocks phosphate- and thyroxine-induced swelling. (c) Substances that destroy H$_2$O$_2$ or act as anti-oxidants might inhibit swelling associated with lipid peroxidation. (d) Swelling per se might render mitochondrial lipids available for rapid peroxidation.

**Electron Transport Chain Inhibitors** — NaCN, 1 mM, which blocks phosphate-induced swelling, ascorbate induced lysis, and GSSG plus GSH-induced lysis, and which has been reported to inhibit lipid peroxidation (18), produces only partial inhibition (10 to 50%) of Fe$^{++}$-induced swelling. Antimycin A blocks phosphate-induced swelling at 0.1 $\mu$M, but 3 to 4 $\mu$M are required to prevent ascorbate induced lysis. Fig. 8 shows that with Fe$^{++}$ even the higher concentration of antimycin A only lengthens the lag period and slows the rate of swelling. The formation of lipid peroxide is delayed, slowed, and the total yield is lowered. Results are similar with Fe$^{++}$ + Fe$^{++}$. The mitochondria were exposed to antimycin A 3 or 4 minutes before the addition of the iron. SN 5949, 3 $\mu$M, and 3 mM amytyl produced no inhibition of Fe$^{++}$ plus Fe$^{++}$-induced swelling.

**Phosphorylation Inhibitors** — DNP in concentrations as high as 0.3 mM had no detectable effect on the swelling or lipid peroxidation produced by Fe$^{++}$. Gramicidin at 1 $\mu$g per ml delayed...
lipoïd peroxide formation but caused swelling. Octyl guanidine at 100 to 200 $\mu$M is without effect against Fe$^{++}$. Oligomycin (1 to 5 $\mu$g per ml) did not consistently reproduce the inhibition reported earlier (1).

**Phosphate and Substrates**—Inorganic phosphate, 5 mM, with or without substrates, seems to prevent the action of Fe$^{++}$, yielding a mitochondrial swelling curve that is nearly identical with that for phosphate + substrate (β-hydroxybutyrate). Possibly there is a specific effect of phosphate on lipid peroxidation in mitochondria, but simple removal of Fe$^{++}$ as insoluble salts must be considered. β-Hydroxybutyrate, succinate, pyruvate, and oxaloacetate had essentially no effect at 2 mM concentration.

**Anti-oxidants**—α-Tocopherol at 10 $\mu$M produced nearly complete inhibition of lipid peroxidation and swelling caused by 50 $\mu$M Fe$^{++}$. Vitamin A, not ordinarily considered an antioxi
dant, showed similar effects at 10 to 100$\mu$M. Vitamin K$_1$ was completely without effect. Fifty $\mu$M Mn$^{++}$, a powerful inhibitor of lipid peroxidation (1, 19), produced nearly complete inhibition of lipid peroxidation and mitochondrial swelling induced by 50 $\mu$M Fe$^{++}$. Serotonin (5-hydroxytryptamine), 100 $\mu$M, also an inhibitor of lipid peroxidation (20), greatly slowed and reduced both lipid peroxide formation and swelling with Fe$^{++}$.

**Catalase, C-Factor, and BSA**—When crystalline catalase was added in concentrations between 50 and 115 $\mu$g per ml, there was usually some delay in the appearance of swelling with either Fe$^{++}$ or Fe$^{+++}$ to Fe$^{++}$ mixtures. The rate was not slowed very much. The appearance of lipid peroxide is delayed a corresponding amount and the total yield was somewhat lower.

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**Surface Active Agents**—Fairly rapid swelling or lysis is caused by 0.1 mM Na oleate, 0.1 mM Na linoleate, 0.3 mM Na lauryl sulfate, 0.05% saponin, and 1 $\mu$g gramicidin, but no TBA color material is formed during this swelling. When lipid peroxides have been previously formed through the action of Fe$^{++}$, these substances do not decrease the yield of color in the TBA method. However, when these substances are present and have produced swelling, the formation of lipid peroxides upon the subsequent addition of Fe$^{++}$ is reduced in the case of saponin and gramicidin, slight in the case of lauryl sulfate, and completely absent with the fatty acids. Undoubtedly the physical state of the mitochondrial lipids is altered drastically so that peroxidation may be inhibited or not lead to malonaldehyde. Formation of undissociated or insoluble iron salts may explain a number of these observations.

**Reduced Glutathione**—Because GSSG shortened the lag period with Fe$^{++}$, and because of similarities between the action of GSH and Fe$^{++}$ on mitochondria, a range of GSH concentrations in combination with iron was tested. Between 1 and 3 mM GSH usually does not have any marked effect on the lag period or rate of swelling with 50 $\mu$M Fe$^{++}$ or Fe$^{+++}$ plus Fe$^{++}$, but the end point or final $D_{50}$ plateau is the low one characteristic for GSSG + GSH instead of that with Fe$^{++}$ (Fig. 9). With these

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**Fig. 9.** The effect of GSH on mitochondrial swelling (A) and lipid peroxide formation (B) with 25 $\mu$M Fe$^{+++} + 25$ $\mu$M Fe$^{++}$.
low concentrations of GSH, the lipid peroxide continues to increase to values several times that seen with Fe++ alone. Higher concentrations of GSH (5 to 10 μM) markedly increase the lag period and slow the rate of swelling. During the long lag there is only a slight rise in lipid peroxide, but a rapid rise is associated with the rapid phase of swelling. When 10 μM GSH is added after the action of iron is complete (Fig. 9), there is an immediate resumption of lipid peroxide formation and the $D_{50}$ of the swelling curve falls until it reaches the plateau characteristic for GSSG + GSH. It appears that high GSH concentrations added initially (before swelling) have lag-producing effects which are not seen if swelling has already occurred. Combination of GSH with Fe+++ ion, as might be expected, gives results similar to GSSG + Fe++. Fe++ ion plays a direct role in the structural alteration of mitochondria, for the damage is directly related to the amount of Fe++ added. Lipid peroxidation is a likely mechanism (15, 19). The exact role of Fe++ in the mechanisms of such reactions may still be uncertain, but several very recent studies increase our knowledge in this area (23-20). In the mitochondrial system the reaction appears to stop when Fe++ becomes depleted. At present some stoichiometric reaction appears likely, but function as an oxidation-reduction catalyst may not be entirely ruled out.

Under certain conditions, lag periods are largely removed when Fe+++ ion is added with the Fe++. There may be an optimal ratio of 5 or 10 to 1 for Fe+++ to Fe++. This is consistent with the hypothesis of function as an oxidation-reduction couple and the earlier demonstration that GSSG and GSH are both required for GSH swelling (22). No requirement for dehydroascorbate has been demonstrated with ascorbate lysis, but this might be because monodehydroascorbate is the member of the oxidation-reduction couple. If a ratio of Fe+++ to Fe++ is important, it probably determines the length of the lag period rather than the rate of swelling. An alternative interpretation is that high concentrations of Fe+++ and a-ketoglutarate shorten the lag period by producing changes which must precede the action of Fe++ ion.

Peroxide formation in unsaturated lipids was demonstrated as a likely cause of Fe++-induced swelling by the close correlation between swelling or lysis and the thiobarbituric color reaction. However, a large production of lipid peroxide does not always precede swelling. Many times it occurs simultaneously. Therefore, the fundamental question is whether lipid peroxidation is the cause or the result of swelling. Our data suggest that it is the cause of a permeability or structural change. Possibly the lipid peroxidation and the structural change are simultaneous and synonymous. Addition of mitochondrial material that has undergone peroxidation to fresh mitochondria does not produce swelling. Most inhibitors prevent lipid peroxidation to exactly the same degree that they inhibit swelling. Any apparent discrepancies between lipid peroxide formation and swelling could be due to several factors: (a) Unsatisfactory methods for detecting early formation of lipid peroxides or early swelling changes. (b) Some swelling supported by endogenous substrate may occur during lag periods with Fe++. (c) Various reactions may destroy part of the lipid peroxides or the malonaldehyde being formed. Several workers (15, 19, 24, 27, 28) have observed lipid peroxide formation in mitochondria under various conditions. These changes are accompanied by alterations in electron transport and phosphorylation enzymes, which are known to represent organized systems in the mitochondrial membranes.

The amount of lipid peroxide formation required to cause permeability changes in the mitochondrial membrane is not large. With low concentrations of Fe++ there is a small amount of thiobarbituric color material formed in the first few minutes and then the reaction stops. Swelling begins at the same time, and the rate of swelling is related to the amount of lipid peroxide which was formed. Interestingly, the swelling ($D_{50}$ change) continues almost as a straight line until the final plateau is reached. No further lipid peroxidation occurs, yet swelling progresses over 30 to 60 minutes. This suggests that the swelling is the result of initial damage and permeability change when the lipid peroxide is formed. It would appear that all of
the mitochondria in the suspension may have their permeability altered to approximately the same degree during the initial action of the Fe++. The amounts of lipid peroxide formation that give moderate rates of swelling with Fe++ are of the same order as those seen with ascorbate and GSH for similar rates of swelling.

The TBA color method unfortunately does not measure lipid peroxides per se, but only those which decompose to release malonaldehyde. However, the correlation between the initiation and the rates of swelling and formation of TBA color material is excellent, although there is no evidence that malonaldehyde per se causes swelling. Making certain assumptions, it is possible to calculate the approximate number of lipid molecules affected for any given rate of swelling. If one assumes a yield of only one malonaldehyde per molecule of lipid, this means that one molecule of lipid in 600 has undergone peroxidation with the amounts of TBA color corresponding to slower rates of swelling, such as seen with 3 to 5 μM Fe++. It should be noted that 10 mmoles of Fe++ yield approximately 1 m mole of malonaldehyde.

The fatty acids known to be present in rat liver mitochondria and most likely to undergo peroxidation with the release of malonaldehyde are arachidonic and docosahexenoic, 22:6 (25, 29, 30). Other substances like sialic acid (31), heme compounds, and pyrimidines are present and can give colored compounds with TBA, but only when the test is run with oxidizing agents present (32).

Swelling per se does not result in lipid peroxide formation, for swelling induced by phosphate, fatty acid, detergents, and aging in KCl is not associated with lipid peroxide formation. A small amount of substrate-induced swelling is not necessary for Fe++ to penetrate and produce its action, as the effect of Fe++ is almost identical in fresh- and in substrate-depleted mitochondria.

Although catalease at higher concentrations produces some delaying effect on lipid peroxide appearance and on swelling, this does not prove that H2O2 is a precursor of lipid peroxide. Presumably catalease would remain outside mitochondrial membranes, so it is interesting that added H2O2 and H2O2 generated with glucose oxidase produces no effect like that of Fe++ (2, 15, 19). Catalase might act as a peroxidase and remove lipid peroxides until becoming inactivated (33). Very recently Neubert, Wojezak, and Lehninger (34) have reported that C-factor is GSH peroxidase and catalase.

The mechanism by which high concentrations of Fe++ inhibit or delay the appearance of TBA color material is unknown. It is noteworthy that similar delays are seen with high ascorbate, GSH, and some other reducing agents. Such substances could react with malonaldehyde, but it is more likely that they react with intermediates in the formation of lipid peroxides, as has been suggested for antioxidants (35). Whatever early steps of lipid peroxidation take place before such reactions, they do not suffice for permeability changes, since swelling is always delayed until TBA color (malonaldehyde) begins to appear.

Although cyanide and antymycin A show inhibitory effects on lipid peroxide formation with Fe++, it is unlikely that electron transport is involved in this process. Electron transport would be completely inhibited at one-tenth of the concentrations which affect lipid peroxidation. These inhibitors might exert their effect by (a) complexing trace metal ions, (b) reacting with nonheme iron in the mitochondrial structure (30), (c) reacting with heme proteins (33), (d) reacting with mitochondrial lipoproteins, (e) serving as anti-oxidants, or (f) keeping the mitochondria impermeable to ascorbate, GSSG + GSH, and Fe++. Antymycin A at 4 μM could not complex all of the iron added in these experiments.

The experiments described here demonstrate a clear similarity between Fe++- induced swelling of mitochondria and that seen with GSSG + GSH or ascorbate, although the details of their action are not identical. On certain points the effect of Fe++ can be studied more quantitatively. The peroxidation of lipids in the mitochondrial membranes occurs rapidly at temperatures below body temperature, especially when GSSG and certain other substances are present. Lipid peroxide changes may be the key to GSH swelling and the requirement of C-factor for contraction (1, 34). The possible relation of these changes to the control of mitochondrial swelling and contraction by electron transport and phosphorylation enzymes must be determined by further studies. Phosphate-induced electron transport supported swelling shows different characteristics, and no peroxidation of lipids has been detected under our conditions. However, Corwin (37) has recently shown that lipid peroxide formation may result from electron transport chain activity if the tissue is deficient in vitamin E. Thus, the possible role of lipid peroxide intermediates in electron transport, phosphorylation, and mitochondrial permeability control is a subject for investigation.

**SUMMARY**

1. In KCl medium, Fe++ ion induces a swelling and lysis of mitochondria which is quite different from electron transport-supported swelling. The light scattering of suspensions falls to a low level, approaching that seen with ascorbate and GSSG + GSH.

2. With 1 to 10 μM Fe++ there is no lag period before swelling. As the Fe++ concentration is increased to 50 μM, the beginning of swelling is delayed as long as 40 to 60 minutes. Fe++ ion, α-ketoglutarate greatly shorten this lag period.

3. Although cyanide and antymycin A partially inhibit Fe++-induced swelling, the concentrations required are higher than those necessary for respiratory chain inhibition. Uncouplers of oxidative phosphorylation, such as DNP, have no effect.

4. Lipid peroxide formation is very well correlated with swelling and lysis. Accelerators and inhibitors of this type of swelling affect lipid peroxide formation in an exactly parallel fashion.

5. Formation of lipid peroxide, possibly in some lipoproteins associated with electron transport and energy transfer or phosphorylation, causes first permeability increase and swelling. Extensive lipid peroxidation leads to lysis and disintegration.

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