Effect of Calcium Chelation on the Ion Content of Liver Mitochondria in Carbon Tetrachloride-poisoned Rats*

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SUMMARY

When toxic doses of CCl₄ were given to rats, there ensued marked accumulations of Ca⁺⁺ and inorganic orthophosphate by whole liver commencing 6 hours after treatment. When these livers were homogenized in 0.25 M sucrose, the Ca⁺⁺ and Pᵢ concentrations of the mitochondria subsequently harvested were elevated several fold, reflecting the higher levels of these ions in the whole tissue. The K⁺ concentration usually declined in the whole tissue and decreased in the mitochondria. In these mitochondria, respiratory rate and respiratory control were markedly depressed. If, on the other hand, the liver samples were homogenized in 0.25 M sucrose containing the Ca⁺⁺ chelator, ethylenediaminetetraacetate (or EGTA or DCTA), the mitochondria often contained much lower amounts of Ca⁺⁺ and Pᵢ, and the respiratory rate and respiratory control were closer to normal levels. Whether a chelator was used or not, the changes in Ca⁺⁺ and K⁺ concentration in the whole tissue or mitochondria did not appear to be closely related to each other. The effect of the chelators on ionic composition appeared to result from the prevention of accumulation of Ca⁺⁺ and Pᵢ by the mitochondria subsequent to the homogenization of the tissue. It is concluded that the major portion of the changes in content of Ca⁺⁺ of isolated liver mitochondria following CCl₄ poisoning which have been reported by others occurred during the isolation of these organelles and must be considered to be artifacts of the isolation procedure. The residual chelator-resistant changes in respiratory function and ionic composition may reflect actual qualitative changes in the mitochondria extant in these organelles in vivo.

It has been reported that among the morphological and biochemical changes in liver brought about by feeding CCl₄ are striking alterations in ionic composition of the whole liver and mitochondria (1–5). Thiers, Reynolds, and Vallee (2) showed that mitochondria isolated from livers of CCl₄-treated animals contained larger amounts of Ca⁺⁺ and lower amounts of K⁺ than did the control mitochondria. In addition, the respiratory rates and phosphorylative capacity of these mitochondria decreased as a function of the Ca⁺⁺:K⁺ ratio (3). The data on ion movement during CCl₄ poisoning have been used to support the concept that the changes observed in mitochondrial transport of these ions in vitro also occur in vivo, perhaps as part of a physiological regulatory process (6). In support of this concept are the extensive studies by Reynolds (4, 7, 8) on the morphology, cytochemistry, and ultrastructure of liver parenchymal cells, in which he concluded that in the latter stages of Ca⁺⁺ accumulation (16 to 24 hours following administration of CCl₄ the mitochondria contained large amounts of Ca⁺⁺ which were actively accumulated.

Bearing on these findings is the recent report that kidney mitochondria obtained from tissue which was calcified as a consequence of parathyroid hormone treatment also contained high levels of Ca⁺⁺ as well as Pᵢ, and exhibited inhibited respiration and phosphorylation, and impaired ion-translocating properties (9, 10). By homogenizing the kidney tissue in the presence of ethylenediaminetetraacetic acid—a technique introduced by Slater and Cleland (11, 12) to prevent Ca⁺⁺ translocation during the isolation of sarcosomes—it was concluded that a major portion of this Ca⁺⁺ was not present within the mitochondrion in situ but rather was sequestered during the isolation of the mitochondria. Because the total Ca⁺⁺ content of the whole liver increased after CCl₄ poisoning (1, 2), and because the behavior of the mitochondria subsequently harvested resembled that of kidney mitochondria following parathyroid hormone administration (9, 10), the possibility was raised that under the former conditions some Ca⁺⁺—perhaps a major portion—was present in the mitochondrion as an artifact of the preparatory procedure. Indeed Rees, Sinha, and Spector (5) had suggested that certain abnormalities in mitochondrial enzyme activities were artifacts arising from exposure of the mitochondria to high concentrations of calcium during isolation of the particles.

The present study was undertaken to evaluate this possibility and to determine quantitatively, if possible, the degree of internal redistribution of Ca⁺⁺ which might have occurred. The data presented below indicate that a major fraction of the Ca⁺⁺ found

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**TABLE I**

**Ion content of whole liver from rats killed at various times after receiving CCl₄**

<table>
<thead>
<tr>
<th>Hours after CCl₄</th>
<th>Ion content (mean ± S.D.)</th>
<th>μmoles/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ca⁺⁺</td>
<td>P₁</td>
</tr>
<tr>
<td>Control</td>
<td>10 ± 2</td>
<td>43 ± 12</td>
</tr>
<tr>
<td>6</td>
<td>18 ± 2*</td>
<td>57 ± 5*</td>
</tr>
<tr>
<td>12</td>
<td>24 ± 18*</td>
<td>51 ± 15*</td>
</tr>
<tr>
<td>24</td>
<td>130 ± 43*</td>
<td>99 ± 9*</td>
</tr>
<tr>
<td>37</td>
<td>237 ± 43*</td>
<td>172 ± 34*</td>
</tr>
<tr>
<td>44</td>
<td>115 ± 47*</td>
<td>90 ± 18*</td>
</tr>
</tbody>
</table>

a p < 0.01.
a p < 0.05.

in the liver mitochondria from CCl₄-fed rats which were isolated from sucrose media that did not contain an adequate level of EDTA or other Ca⁺⁺ chelator was sequestered post mortem and should not be attributed to an action of CCl₄ on transport in vivo. Likewise, some loss in respiratory function appeared to be secondary to this movement of Ca⁺⁺. The data also show that CCl₄ treatment decreased the content of K⁺ in the whole liver and mitochondria and that this change was not directly related to the accumulation of Ca⁺⁺.

**METHODS**

White, male rats of the A. R. Schmidt or Holtzman strains, usually weighing between 200 and 300 g, were treated orally with a 1:1 mixture of CCl₄ and mineral oil at a level of either 0.5 or 0.25 ml/100 g of body weight as specified in the text below. In early studies the control animals received an equal volume of mineral oil only, but, since these animals were indistinguishable from untreated ones, in the later studies this practice was discontinued. The animals were killed by decapitation, and their livers (unperfused) were chilled and rinsed in cold sucrose. Liver samples were homogenized in 4 parts (w/v) of ice-cold 0.25 M sucrose with no other addition, or containing specified concentrations of one of the following chelators: EDTA, EGTA, or DCTA. Unless otherwise specified, these chelators were adjusted to pH 7.4 and were present as sodium salts. No effort was made to decrease the concentration of the sucrose in order to maintain toxicity when it contained chelator, since in preliminary studies this adjustment had no appreciable effect on the separation or function of the isolated mitochondria. Debris and nuclei were removed by centrifugation for 10 min at 1,000 g. The mitochondrial pellet was obtained after 10 min at 6,400 g. The mitochondria were twice washed by resuspending them in one-fourth the original volume of 0.25 M sucrose (no chelator) and centrifuging for 10 min at 6,400 g. The final pellet was resuspended in 0.25 M sucrose. When needed, the microsomal and cell supernatant fractions were obtained by centrifuging the supernatant fluid above the original mitochondrial pellet at 105,000 g for 2 hours. All operations were conducted between 0⁰ and 3⁰.

EDTA and EGTA were "reagent grade" products of Sigma and J. T. Baker, respectively. DCTA, "practical grade," was obtained from the latter firm and was purified by crystallizing it as the free acid.

Respiration and respiratory control were measured with succinate and ADP with an oscillating platinum electrode as described earlier (9). Metals were assayed by atomic absorption spectrometry in trichloracetic acid extracts of whole tissue or subcellular fractions. P₁ was determined by the method of Chen, Toribara, and Warner (13), and protein by the method of Lowry et al. (14).

**RESULTS**

Patterns of Ionic Change in Liver and Liver Mitochondria Produced by CCl₄ Poisoning, and Effect of Chelating Agents—The results of CCl₄ poisoning on the ion content of whole liver in one specific study are listed in Table I. Major increases in Ca⁺⁺, P₁, and Na⁺ content and a decrease in K⁺ content occurred by the 6th hour after treatment. Ca⁺⁺ and P₁ concentrations continued to increase and were, respectively, 24- and 4-fold higher than the control level 37 hours after treatment, after which they decreased and appeared to return toward the control values. The K⁺ concentration decreased one-third by the 12th hour and remained depressed at that level throughout the remainder of the study. The Na⁺ concentration reached a maximum by the 24th hour and then decreased, but was still more than twice the control concentration when the study was terminated. The Mg⁺⁺ concentration increased to a small but significant extent in the 12- to 37-hour study periods. There did not seem to be any consistent quantitative relationship between K⁺ loss and Na⁺, Ca⁺⁺, or P₁ gain, or between the gain in Na⁺ and Ca⁺⁺. On the other hand, P₁ and Ca⁺⁺ were closely correlated, with about 0.5 mole of P₁ gained per mole of Ca⁺⁺.

The concentrations of these ions in the mitochondria isolated from these liver specimens are listed in Table II. When homogenization was conducted in 0.25 M sucrose only, that is, in the absence of a chelator such as EDTA, the changes in ionic composition following CCl₄ usually reflected the changes which occurred in the whole tissue. For example, Ca⁺⁺ and P₁ concentrations increased to maximum values at the 37th hour, at which time the concentrations of these ions in the whole liver were also maximum. K⁺ content decreased by the 6th hour, and was depressed throughout the remainder of the study. Mg⁺⁺ content changed little, if at all. One interesting discrepancy between the ionic composition of the mitochondria and that of whole tissue was noted in the case of Na⁺ concentration. Whereas the mitochondrial content of Na⁺ increased in the 6-, 12-, and 24-hour periods to a degree which closely paralleled a
The experimental design and the manner of statistical analysis for changes in ion content from the control values were those described in Table I. Evaluation of the effect of EDTA on the ion content of each sample was performed by Student's t test for correlated samples.

Quite different data were obtained when samples of these same livers were homogenized in 0.25 M sucrose containing 0.01 M EDTA. Table II (columns headed "+EDTA") shows that this treatment yielded mitochondria from control tissue which contained slightly less Ca++, Pi, and Mg++ and the same amount of K+. The large increase in Na+ was due to the use of the sodium salt of EDTA (see below). In the case of the experimental mitochondria, however, in every instance these organelles contained appreciably less Ca++ and P1 than the mitochondria harvested without EDTA although the concentrations of these ions if the NH₄⁺ salt was used.

Two additional studies performed in the manner of those described in Tables I and II fully confirmed the effect of chelator on yielding mitochondria with low levels of calcium and phosphate. These experiments also confirmed that there was no obvious relationship between K+ and Ca++ content of the mitochondria.

In another experiment, it was found that the calcium contents of the mitochondria harvested in the presence of the Na+, K+, or NH₄⁺ salts of EDTA were identical, showing that the lowering of calcium content was not related to the cationic form of the chelator. On the other hand, the isolated mitochondria contained an elevated level of either K+ or Na+, depending upon which form of the EDTA was used, and had a normal level of these ions if the NH₄⁺ salt was used.

The liver mitochondria from the CCl₄-poisoned rats also displayed inhibited respiration and loss of respiratory control (Table III). When chelator was omitted during homogenization, the mitochondria from CCl₄-fed animals contained several times greater concentrations of Ca++ and P1 than the mitochondria prepared in the presence of EDTA. The K+ concentration was generally unaffected by treatment with chelator. The increase in Na+ content produced by the treatment with CCl₄ persisted when EDTA was used.

<table>
<thead>
<tr>
<th>Hours after CCl₄</th>
<th>Ion content</th>
<th>Respiration</th>
<th>Respiratory control ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Ca++</td>
<td>P1</td>
<td>K+</td>
</tr>
<tr>
<td>-EDTA</td>
<td>29 ± 8</td>
<td>24 ± 11a</td>
<td>17 ± 4</td>
</tr>
<tr>
<td>+EDTA</td>
<td>42 ± 12b</td>
<td>36 ± 3</td>
<td>29 ± 2b,a</td>
</tr>
<tr>
<td>6</td>
<td>53 ± 8</td>
<td>42 ± 12b</td>
<td>36 ± 3</td>
</tr>
<tr>
<td>12</td>
<td>79 ± 10b</td>
<td>42 ± 8b,a</td>
<td>32 ± 4</td>
</tr>
<tr>
<td>24</td>
<td>145 ± 25b</td>
<td>75 ± 20b</td>
<td>65 ± 8b</td>
</tr>
<tr>
<td>37</td>
<td>227 ± 70b</td>
<td>46 ± 19a,b</td>
<td>148 ± 30b</td>
</tr>
<tr>
<td>44</td>
<td>232 ± 42b</td>
<td>42 ± 6b</td>
<td>92 ± 20b</td>
</tr>
<tr>
<td>+EDTA</td>
<td>42 ± 6b</td>
<td>92 ± 20b</td>
<td>21 ± 17c,a</td>
</tr>
</tbody>
</table>

*Significance in change in ion content due to EDTA: p < 0.01.
*Significance in change in ion content from control levels: p < 0.05.
*Significance in change in ion content from control values: p < 0.05.
of the CCl₄ mitochondria, it should be noted that EDTA and EGTA by themselves had a deleterious effect on the respiratory rate and respiratory control of the normal mitochondria. DCTA, on the other hand, stimulated respiration of the control mitochondria without lowering respiratory control. Since Chance had shown that Mg⁺⁺ could stimulate respiration of EDTA-treated mitochondria (15), this ion, alone or with ATP and succinate, was included together with the chelator during homogenization. These combinations had no effect over that of the chelator alone.

Studies on Mode of Action of Chelating Agents as Related to Ca⁺⁺ and P₄ Content of Mitochondria—In view of the known chelating properties of EDTA, we hypothesized that the chelator functioned in one of three ways: (a) by altering the characteristics of the mitochondrion so that Ca⁺⁺-containing mitochondria were either destroyed or else sedimented in a different fraction during differential centrifugation, e.g. debris or microsomes; (b) by removing from the mitochondrion Ca⁺⁺ which had been deposited in situ; or (c) by preventing extramitochondrial Ca⁺⁺ from entering the mitochondrion following homogenization of the tissue. Each of these possibilities was examined, with the evidence favoring the last mode of action.

It was determined first whether the Ca⁺⁺ in the liver mitochondria from the CCl₄-fed animals harvested without chelator was confined to a small portion of the total mitochondrial fraction or was instead distributed in a general fashion. Fig. 1 portrays the results from one study, in which these mitochondria were partially sedimented through a linear sucrose gradient (0.25 M to 1.75 M). The mitochondria (defined by protein analysis) migrated as a broad band which spread over a large portion of the gradient, indicating by this behavior their heterogeneity. Ca⁺⁺ was distributed in a similar manner, suggesting that it was associated with the majority of the mitochondria. Hence, the reduction in Ca⁺⁺ content produced by chelator, often to a small fraction of that when chelator was not used, could not be attributed to loss or destruction of a specific Ca⁺⁺-containing fraction.

In order to determine the effect of chelator on the distribution of Ca⁺⁺ within the various sedimentable fractions of the cell, livers from normal and CCl₄-fed rats were homogenized and separated by differential centrifugation as described above. Table IV shows that, in the normal liver homogenized without EDTA, Ca⁺⁺ was present in greatest amount in the debris plus nuclear fraction, followed by the supernatant, microsomal, mitochondrial, and mitochondrial "wash" fractions, in that order. Inclusion of EDTA in the homogenizing medium had no effect on the Ca⁺⁺ content of the debris plus nuclear fraction, but this ion was decreased in the mitochondrial and microsomal fractions and increased in the cell supernatant fraction. CCl₄ treatment led to major increases in Ca⁺⁺ content in all fractions, with the debris plus nuclear fraction containing over two-thirds and the mitochondrial fraction containing about 10% of the total amount. When EDTA was present during the homogenization, the Ca⁺⁺ content of all of the particulate fractions was drastically lowered, and over 75% of the Ca⁺⁺ of the whole liver was found in the supernatant fraction. The data in this and the preceding paragraph, taken together, make it unlikely that the chelator functioned in accordance with Possibility a.

Data which indicate that under our experimental conditions EDTA was unable to remove the major content of Ca⁺⁺ contained in mitochondria (Possibility b) are shown in Fig. 2. Livers from control and CCl₄-fed rats were homogenized in 0.25 M sucrose. After the debris plus nuclear fraction was removed, the mitochondrial pellet was obtained and without

![Figure 1](http://www.jbc.org/)
FIG. 2. The effect of suspension in EDTA on the Ca++ content of liver mitochondria from control and CCl4-fed rats. The animals receiving \( \text{CCl}_4 \) (0.25 ml of \( \text{CCl}_4 \) in mineral oil per 100 g of body weight) were killed 18 hours after treatment. Other details are listed in the text.

Additional washing was resuspended in 0.25 M sucrose alone or in sucrose containing 0.01 and 0.03 M EDTA in a final volume equal to that of the original homogenate. These suspensions were shaken gently and continuously at 0\(^\circ\) and were sampled over a 2-hour period. Experimental livers homogenized without chelator yielded mitochondria with a 6-fold increase in Ca++ concentration compared to the control tissue. There was an initial, rapid loss of about 15% of the Ca++ from the experimental mitochondria, followed by a gradual loss at an over-all rate which was little influenced by the concentration or presence of EDTA. At the end of the study, for example, the mitochondria from the CCl4-treated animals suspended in EDTA contained one-third less Ca++ than at the start of the study, but this level was somewhat greater than in the mitochondria suspended in sucrose alone. Moreover, there was no discernible effect of the chelator on the levels of Ca++ in the control mitochondria. These data seem to rule out Possibility b.

The data suggest, by elimination, that EDTA functioned according to Possibility c, by preventing the uptake of extramitochondrial Ca++ by the mitochondrion subsequent to homogenization of the tissue. That normal mitochondria freed from the liver cell could sequester Ca++ under the conditions of homogenization (0–3\(^\circ\), no added substrate) is indicated by the data shown in Fig. 3. Low levels of CaCl\(_2\) (0.5 to 2.0 mM) were added to 0.25 M sucrose, and the liver was homogenized. The initial mitochondrial pellet was resuspended and washed in 0.25 M sucrose only, so that the only intentional exposure of the mitochondria to Ca++ was during the initial part of the isolation procedure. As indicated in the figure, the concentration of Ca++ in the isolated mitochondria was a direct function of the concentration of the Ca++ in the homogenizing medium. The concentration to Ca++ appeared to be \( P_{1} \), since its accumulation by the mitochondrion was proportional to that of Ca++. The concentration of the other ions measured did not change appreciably.

Additional evidence supporting the concept that the chelator functioned by complexation of Ca++ and preventing its entry and that of \( P_{1} \) into the mitochondrion during homogenization is shown in Fig. 4. In these experiments, livers from normal and

![Fig. 3. The accumulation in vitro of Ca++ and \( P_{1} \) and the movement of other ions in liver mitochondria during the isolation of the mitochondria. The livers from six normal rats were pooled and homogenized in 0.25 M sucrose alone or containing either 0.5, 1.0, or 2.0 mM CaCl\(_2\), and mitochondria were prepared as described in the text.](http://www.jbc.org/)
CCl₄ rate were homogenized in different concentrations of EDTA or DCTA. At concentrations of 5.0 or 10 mM, both chelators were maximally effective in yielding mitochondria with low levels of Ca++ and P. At 2.5 mM, however, EDTA was more effective than DCTA. This result seemed to be related to the total amount of Ca++ present in the tissue samples, which was 89 μmoles in the EDTA study and 118 μmoles when DCTA was used. Since, at a concentration of chelator of 2.5 mM, a total of 80 μmoles of chelator were added, it appeared that the effective concentration of the chelator was determined by the amount of complex-forming calcium in the tissue sample.

**DISCUSSION**

The mechanism of liver damage by CCl₄ has been the subject of extensive investigation in a number of laboratories. Several hypotheses have been advanced to explain the action of the drug, including (a) direct attack upon the lipid membranes of the cell (3), (b) conversion to a toxic split product and consequent damage to membrane components (16, 17), and (c) damage to cellular structures as a consequence of intracellular shifts of ions, notably calcium (1). As yet no single mechanism of action of CCl₄ has been accepted to explain the assortment of biochemical and morphological changes which are observed (1). Following the isolation of liver mitochondria from CCl₄-poisoned rats and the demonstration that these organelles contained a high content of Ca++, a low content of K⁺, and a loss of respiratory function (2, 3), Reynolds conducted a careful study of the morphological and biochemical changes in liver tissue (4, 7, 8). He reported that there was rapid accumulation and release of Ca++ by the liver cell within a 2-hour period after poisoning, followed by a sustained accumulation of Ca++ beginning several hours later. The earlier rise in liver Ca++ content has been confirmed by Smuckler (14), but not by Judah, Ahmed, and McLean (1). The accretion of Ca++ during the later time periods has been related to the appearance of cation-binding sites (8) and deposition of electron-dense material, presumably calcium phosphate. This same material has been assumed to be accreted by the mitochondria during the earlier periods (4).

These data on the mitochondrial accumulation of Ca++ following CCl₄ poisoning have been extended to the general question of ion movement by cells, and it has been postulated that mitochondria play a physiological role in the cellular translocation of ions (6). As mentioned above, based on our previous experience with the post-mortem accumulation of Ca++ by kidney mitochondria following treatment of the intact animal with parathyroid hormone (10), the quantitative aspects of liver mitochondrial Ca++ content seemed questionable. From the viewpoint of interpreting the results as they relate to the primary actions of CCl₄ on the liver and to the somewhat more general question of ion translocation, it seemed worthwhile to determine whether or not any part of the reported increment in Ca++ content of the liver mitochondria occurred as an artifact of isolation of the mitochondria. The present report shows that in the cases of Ca++ and P, the final concentrations of these ions in the isolated mitochondria may bear little relationship to the concentrations as they exist in vivo.

The changes in Ca++ content of whole liver following CCl₄ treatment were qualitatively similar to those reported by Judah, Ahmed, and McLean (1) and Reynolds, Thiers, and Vallee (2, 3). Although, in the original studies of the latter workers (3), an increase in P did not occur, Reynolds (8) subsequently observed a large increase in this ion, which the present study confirms. The K⁺ content of whole liver did not decrease in the studies of Reynolds, Thiers, and Vallee (2), and we have confirmed this observation in some studies, but in other experiments we have noted a large loss of K⁺ (Tables I and II).

When the mitochondria were isolated by homogenizing the liver in 0.25 M sucrose, the increased Ca++ and decreased K⁺ contents and depressed respiratory rates of the experimental mitochondria were similar to the changes reported by Reynolds, Thiers, and Vallee (2, 3). When, on the other hand, an adequate level of EDTA or other calcium-chelating agent was present during homogenization, the Ca++ and P contents were always markedly lower and respiratory function was improved. Moreover, since the K⁺ content of the mitochondria harvested in the presence or absence of chelator was usually unchanged, it is clear that no obvious inverse relationship exists between Ca++ and K⁺ content in the mitochondria.

Based on the Ca++-lowering action of EDTA when it was included in the medium during the preparation of heart sarcomeres, Slater and Cleland (11) concluded that the high calcium content and instability of sarcomeres isolated in the absence of the chelator were artifactual. Before a similar conclusion could be drawn in the case of the elevated Ca++ content of liver mitochondria after CCl₄ poisoning, it was necessary to determine whether or not the mode of action of the chelator in the present study was equivalent to that in normal heart tissue. This determination was particularly required because of the morphological and metabolic changes in the mitochondrion caused by CCl₄ (1, 4, 7, 8, 16, 17). That the chelator lowered the Ca++ content of isolated mitochondria through complexation with extramitochondrial Ca++ and preventing its accumulation by the mitochondrion during the isolation procedure—the action assumed to occur by Slater and Cleland (11)—is supported by the following evidence.

1. The liver mitochondria were capable of rapid accumulation of Ca++ during the fractionation procedure when this ion was present in the medium in which the whole liver was homogenized. This confirms the similar observation of Reynolds, Thiers, and Vallee (3).

2. Three different chelators and different cationic forms of one chelator functioned in a qualitatively similar manner, suggesting that the factor in common was the Ca++-binding property of these compounds.

3. The concentration of chelator effective in yielding low Ca++ mitochondria was related to the total amount of Ca++ in the whole tissue.

4. The chelator had relatively little influence on the removal of predeposited Ca++ from normal mitochondria or mitochondria from CCl₄-fed animals, even during extended periods of exposure.

5. Since Ca++ was distributed in a general fashion throughout the mitochondrial fraction, destruction by chelator of a possible mitochondrial fraction, small in amount but heavily loaded with Ca++, could not account for the lowering of the Ca++ content of the mitochondria.

6. Inclusion of chelator during homogenization caused the majority of the Ca++ of the tissue to appear in the supernatant fraction, rather than in the debris and mitochondria, as occurred when chelator was omitted. This finding would rule out the possibility that the chelator slightly altered the sedimenting properties of the mitochondrion so that the calcium-containing...
particles would sediment in the fraction just ahead of or just after the mitochondria (i.e. debris or microsomes).

It is apparent, therefore, that the earlier data (2, 3) on the large scale accumulation of Ca++ and the apparent inverse stoichiometric relationship between Ca++ gain and K+ loss in the mitochondria from CCl4-poisoned rats are misleading, since the reported changes in Ca++ content most likely occurred in large measure in vitro, not in vivo as assumed. On the other hand, since the experimental mitochondria contained higher than control levels of Ca++ and Pi and lower levels of K+ and respiratory function even when harvested in the presence of chelator, our results resemble the earlier data (2, 3) qualitatively, if not quantitatively. Whether the present data when chelator was used reflect the ionic composition of the mitochondria as they exist in vivo, or instead mean that the chelator as used was only partially effective in preventing artifactual translocation of Ca++ and Pi, must yet be determined.

REFERENCES

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