HEPATIC ORGANELLE INTERACTION

IV. Mechanism of Succinate Enhancement of Formaldehyde Accumulation from Endoplasmic Reticulum N-Dealkylations

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ABSTRACT

Further evidence for organelle interaction during drug metabolism by the liver is presented. The apparent stimulation by succinate of formaldehyde accumulation in the medium, which was reported to occur with liver slices and homogenates as well as with mitochondria plus microsomes, has been shown to be the result of succinate inhibition of mitochondrial aldehyde dehydrogenase. The mechanism of succinate inhibition is shown to be by reverse electron transport, and an increase in the NADH to NAD+ ratio in the mitochondria; the aldehyde dehydrogenase requires the oxidized form of the pyridine nucleotide as its cofactor.

Studies on in vitro N-demethylation by liver microsomes and endoplasmic reticulum segments which cosediment with the mitochondria indicate that formaldehyde produced by the mixed function oxidase is handled differently from formaldehyde added to the medium. The latter is mainly retained in the medium containing 5 mM semicarbazide, while the generated formaldehyde is more than 50% consumed by the mitochondria.

Electron microscopy has indicated that the microsomes and the endoplasmic reticulum fragments have a tendency to align themselves close to the mitochondria when present in the same medium. Consequently, it is possible that formaldehyde released to the medium adjacent to the mitochondria, as by N-demethylation, would be exposed to semicarbazide for shorter periods than that added directly to the medium. In agreement with this suggestion, complexing of formaldehyde with semicarbazide was observed spectroscopically not to be an extremely rapid reaction even at 37°C. This is believed to be the reason for the greater extent of consumption of formaldehyde generated by the endoplasmic reticulum.

In previous papers in this series (4, 6, 7, 20) we have accumulated evidence for an interaction between two hepatocellular organelles, the endoplasmic reticulum and the mitochondria. Evidence for an interaction during drug metabolism was first shown in intact cells of liver slices by biochemical (6, 7) and morphological (20) examination. Functional interaction was suggested as a coopera-
tive effect between the mitochondria and endoplasmic reticulum in the metabolism of drugs by the latter organelle, and was manifest by an apparent increase in the rate of N-dealkylation when both organelles were combined in the presence of succinate, and by an apparent decrease in N-dealkylation in the presence of oxaloacetate (4, 20).

This communication contains data amending our earlier interpretation that the increased formaldehyde levels obtained in the presence of sodium succinate was an indication of a synergistic action of mitochondria on microsomal N-dealkylation. The enhanced amount of formaldehyde is shown to be due to an inhibition of mitochondrial metabolism of the aldehyde by succinate. The mechanism of the succinate inhibition is shown to be due to an alteration of the intramitochondrial NAD⁺ level. This process, which can occur in intact cells (4), is discussed as a potential source of cell toxicity.

MATERIALS AND METHODS
Male Sprague-Dawley rats (250-350 g, Charles River, Breeding Laboratories, Wilmington, Mass.) were maintained on Purina lab chow (Ralston Purina Co., St. Louis, Mo.) ad libitum. The rats were killed by decapitation, and the livers were removed, chilled quickly in ice cold 0.9% NaCl, and perfused with fresh ice-cold 0.9% NaCl through the hepatic veins to remove blood. After very gentle homogenization (one pass with a Potter-Elvehjem glass-Teflon homogenizer) in 0.25 M sucrose, 10 mM Tris HCl, pH 7.5, the mitochondrial fraction was isolated essentially by the method of Johnson and Lardy (13). The homogenate was centrifuged at 600 g for 10 min in a Sorvall refrigerated centrifuge (Du Pont Instruments, Sorvall Operations, Newtown, Conn.) at 4°C. The pellet was gently resuspended in fresh buffered sucrose to the previous volume, using a glass rod with rubber policeman. After another centrifugation at 600 g for 10 min, the supernates were combined and centrifuged at 6,500 g for 15 min to sediment mitochondria. The pellet was resuspended to half the original volume in buffered sucrose and resedimented at 6,500 g for 10 min.

This washed mitochondrial pellet was used in subsequent studies. The supernate of the 15-min centrifugation was centrifuged at 12,000 g for 15 min to remove broken and light mitochondria before preparation of the microsomes; after removal of the pellet, dry CaCl₂ was added to the supernate to a final concentration of 8 mM, and the microsomes were sedimented by centrifugation for 15 min at 18,000 g (5). The microsomal pellet was washed once in an equal vol of 0.15 M KCl before use.

Spectra were recorded on an Aminco DW2 spectrophotometer (American Instrument Co., Travenol Laboratories, Inc., Silver Spring, Md.) at room temperature (22°C). Endoplasmic reticulum fragments in the mitochondrial fraction were detected by electron microscopy (microsomal vesicles were rarely seen) and were quantitated by measuring the cytochrome P-450 content and glucose 6-phosphatase activity relative to isolated microsomes. For P-450 content, the mitochondria were suspended in the buffered sucrose to 3 mg protein/ml, and 0.5 mM KCN was added. Carbon monoxide and dithionite were added to the sample cuvette. The cyanide prevented spectral overlap of the 450-nm peak of P-450 with cytochrome oxidase in the reference cuvette. With this measurement the endoplasmic reticulum content in the washed mitochondrial fraction was found to range from 5 to 15% on a protein basis. Glucose 6-phosphatase measurements (9) gave the same results when run in parallel in three experiments, so for most determinations the easier spectral measurements were performed.

Aminopyrine N-dealkylations were assayed either in 50 mM Tris, pH 7.5, containing 5 mM MgCl₂, or in "perfusion fluid" (7), an iso-osmotic medium containing 125 mM NaCl, 6 mM KCl, 12 mM Na₂HPO₄, 3 mM NaH₂PO₄, and 5 mM MgCl₂, adjusted to pH 7.4 with 1 M HCl. Drug substrates were added at 8 mM concentration, and the reactions were started by adding 1 mM NADPH, and were run for 6 or 8 min at 37°C as specified. Semicarbazide, when present, was buffered to pH 7.5 before addition, and unless specified was 5 mM. Sodium succinate, when present, was added to a final concentration of 15 mM; 15 mM was found to be slightly inhibitory, and 5 mM was suboptimal for alterations of aldehyde levels. Total incubation vol was 3 ml. N-dealkylation was measured by the formaldehyde formed using the Nash procedure (21), after stopping the reaction with one-third vol of 15% trichloroacetic acid. Similar methodology was used to measure formaldehyde disappearance.

Intramitochondrial pyridine nucleotides were determined by the method of Estabrook and Maitra (10). Depletion of mitochondrial NAD⁺ by swelling in phosphate and reincorporation of NAD⁺ into mitochondria was performed by the method of Hunter et al. (12). Protein was measured by a biuret procedure.

RESULTS
In the third paper of this series (20), increasing amounts of washed mitochondria added to a microsome-containing medium caused increased aminopyrine N-dealkylation above the level obtained with microsomes alone, with maximal activity at 4-mg mitochondrial protein per milligram added microsomes; this enhancement was obtained only in the presence of succinate. In those studies, it was assumed that the washed mitochondrial fraction did not contain any endoplasmic reticulum fragments because there was no observed aminopyrine N-demethylation activity in that frac-
Figure 1. Electron micrographs of isolated mitochondria and their associated rough endoplasmic reticulum fragments. Mitochondria were prepared as described in the Materials and Methods section, and were suspended in a perfusion medium at 0°C to 5 mg/ml. 2 vol of chilled Karnovsky's reagent (1:1 paraformaldehyde glutaraldehyde) were added, and the mixture was refrigerated overnight. The mitochondria were then washed two times as described earlier. Two different preparations are shown. x 50,000.
tion. However, in this study, electron microscope examination of the mitochondrial fraction (6,500 g pellet) has revealed the presence of segments of rough endoplasmic reticulum (Fig. 1). The biochemical presence of these fragments was further confirmed and quantitated (see Materials and Methods) at 5–15% of the mitochondrial protein. Consequently, the effect of addition of mitochondria to microsomes on aminopyrine N-dealkylation was reexamined, correcting for the additional endoplasmic reticulum (microsomal) protein in the mitochondrial fraction, and keeping the total content of microsomal protein as close to 1 mg/ml as possible (Fig. 2). When this was done, it was found that in the absence of succinate the amount of formaldehyde accumulating actually decreased with increasing mitochondrial concentration, instead of remaining constant. At 4 mg mitochondria per milligram of endoplasmic reticulum protein, formaldehyde accumulation was only 25% of that in the absence of mitochondria. The addition of sodium succinate partially reversed the mitochondrial inhibition of formaldehyde accumulation. Thus, it appears that, in the presence of excess mitochondria, either less formaldehyde is formed by the microsomes or the semicarbazide (5 mM) is unable to completely complex that which is formed, resulting in either evaporation or further degradation of the aldehyde; in liver homogenates, where the ratio of mitochondria to endoplasmic reticulum protein is 1:3:1, this amount (5 mM) of semicarbazide was found to prevent disappearance to exogenous formaldehyde (20). However, in the experiment described above, much higher amounts of mitochondria were used. Since mitochondria are known to be able to oxidize aldehydes such as formaldehyde (26), the possibility was investigated that mitochondrial consumption of formaldehyde was the key to the succinate effect. The questions which had to be answered were: (a) does semicarbazide completely complex exogenous formaldehyde, and, if so, (b) is microsome-produced formaldehyde handled differently from exogenously added formaldehyde, and (c) how does succinate exert its action?

The $K_m$ for formaldehyde consumption, based upon oxygen uptake, by rat liver mitochondria has been reported as 0.8 mM (25). In the presence of 0.1 mM formaldehyde the initial rate of disappearance is about 2–5 nmol/min/mg mitochondria (Fig. 3) at 37°C. In the absence of additions, about 50% of a 0.1 mM solution of formaldehyde disappeared during the incubation (Fig. 3), as compared with only 13% in the presence of semicarbazide, up to 20% disappeared in the presence of succinate, and 6% disappeared in the presence of both chemicals. Little difference in the extent of formaldehyde consumption was observed when the formaldehyde was added dropwise over the assay period or added all at once before or after the addition of mitochondria. From these results it would appear that semicarbazide complexing of formaldehyde is effective but not complete, i.e., is reversible, and that some uncomplexed formaldehyde is consumed by the mitochondria.

It was found that formaldehyde does not have an absorption spectrum in the ultraviolet region above 200 nm, while semicarbazide has an absorption peak at 204 nm. When combined, however, a large peak forms at 235 nm, while the smaller 204-nm semicarbazide peak disappears. When 0.1 mM formaldehyde was added to the organelle-free medium containing 5 mM semicarbazide, the halftime for the complex formation (absissa intercept of double reciprocal plot) was found to be 13 min at 23°C. The magnitude of absorption at 10 min was about the same (Fig. 4) when the formal-
Dehyde was added in five equal aliquots at 1-min intervals to equal 0.1 mM, or was added all at once, in agreement with the above similarity in formaldehyde consumption. Doubling the semicarbazide concentration to 10 mM did not affect appreciably the extent of consumption. Comparison of results in Fig. 3 and Fig. 2 indicates that formaldehyde generated by aminopyrine demethylation is handled differently from exogenously administered formaldehyde. In Fig. 2, for example, the amount of formaldehyde produced in 6 min by the microsomes alone approximated about half the amount of exogenous formaldehyde added in Fig. 3 with mitochondria alone (5 mg/ml). Yet the mitochondria alone only consumed 13% of the added formaldehyde in the presence of semicarbazide, as compared with 75% of the generated formaldehyde (Fig. 2) in the presence of comparable amounts of mitochondria. In the presence of both semicarbazide and succinate, mitochondria only consumed about 6% of the exogenous formaldehyde (Fig. 3), but still consumed about 40% (4:1 ratio) of the generated formaldehyde (Fig. 2). Clearly then, generated formaldehyde is handled differently from added formaldehyde, or the endoplasmic reticulum fragments associated with the mitochondria do not N-demethylate aminopyrine in the absence of succinate; at a mitochondria to microsomal ratio of 4:1, 40% of the microsomal protein was endoplasmic reticulum fragments associated with mitochondria (10% in this study).

In order to test whether the mitochondrial-associated endoplasmic reticulum fragments actively N-dealkylate aminopyrine, the mitochondria were again resuspended and washed. This and

**FIGURE 3** Mitochondrial consumption of exogenous formaldehyde and the effects of succinate (succ) and semicarbazide (sc). 5 mg of mitochondrial protein was added per milliliter of perfusion medium in the presence and absence of 5 mM semicarbazide, at 37°C. Formaldehyde was added to 0.1 mM, and incubation was carried out for 6 min. The reaction was stopped with one-third vol of 15% trichloroacetic acid, and the formaldehyde content was measured. Similar effects were obtained by addition of mitochondria to start the reaction, or on dropwise addition of formaldehyde to 0.1 mM over 5 min. Control = C.

**FIGURE 4** Complexing of formaldehyde with semicarbazide observed spectrophotometrically. The cuvettes contained 3 ml of 0.1 M Tris, pH 7.5, containing 5 mM semicarbazide at room temperature. The reaction was initiated by addition of 25 μl of 12 mM formaldehyde to the cuvette (a) or 5 μl of 12 mM formaldehyde at 1-min intervals at zero, 1, 2, 3, and 4 min (b). The absorption change was measured between 231 nm and 265 nm.
gentle homogenization removed from the mitochondria endoplasmic reticulum fragments which had the same specific N-demethylation activity as the regular microsomal fraction; the resultant mitochondria were devoid of aminopyrine demethylase activity as previously reported (20), but lacked the proper ADP to O ratios. Furthermore, the N-dealkylation activity was examined in the untreated mitochondrial fraction itself in the presence and absence of inhibitors of mitochondrial processes, without further addition of microsomes. In Table I it is seen that, indeed, even while in association with mitochondria the endoplasmic reticulum fragments do N-dealkylate aminopyrine, and, as suspected from Figs. 2 and 3, the generated formaldehyde is handled differently from exogenous formaldehyde, i.e., the generated formaldehyde does not extensively accumulate in the assay medium.

Table II shows the influence of specific mitochondrial inhibitors on N-dealkylation. 10 μM Rotenone and 1 mM cyanide inhibited the microsomal N-dealkylation alone, as would be expected since both agents bind to cytochrome P-450 (24), so activity in Table II is expressed as percent of microsomes alone in the presence of the respective inhibitors. In the absence of inhibitors of mitochondrial electron transport, succinate elevated the rate of formaldehyde accumulation to 80% of the activity of microsomes alone. Rotenone addition (1 μM) exerted a succinate-like effect, increasing the formaldehyde accumulation rate to almost that in the presence of succinate. However, in the presence of Rotenone, the stimulation by succinate was lost. Antimycin A, another inhibitor of mitochondrial electron transport, did not enhance formaldehyde accumulation, nor did it prevent a succinate-mediated enhancement, at 0.6 μM concentrations. At a level of 1 μM, however, antimycin A caused some inhibition of the response to succinate (not shown). Cyanide, at levels around 0.3 mM, did not inhibit microsomal N-dealkyla-

| Table I |
|----------|
| **Formaldehyde Accumulation from Aminopyrine** |
| N-Dealkylation by Mitochondria-Associated Endoplasmic Reticulum Fragments |

| Additions | nmol/min/ mg | % of ms alone |
|-----------|--------------|---------------|
| Mito + semicarbazide + succinate | 9.18 | 71 |
| Mito + semicarbazide | 6.12 | 47 |
| Mito + succinate | 6.88 | 53 |
| Mito | 1.58 | 12 |

Microsomes (ms) alone produced 13 nmol HCHO/min/mg. 6.11-mg mitochondrial fraction protein/milliliter was used. Endoplasmic reticulum fragments - 16.5% of total protein of mitochondrial fraction (mito). Assay time was 6 min.

| Table II |
|----------|
| **Effect of Different Inhibitors of Mitochondrial Electron Transport on Endoplasmic Reticulum Aminopyrine N-Dealkylation** |

| Inhibitor Additions | Succinate | Combined organelles* | Ms alone† | % of ms alone§ |
|---------------------|-----------|----------------------|----------|---------------|
| None                | -         | 4.99                 | 7.8      | 64            |
| +                   |           | 6.33                 | 7.9      | 80            |
| 1 μM rotenone       | -         | 5.62                 | 7.4      | 76            |
| +                   |           | 6.11                 | 7.8      | 78            |
| 10 μM rotenone      | -         | 4.90                 | 6.0      | 82            |
| +                   |           | 5.26                 | 6.1      | 87            |
| 0.6 μM antimycin A  | -         | 4.99                 | 7.2      | 69            |
| +                   |           | 5.93                 | 7.4      | 80            |
| 1 mM KCN            | -         | 3.70                 | 5.7      | 65            |
| +                   |           | 3.83                 | 5.6      | 69            |

* Mitochondria supplemented with microsomes to a mitochondrial:microsomal ratio of 4.2:1. 5 mM semicarbazide present in all assays. Assay time, 6 min.
† Ms = microsomes.
§ Inhibitors ± succinate were also added to microsomes alone as a control.
tion, but still prevented the enhancement afforded by succinate (not shown). Oligomycin (1 μM), an inhibitor of phosphorylation but not of oxidation and electron transport, was without effect on formaldehyde accumulation in the absence of succinate, but did prevent the succinate-mediated enhancement. From these results it would appear that the succinate effect is energy requiring and involves reverse electron transport (18). Since the mitochondrial aldehyde dehydrogenase is known to be NAD⁺-dependent (25), it seemed reasonable to hypothesize that the succinate-mediated enhancement of formaldehyde accumulation was the result of inhibition of this enzyme by decreasing the level of intramitochondrial NAD⁺ (by reducing it).

The suggestion was strengthened by the observations that in the medium containing 0.1 mM formaldehyde the addition of 1 μM Rotenone, like succinate, strongly prevented the rapid disappearance of formaldehyde with time while antimycin A (1 μM) and oligomycin (1 μM) were without effect on formaldehyde disappearance during the assay period. Measurement of intramitochondrial pyridine nucleotides (Table III) after incubation at 37°C for 6 min in perfusion medium indicated that the presence of succinate caused a considerable reduction of NAD⁺ to NADH, while antimycin A had only a small effect on the NAD⁺ level. However, as would be expected, when antimycin A was present it prevented succinate-mediated reduction of NAD⁺ to a considerable extent (Table III).

To further test the hypothesis that mitochondrial aldehyde dehydrogenase is responsible for the influence of succinate on N-demethylase activity, the relationship between intramitochondrial NAD⁺ level and formaldehyde consumption, as well as accumulation, during N-dealkylation was measured. Although rat liver mitochondria are slightly permeable to NAD⁺ (11) and NADH (1), they are maximally permeable only when swollen, as in the presence of 20 mM phosphate buffer (12). This latter technique was used to deplete the mitochondria of NAD⁺ and to elevate their NAD⁺ levels. In Table IV, it is shown that the rate of formaldehyde consumption by liver mitochondria is directly related to the level of the oxidized pyridine nucleotide in the mitochondria. Swelling in phosphate buffer greatly depleted the mitochondria of NAD⁺ and drastically slowed the rate of

### Table III

| Inclusions | NAD⁺ | NADH |
|------------|------|------|
| None       | 3.61 | 0.19 |
| 10 mM succinate | 1.06 | 2.21 |
| 1 μM antimycin A | 2.99 | 0.15 |
| 1 μM antimycin A + 10 mM succinate | 2.56 | 1.05 |

Mitochondria were incubated 10 mg/3 ml in perfusion medium with and without inclusions for 6 min at 37°C before centrifugation at 6,500 g for 10 min. They were then assayed for NAD⁺ and NADH according to methods of Estabrook and Maitra (10).

### Table IV

| Mitochondrial conditions | NAD⁺ | HCHO consumption | APD |
|--------------------------|------|------------------|-----|
| Untreated                | 5.06 | 14               | 4.11 6.93 |
| Depleted                 | 0.90 | 3                | 5.22 6.84 |
| Repleted †               | 8.73 | 22               | 3.67 6.63 |

* The amount of NADH was generally negligible, less than 0.1–0.3 nmol/mg.
† Aminopyrine N-demethylase (APD) activity was measured using a preparation other than that of the rest of the table, but was handled in the same manner. Activity in nmol/min/mg microsomal protein; assay used 3 mg mitochondria per milliliter. Microsomes of this preparation had APD activity of 7.8 nmol/mg/min. Medium contained 5 mM semicarbazide.
§ 0.1 mM formaldehyde in perfusion medium was incubated with 3-mg mitochondrial protein per milliliter at 37°C in the absence of semicarbazide and other additions. Mitochondria alone used.
¶ Mitochondria were depleted of NAD⁺ level by swelling in 20 mM potassium phosphate buffer, pH 7.4 for 15 min at 30°C; to 2.4 ml of mitochondria in 0.25 M sucrose, 10 mM Tris was added 0.6 ml of 0.1 M phosphate buffer. The mitochondria were centrifuged for 10 min at 6,500 g, and the resultant pellets were resuspended in fresh sucrose-Tris (12).
‖ For repletion of NAD⁺ levels, some of the depleted mitochondria, mentioned in the preceding paragraph, were suspended in 0.25 M sucrose containing 0.022 M Tris, 5 mM potassium phosphate, pH 7.4, 2.0 mM NAD⁺, and 2.5 mM ATP. The suspension was chilled on ice for 15 min before centrifugation and was suspended in fresh 0.25 M sucrose, 10 mM Tris, pH 7.5.

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formaldehyde consumption, while an increase in the mitochondrial NAD\(^+\) level greatly enhanced formaldehyde consumption (Table IV). These treatments similarly affected accumulation of formaldehyde during aminopyrine N-dealkylation by associated endoplasmic reticulum fragments. In the data shown in Table IV, the rate of formaldehyde accumulation was 67% of that of the microsomes alone when NAD\(^+\) depleted mitochondria and their associated endoplasmic reticulum fragments were used. After repletion with NAD\(^+\) the rate of formaldehyde accumulation was only 47% of that of the isolated microsomes, due to elevated NAD\(^+\) levels. Subsequent addition of succinate pushed the rate of accumulation to about 85% of the microsomes in both cases. In normal mitochondria, formaldehyde accumulation during N-dealkylation by the associated endoplasmic reticulum fragments rarely exceeded 60% in the presence of semicarbazide, and in the presence of both succinate and semicarbazide it was elevated to 70–80%.

**DISCUSSION**

The data in this paper show that the observed stimulation by succinate of formaldehyde accumulation during liver microsomal N-dealkylation reactions in the presence of hepatic mitochondria occurs via inhibition of the mitochondrial NAD\(^+\)-dependent aldehyde dehydrogenase. The mechanism of stimulation is due to reduction of the intramitochondrial NAD\(^+\), which inhibits the action of the aldehyde dehydrogenase. Rotenone, which blocks the intramitochondrial NADH oxidase (18), enhances the rate of formaldehyde accumulation as does succinate, because they both cause an accumulation of reducing equivalents in the NAD\(^+\) pool (17).

The data in this paper also show evidence for a physiological interaction between the hepatic endoplasmic reticulum and the hepatic mitochondria during drug metabolism. The interaction involves a sequential processing of a drug by the endoplasmic reticulum and potentially toxic aldehyde metabolites by the mitochondria. In an earlier report (20) it was shown that drugs affect the physical relationship of the mitochondria and endoplasmic reticulum. Similar close association has also been observed after in vivo treatment with steroids such as progesterone (14), and in Morris hepatoma cells (16). As in the current report with liver, endoplasmic reticulum fragments were found to be present in mouse oocyte mitochondrial fractions (23), and continuities were actually observed. A similar observation of associations between hepatic mitochondria and rough endoplasmic reticulum fragments was made by Lewis and Tata (19). Such fragments were suggested as representing functional units, perhaps serving for heme incorporation into the endoplasmic reticulum (8). These suggestions appear to be extensions of two earlier hypotheses: (a) Membrane connections are residues of mitochondrial formation (22), and (b) membrane connections serve as channels to and from the mitochondria (2, 15, 23).

Although continuities have been observed occasionally in the current study, the interactions described in this paper represent still a different type of functional interaction, where the duration of potentially cytotoxic aldehydes in the cytoplasm generated during mixed function oxidations is minimized by arrangement of the endoplasmic reticulum in juxtaposition with the mitochondria. The resultant close proximity was most apparent when the duration of externally added formaldehyde was compared with that of formaldehyde generated by aminopyrine dealkylation; even when the formaldehyde was added at a rate approximating its generation, only about 15% was consumed vs. 54% of that generated, in the presence of semicarbazide (Table I).

Whether such arrangement of the endoplasmic reticulum adjacent to mitochondria (see Fig. 1 and reference 20) is fortuitous or is a response to production of potentially toxic aldehydes cannot at present be answered. Certainly, the fact that a two- to three-fold increase in accumulation of formaldehyde in the medium bathing intact liver cells (in slices) was observed on addition of succinate (6) indicates that the mitochondrial aldehyde dehydrogenase represents the major route of formaldehyde detoxification and that succinate can block this pathway.

The observation that succinate can shut off mitochondrial formaldehyde consumption and that generated formaldehyde then appears outside of the mitochondria may have other ramifications. Under certain conditions in vivo, hepatic toxicity could be exacerbated by agents which either block NADH oxidase or cause an increase in the hepatic cell succinate level. On the other hand, one would expect a protective action by agents which enhance the rate of mitochondrial NADH oxidation, e.g., oxaloacetate. In an earlier study we showed an
inhibition of the rate of formaldehyde accumulation in the presence of oxaloacetate with liver slices, when semicarbazide was not used (6). In subsequent studies (20) semicarbazide was added, and the inhibitory action of oxaloacetate was masked by its ability to complex semicarbazide. Preliminary studies have shown that oxaloacetate enhances the rate of mitochondrial aldehyde oxidation. Whether agents capable of enhancing and inhibiting mitochondrial aldehyde dehydrogenase will prove useful for modification of effects of toxic agents must await further investigation.

The mechanism of the succinate stimulation of formaldehyde accumulation is by virtue of the succinate capacity to engage in reverse electron transport. It was shown that succinate causes a reduction of intramitochondrial NAD⁺ to NADH.

In a subsequent paper in which the biochemical properties of the intramitochondrial aldehyde dehydrogenase are described (3), the enzyme’s requirement for NAD⁺ is shown.

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