Studies on the Effects of Coenzyme A—SH: Acetyl Coenzyme A, Nicotinamide Adenine Dinucleotide: Reduced Nicotinamide Adenine Dinucleotide, and Adenosine Diphosphate: Adenosine Triphosphate Ratios on the Interconversion of Active and Inactive Pyruvate Dehydrogenase in Isolated Rat Heart Mitochondria

(Received for publication, May 6, 1976)

RICHARD G. HANSFORD

From the Laboratory of Molecular Aging, Gerontology Research Center, National Institute on Aging, National Institutes of Health, Baltimore City Hospitals, Baltimore, Maryland 21224

The content of coenzyme A—SH (CoASH) and acetyl-CoA of suspensions of rat heart mitochondria was stabilized by the addition of DL-carnitine and acetyl-DL-carnitine, in the presence of the respiratory inhibitor rotenone. The mitochondrial content of NAD+ and NADH was similarly stabilized by the addition of acetoacetate and DL-3-hydroxybutyrate, and the content of ADP and ATP was imposed by the addition of these nucleotides to the mitochondrial suspension, in the presence of uncoupling agent and oligomycin, to inhibit ATPase. Under these conditions, mitochondrial CoASH/acetyl-CoA, NAD+/NADH, and ADP/ATP ratios could be varied independently, and the effect on the interconversion of active and inactive pyruvate dehydrogenase could be studied. Decreases in both CoASH/acetyl-CoA and NAD+/NADH ratios were shown to be inhibitory to the steady state activity of pyruvate dehydrogenase, and this effect is described at three different ADP/ATP ratios and different concentrations of added MgCl₂. A new steady state level of activity was achieved within 10 min of a change in either CoASH/acetyl-CoA or NAD+/NADH ratio; the rate of inactivation was much higher than the rate of reactivation under these conditions. Effects of CoASH/acetyl-CoA and NAD+/NADH may be additive but are still quantitatively lesser than the changes in activity of pyruvate dehydrogenase induced by changes in ADP/ATP ratio. The variation in activity of pyruvate dehydrogenase with ADP/ATP ratio is described in the absence of changes in the other two ratios, conditions which were not met in earlier studies which employed the oxidation of different substrates to generate changes in all three ratios.
enforced by a suitable added “buffer.” In this way each ratio can be manipulated at will, independently of the others.

**EXPERIMENTAL PROCEDURE**

Preparation of Mitochondria—Mitochondria were prepared from the hearts of 6-month male Wistar-derived rats from the Gerontology Research Center aging colony. The method was substantially that described by Chappell and Hansford (12) and employed 5 mg of the proteolytic enzyme pancreatic per heart. Typically, either one or two hearts were used, and the yield was 30 to 55 mg of mitochondrial protein per heart.

Incubation of Mitochondria—The conditions of incubation were varied and are described in detail in the appropriate legend or table headings. Activites described as “maximal” were obtained by adding 100 µl of mitochondrial suspension (1.0 to 3 mg of protein) to 2 ml of medium comprising 0.12 mM KCl, 20 mM potassium Tes, 1.5 mM Tris/phosphate, 0.5 µM FCCP, 5 µg/ml of oligomycin, 5.5 mM MgCl₂, 3 units/ml of dialyzed adenylate kinase, 0.5 mM ADP, 6.5 mM AMP, 6.5 mM dl-carnitine, and 12.5 mM acetoacetate. The temperature was 22°C and the final pH 7.2.

Termination of Incubations and Estimation of Pyruvate Dehydrogenase Activity—At the times indicated in the appropriate legends and table headings, 50-µl aliquots of the mitochondrial suspension were withdrawn and expelled into 250 µl of ice-cold “stopping solution” of the following composition: 0.05 M potassium Hepes, 2 mM potassium EDTA, 25 mM NaF, 0.5 mM (β,γ-methylene)adenosine triphosphate, 1 mM diethiothreitol, and 0.1% (v/v) Triton X-100, and of pH 7.1. Immediately after mixing, a 200-µl portion of the solubilized mitochondria was withdrawn and added to a cuvette containing 0.8 ml of pyruvate dehydrogenase assay mix, at 25°C. This mix comprised 40 mM potassium Hepes, pH 7.1, 0.8 mM MgCl₂, 0.18 mM thiamin pyrophosphate, 2 mM sodium pyruvate, 2 mM NAD, 0.08 mM CoASH, 0.8 mM diethiothreitol, and 2 µg/ml of rotenone. Pyruvate dehydrogenase activity was measured by the rate of increase in A₅₅₀, with the use of a Gilford recording spectrophotometer and a full scale absorbance of 0.2. Controls indicated no lactate dehydrogenase activity under these conditions and a rate of rotenone-insensitive NADH oxidase of less than 10% of the lowest rates recorded.

Duplicate samplings were made from each incubation, except for the time course experiments, where the data plotted are the results from single samplings at each time point.

Estimation of ADP and ATP in Mitochondrial Incubations—Immediately before sampling for pyruvate dehydrogenase activity a 1-ml portion of the incubation was removed and expelled into 1.3 ml of 8% (v/v) HClO₄. After removal of denatured protein and neutralization, the extracts were used for the determination of ADP and ATP, as described by Williamson and Cockey (13). Estimation of CoASH, Acetyl-CoA, NAD⁺, and NADH—CoASH and acetyl-CoA were estimated in neutralized HClO₄ extracts of mitochondrial suspensions by means of the kinetic method of Allred and Guy (14) as described by Hansford (15). NAD⁺ was estimated in neutralized HClO₄ extracts by fluorometry as described by Williamson and Cockey (13), and NADH was estimated in neutralized KOH extracts as described by the same authors (13).

Reagents and Enzymes—Reagents were of the highest purity available. The uncoupling agent FCCP was from Pierce Chemical Company; lithium acetate, sodium DL-3-hydroxybutyrate, and acetyl-L-carnitine hydrochloride was from Sigma; dl-carnitine hydrochloride and acetyl-CoA were from Calbiochem; (β,γ-methylene)adenosine triphosphate and coenzyme A were from P-L Biochemicals, Inc. All enzymes were from Boehringer Mannheim Corp., N.Y.

**RESULTS**

Establishment of Conditions which Stabilize Mitochondrial NAD⁺/NADH, CoASH/Acetyl-CoA, and ADP/ATP Ratios—It was realized that it was important to evolve conditions which would stabilize the mitochondrial content of activators and inhibitors of the pyruvate dehydrogenase phosphorylation and dephosphorylation reactions. Table I illustrates the approach to stabilizing the mitochondrial CoASH/acetyl-CoA ratio. By comparing the two experiments, which employed different mitochondrial suspensions, it is evident that the mitochondrial CoASH/acetyl-CoA ratio can be systematically and reproducibly varied by varying the proportions of DL-carnitine and acetyl-DL-carnitine added to the suspension. This was expected, as the carnitine acetyltransferase (EC 2.3.1.7) would reasonably be expected to be active enough to maintain equilibrium in the nonrespiring system. The NAD⁺/NADH ratio differed in the two experiments, but did not affect the carnitine acetyltransferase equilibrium.

Table II presents the results of two experiments, employing different mitochondrial suspensions, in which the ratio of added acetateacetate/DL-3-hydroxybutyrate was varied, and mitochondrial NAD⁺ and NADH were measured. The results testify to a near-equilibrium at the 3-hydroxybutyrate dehydrogenase reaction and show that the acetateacetate/3-hydroxybutyrurate couple can be used to impose mitochondrial ratios of NAD⁺/NADH in the range of interest. It was predicted that the NAD⁺/NADH ratios would not be affected by the difference in DL-carnitine/acetyl-DL-carnitine ratio in the two experiments represented in Table II, and this was the case.

Mitochondrial ADP and ATP were not measured, but there is a good reason to believe that in the presence of oligomycin and the uncoupling agent FCCP, the matrix ADP/ATP ratio approximates that in the incubation medium. This is because the carnitine acetyltransferase can be manipulated at will, independently of the others. Enforced by a suitable added “buffer.” In this way each ratio can be manipulated at will, independently of the others.

**Table I**

| Added dl-carnitine/ acetyl-DL-carnitine | Mitochondrial CoASH | Mitochondrial acetyl-CoA | Ratio CoASH/ acetyl-CoA |
|----------------------------------------|----------------------|--------------------------|-------------------------|
| A                                      |                      |                          |                         |
| 0.20                                   | 0.15 ± 0.03          | 1.26 ± 0.04              | 0.12                    |
| 0.70                                   | 0.32 ± 0.03          | 1.09 ± 0.05              | 0.29                    |
| 2.7                                    | 0.38 ± 0.0         | 0.58 ± 0.01              | 1.7                     |
| 11.5                                   | 1.23 ± 0.11          | 0.29 ± 0.01              | 4.6                     |
| B                                      |                      |                          |                         |
| 0.20                                   | 0.23 ± 0.05          | 1.42 ± 0.08              | 0.16                    |
| 0.70                                   | 0.36 ± 0.03          | 1.20 ± 0.00              | 0.29                    |
| 2.7                                    | 1.01 ± 0.1           | 0.61 ± 0.02              | 1.7                     |
| 11.5                                   | 1.39 ± 0.01          | 0.28 ± 0.02              | 4.9                     |

*The abbreviations used are: Tes, N-tris(hydroxymethyl)methyl-2-aminoethanesulfonate; FCCP, carbonyl cyanide p-trifluoromethoxyphenylhydrazone; Hepes, N-2-hydroxyethylpiperezine-N'-2-ethanesulfonate.
the mitochondrial ATPase is almost totally inactivated by the oligomycin, at the high concentration used here (3 to 6 \( \mu \)g of oligomycin/mg of protein), while the adenine nucleotide translocase is still fully active. Moreover, it follows from the studies of Klingenberg (16) that in the presence of the uncoupling agent, the translocase will interact with ADP and ATP with equal affinity, as the membrane potential which normally favors ADP uptake is diminished by the uncoupler. This results in the matrix ADP/ATP ratio corresponding with that of the surrounding medium. Finally, phosphorylation of mitochondrial matrix ADP by substrate-level phosphorylation, otherwise possible in the presence of uncoupling agent and oligomycin, is ruled out by the absence of respiration in the presence of rotenone.

It was established in early studies that pyruvate dehydrogenase was extremely sensitive to inhibition by ATP if MgCl₂ was omitted from the incubation medium or EDTA was included. It was thought that this probably reflected mitochondrial Mg²⁺-loss, previously described for heart mitochondria incubated with uncoupling agent (17), and most of the subsequent work was done with added MgCl₂. This meant the action of mitochondrial adenylate kinase on the added adenine nucleotide could not be avoided. To make incubation conditions reproducible, commercial purified adenylate kinase was added to 39 ml of a medium comprising 0.12 M KCl, 20 mM potassium Tes, 5 mM Tris/phosphate, 0.5 \( \mu \)M FCCP, 0.25 \( \mu \)g/ml of oligomycin, 2 mM ADP, and 2 mM ATP. The final pH was 7.2 and the temperature 22°C. Five minutes after the addition of the mitochondria, 100 \( \mu \)g of rotenone, 213 \( \mu \)mol of DL-carnitine, 78 \( \mu \)mol of acetyl-DL-carnitine, 250 \( \mu \)mol of DL-3-hydroxybutyrate, and 5 \( \mu \)mol of acetoacetate were added. Ten minutes after these additions, two 2-ml samples of the suspension were expelled into HClO₄, as described for Table I, for the estimation of NAD⁺, and two 2-ml samples were expelled into 1-ml portions of 0.5 M ethanolic KOH, for the estimation of NADH. Immediately after sampling 16.2 \( \mu \)mol of acetoacetate were added to the suspension, a further 10 min was allowed to elapse, and sampling was carried out as described above. The experiment was completed by the further additions of 62 and 161 \( \mu \)mol of acetoacetate and appropriate sampling.

B. Conditions were the same as in A, except that 57 \( \mu \)g of mitochondrial protein were used and the additions of DL-carnitine and acetyl-DL-carnitine were 119 \( \mu \)mol and 172 \( \mu \)mol, respectively.

| Added acetocetate/DL-3-hydroxybutyrate | Mitochondrial NAD⁺ | Mitochondrial NADH | Ratio NAD⁺/NADH |
|-------------------------------------|--------------------|--------------------|----------------|
| A                                   |                    |                    |                |
| 0.02                                | 3.15 ± 0.05        | 3.05 ± 0.05        | 1.0           |
| 0.10                                | 4.55 ± 0.05        | 1.65 ± 0.15        | 2.8           |
| 0.50                                | 4.85 ± 0.05        | 0.67 ± 0            | 7.2           |
| 2.0                                 | 4.90 ± 0.10        | 0.39 ± 0.10        | 12.6          |
| B                                   |                    |                    |                |
| 0.02                                | 3.17 ± 0.03        | 3.02 ± 0.07        | 1.1           |
| 0.10                                | 4.71 ± 0.02        | 1.41 ± 0.11        | 3.3           |
| 0.50                                | 5.20 ± 0.04        | 0.76 ± 0.06        | 6.8           |
| 2.0                                 | 5.23 ± 0.05        | 0.58 ± 0.03        | 9.0           |

The final pH was 7.2, and the temperature 22°C. Three minutes after the mitochondria, 10 \( \mu \)g of rotenone, 15 \( \mu \)mol of ADP, 66 \( \mu \)mol of acetoacetate, 33 \( \mu \)mol of DL-3-hydroxybutyrate, and 0.7 \( \mu \)mol of acetyl-DL-carnitine were added. At the times indicated on the figure, 50-\mu l aliquots of the mitochondrial incubation were withdrawn and quenched in 0.2 ml of "stopping solution." The composition of this solution and details of the estimation of pyruvate dehydrogenase activity are given under "Experimental Procedure." At the points indicated 5 \( \mu \)mol of DL-carnitine and 46 \( \mu \)mol of acetyl-DL-carnitine were added to the mitochondrial incubation. The maximal activity of the preparation was 65 nmol/min/mg of protein.

The effect of the ratio of added acetocetate/DL-3-hydroxybutyrate on the ratio of mitochondrial NAD⁺/NADH is shown in Table II. The reversibility and time course of the effect of mitochondrial CoASH/acetyl-CoA ratio on the activity of pyruvate dehydrogenase is shown in Fig. 1. A mitochondria suspension (36 mg of protein/ml) 0.2 ml was added to 4 ml of a medium comprising 0.12 M KCl, 20 mM potassium Tes, 5 mM Tris/phosphate, 0.5 \( \mu \)M FCCP, 5 \( \mu \)g/ml of oligomycin, 5.0 mM MgCl₂, and 3 units/ml of dialyzed adenylate kinase. The final pH was 7.2, and the temperature 22°C. Three minutes after the mitochondria, 10 \( \mu \)g of rotenone, 15 \( \mu \)mol of ADP, 66 \( \mu \)mol of acetocetate, 33 \( \mu \)mol of DL-3-hydroxybutyrate, and 0.7 \( \mu \)mol of acetyl-DL-carnitine were added. At the times indicated on the figure, 50-\mu l aliquots of the mitochondrial incubation were withdrawn and quenched in 0.2 ml of "stopping solution." The composition of this solution and details of the estimation of pyruvate dehydrogenase activity are given under "Experimental Procedure." At the points indicated 5 \( \mu \)mol of DL-carnitine and 46 \( \mu \)mol of acetyl-DL-carnitine were added to the mitochondrial incubation. The maximal activity of the preparation was 65 nmol/min/mg of protein.

The ratio of ADP/ATP thus determined is taken to approximate the ratio in the mitochondrial matrix, for the reasons given above.

The time course and reversibility of effects of NAD⁺/NADH and CoASH/acetyl-CoA on pyruvate dehydrogenase activity.—In Fig. 1 mitochondrial pyruvate dehydrogenase is activated by the addition of DL-carnitine to give a ratio of DL-carnitine/acetyl-DL-carnitine of 11.5 and, by inference from Table I, a mitochondrial CoASH/acetyl-CoA of approximately 5. Other important conditions are stabilized as described in the figure legend and so the activation can be attributed to the change in CoASH/acetyl-CoA ratio alone. The half-time of the activation is approximately 1.5 min (at 22°C). The subsequent addition of acetyl-DL-carnitine to give a mitochondrial CoASH/acetyl-CoA ratio of approximately 0.14 results in a very rapid inactivation of pyruvate dehydrogenase with a half-time which was not properly measured, but which is less than 20 s.

An analogous experiment shown in Fig. 2 reveals an activation with a half-time of about 2 min on adding acetocetate to generate a mitochondrial NAD⁺/NADH of approximately 11 (from Table II) and a subsequent inhibition with a half-time of less than 20 s on adding DL-3-hydroxybutyrate to give a mitochondrial NAD⁺/NADH of approximately 7. Again, other mitochondrial parameters of known regulatory significance were stabilized during this experiment. It was concluded from these two experiments that a period of 10 min should be sufficient in the following experiments to achieve a steady state level of pyruvate dehydrogenase activity.

Dependence of steady state pyruvate dehydrogenase activity on ratio ADP/ATP—The effect of mitochondrial ADP/ATP ratio on the interconversion of active and inactive pyruvate dehydrogenase has been the subject of several studies.
CoA/Acetyl-CoA, NAD+/NADH Ratios, and Heart Pyruvate Dehydrogenase

Comparison of Figs. 3 and 4 made it clear that the shape of the dependency of activity upon ADP/ATP was critically dependent on the incubation medium [Mg2+] and suggested investigation of higher concentrations. Repetition of the experiment of Fig. 4 at 8.6 mM MgCl2 in fact gave somewhat similar results, and these are not presented. Also not shown is a repetition of the experiment of Fig. 4 in the presence of 0.5 mM dichloroacetate, as a pyruvate analogue. This had the effect of greatly increasing pyruvate dehydrogenase activity over the range of ADP/ATP from 0.38 to 0.56. Thus at ratios of 0.38 and 0.56, activities were 12 and 23 nmol/min/mg, respectively, in the absence of dichloroacetate, and 22 and 70 nmol/min/mg, respectively, in its presence.

Dependence of Steady State Pyruvate Dehydrogenase Activity on CoASH/Acetyl-CoA and NAD+/NADH Ratios—Fig. 5 presents the results of an experiment in which mitochondrial CoASH/acetyl-CoA and NAD+/NADH ratios were varied as described for the control experiments of Tables I and II, and pyruvate dehydrogenase activity was estimated after being allowed 10 min to achieve a steady state (see Figs. 1, 2). An ADP/ATP ratio of 3.5 was chosen as being a plausible matrix ratio during state 3 (plus ADP) substrate oxidation by isolated mitochondria. The mitochondrial ADP/ATP ratio in the intact heart is not available. It is seen that changes in either CoASH/acetyl-CoA or NAD+/NADH ratio alone can result in more than 2-fold changes in enzyme activity, and that simultaneous changes in both ratios can result in a 4-fold change. This
is consistent with the finding by Pettit et al. (8) of summation in the effects of NADH and acetyl-CoA on the purified pyruvate dehydrogenase plus kinase. Fig. 6 presents the results of a study of the same format, but using an ADP/ATP ratio of 1.1. It is seen that under these conditions an appreciable dependency upon CoASH/acetyl-CoA ratio is only evident when the nicotinamide nucleotide is most highly oxidized, i.e. where NAD+/NADH = 1. Under all other conditions pyruvate dehydrogenase is extremely inactive. Finally, it was desired to do this experiment with an ADP/ATP ratio of less than unity, to determine if a carboxylate were added. This seemed stimulation by ADP (state 3). However, it was clear from Fig. 4 that all activities would be very low at ADP/ATP ratios much reasonable, as pyruvate is present during controlled (state 4) oxidation by isolated mitochondria. Two 50-μl samples were withdrawn from each incubation and expelled into 1.3 ml of 8% (v/v) HClO₄, for subsequent analysis of ADP and ATP. Activity is plotted versus the ADP/ATP ratio in the presence of 4.3 mM MgCl₂ and adenylate kinase. Fig. 6 presents the results of a study of the same format, but using an ADP/ATP ratio of 3.5. Each of 16 incubations was conducted as follows: 0.1 ml of mitochondrial suspension (15 mg/ml) was added to 2 ml of medium comprising 0.12 M KCl, 20 mM potassium isotobutyrate, 5 mM Tris/phosphate, 0.5 μM FCCP, 5 μg/ml of oligomycin, 5.5 mM MgCl₂, and 3 units/ml of dialyzed adenylate kinase. After 3 min of preincubation, 10 μg of rotenone was added, together with 1.75 μmol of ADP, 0.58 μmol of ATP, and 5.2 μmol of AMP. In addition, acetoacetate and Δl,3-hydroxybutyrate were added to a total of 50 μmol and in one of the ratios given in Table II. Δl-Carnitine and acetyl-Δl-carnitine were added to a total of 19 μmol and in one of the ratios given in Table I. Sampling was in duplicate, 10 min after these additions had been made. Alternate incubations were also sampled for the estimation of ADP and ATP. Activities shown are the means of duplicate determinations. Mitochondrial NAD+/NADH ratios and CoASH/acetyl-CoA ratios are inferred from the data of Tables I and II. The ratio of ADP/ATP found on sampling the incubations was 3.51 ± 0.04. The maximal activity of this preparation was 85 nmol/min/mg of protein. O-O, CoASH/acetyl-CoA = 0.14; □-□, CoASH/acetyl-CoA = 0.29; Δ-Δ, CoASH/acetyl-CoA = 1.7; O-O, CoASH/acetyl-CoA = 4.8.

Finally, experiments of the general design of those shown in Figs. 5, 6, and 7 were carried out in an incubation medium containing 1 mM EDTA, instead of added MgCl₂. With ADP the only added nucleotide (and negligible ATP found on assay), the dependency on CoASH/acetyl-CoA and NAD+/NADH ratios closely resembled that shown in Fig. 5 (results not presented). With an ADP/ATP ratio of 10, the results closely resembled those presented in Fig. 6 (results not presented). These results are consistent with the shift in the dependency on ADP/ATP ratio on adding MgCl₂, which is evident from a comparison of Figs. 3 and 4.
The present results demonstrate that the effects of CoASH/acytetyl-CoA and NAD+/NADH ratios on pyruvate dehydrogenase interconversion which were shown by Pettit et al. (8) at the level of the purified enzyme also apply to the enzyme in its mitochondrial milieu. Previously this information was not available for heart mitochondria. Thus, in the very recent study by Kerbev et al. (7) there are anomalies in the correlation between pyruvate dehydrogenase activity and the inverse of the mitochondrial ATP content, and these are cited as evidence for a modulation of pyruvate dehydrogenase interconversion by CoASH/acyetyl-CoA and NAD+/NADH ratios. This is plausible, but not clear, and the different effects of CoASH/acyetyl-CoA and NAD+/NADH ratios are not segregated. Thus, addition of octanoate to respiring mitochondria caused greater inactivation of pyruvate dehydrogenase than expected on the grounds of mitochondrial ATP content, but caused large decreases in both CoASH/acyetyl-CoA and NAD+/NADH ratios. In liver the picture is clearer, in that similar anomalies in the correlation of pyruvate dehydrogenase activity with the mitochondrial ATP/ADP ratio were originally noted by Taylor et al. (5), and a partial resolution of the effects of NAD+/NADH and CoASH/acyetyl-CoA ratios was subsequently made by Batenburg and Olson (11). Very recently, the latter authors (28) have reported experiments using mixtures of 3 hydroxybutyrate and acetoacetate and mixtures of octanoate and carnitine. Though these experiments establish an effect of CoASH/acyetyl-CoA and NAD+/NADH ratios on pyruvate dehydrogenase interconversion in rat liver, they are quantitatively equivocal, as the mitochondrial will oxidize the 3-hydroxybutyrate in the absence of rotenone and will progressively acylate the carnitine in the presence of octanoate. Thus CoASH/acyetyl-CoA and NAD+/NADH ratios will change during the course of the experiment and, judged from Figs. 1 and 2 of the present paper, alterations in pyruvate dehydrogenase activity will lag behind these changes.

The present study also documents the effect of the ADP/ATP ratio upon pyruvate dehydrogenase activity. Previously published experiments have altered ADP/ATP at the same time as NAD+/NADH and CoASH/acyetyl-CoA ratios, by varying respiratory substrates (5-7). An exception is the study of rabbit heart mitochondria by Chiang and Sacktor (21) where sodium nucleotides were added in the absence of substrate. However, the object of that study differed from that of the present one, in that it dealt with rates of inactivation or reactivation, and NaF was added to inhibit phosphatase. The present study deals with steady state levels of active pyruvate dehydrogenase, a resultant of the balance of kinase and phosphatase. The dependency on ADP/ATP which is obtained (Figs. 3 and 4) is markedly affected by the presence of MgCl₂, though only in the range 0 to 4.3 mM added MgCl₂ and not above that (results not shown). Free [Mg²⁺] will be considerably less than 4.3 mM, as there is 3.8 mM total adenine nucleotide present in these studies. Arguments based on the aconitase equilibrium and on the binding of Mg²⁺ to known ligands have suggested about 1 mM free Mg²⁺ in a number of tissues (24), and so these experimental conditions were thought reasonable. The dependency of pyruvate dehydrogenase activity upon ADP/ATP ratio was also affected by the presence of the carboxylates isobutyrate and dichloroacetate. Pyruvate could not be added in these studies, being incompatible with the buffering of NAD+/NADH and CoASH/acyetyl-CoA ratios. However, studies are in progress which utilize pyruvate as respiratory substrate and attempt to correlate the inactivation of pyruvate dehydrogenase seen on adding palmitoylcarnitine, with the changes which occur in the modifier ratios discussed in this paper.
Acknowledgments—I should like to thank Miss Oso Lescure for expert technical assistance and Dr. Bertram Sacktor for his interest in this work.

REFERENCES
1. Linn, T. C., Pettit, F. H., and Reed, L. J. (1969) Proc. Natl. Acad. Sci. U. S. A. 62, 234-241
2. Wieland, O., and Siess, E. (1970) Proc. Natl. Acad. Sci. U. S. A. 65, 941-954
3. Denton, R. M., Randle, P. J., Bridges, B. J., Cooper, R. H., Kerbey, A. L., Pask, H. T., Severson, D. L., Stansbie, D., and Whitehouse, S. (1975) Mol. Cell. Biochem. 9, 27-53
4. Hucho, F., Randall, D. D., Roche, T. E., Burgett, M. W., Pelley, J. W., and Reed, L. J. (1972) Arch. Biochem. Biophys. 151, 328-340
5. Taylor, S. L., Mukherjee, C., and Jungas, R. L. (1975) J. Biol. Chem. 250, 2028-2035
6. Wieland, O. H., and Portenhauser, R. (1974) Eur. J. Biochem. 45, 577-588
7. Kerbey, A. L., Randle, P. J., Cooper, R. H., Whitehouse, S., Pask, H. T., and Denton, R. M. (1976) Biochem. J. 154, 327-348
8. Pettit, F. H., Pelley, J. W., and Reed, L. J. (1975) Biochem. Biophys. Res. Commun. 65, 575-582
9. Cooper, R. H., Randle, P. J., and Denton, R. M. (1975) Nature 257, 808-809
10. Garland, P. R., Newsholme, F. A., and Randle, P. J. (1964) Biochem. J. 93, 665-678
11. Batenberg, J. J., and Olson, M. S. (1976) Biochem. Biophys. Res. Commun. 66, 535-540
12. Chappell, J. B., and Hansford, R. G. (1969) in Subcellular Components (Dimie, G. D., ed) 2nd Ed., pp. 77-91, Butterworths, London
13. Williamson, J. R., and Corkey, B. E. (1966) Methods Enzymol. 13, 434-513
14. Allred, J. B., and Guy, D. G. (1969) Anal. Biochem. 29, 293-299
15. Hansford, R. G. (1974) Biochem. J. 142, 509-519
16. Klingenberg, M. (1979) in Essays in Biochemistry (Campbell, P., and Dickens, F., eds) Vol. 6, pp. 119-159, Academic Press, New York
17. Schuster, S. M., and Olson, M. S. (1972) J. Biol. Chem. 247, 5088-5094
18. Heidt, H. W., Klingenberg, M., and Milovancev, M. (1972) Eur. J. Biochem. 30, 434-440
19. Davis, E. J., and Lumeng, L. (1975) J. Biol. Chem. 250, 2275-2282
20. Whitehouse, S., Cooper, R. H., and Randle, P. J. (1974) Biochem. J. 141, 761-774
21. Chiang, P. K., and Sacktor, B. (1975) J. Biol. Chem. 250, 3399-3406
22. Roche, T. E., and Reed, L. J. (1974) Biochem. Biophys. Res. Commun. 59, 1341-1348
23. Batenburg, J. J., and Olson, M. S. (1976) J. Biol. Chem. 251, 1364-1370
24. Veloso, D., Guynn, R. W., Oskarsson, M., and Veech, R. L. (1973) J. Biol. Chem. 248, 4811-4819
Studies on the effects of coenzyme A-SH: acetyl coenzyme A, nicotinamide adenine dinucleotide: reduced nicotinamide adenine dinucleotide, and adenosine diphosphate: adenosine triphosphate ratios on the interconversion of active and inactive pyruvate dehydrogenase in isolated rat heart mitochondria.

R G Hansford

J. Biol. Chem. 1976, 251:5483-5489.

Access the most updated version of this article at http://www.jbc.org/content/251/18/5483

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/251/18/5483.full.html#ref-list-1