Multiple Molecular Forms of Malic and Lactic Dehydrogenases during Development*

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Lactic dehydrogenase has been shown to exist in multiple molecular forms within a single tissue in several different species (2-15). It is now fairly well established that the lactic dehydrogenase molecule is a tetramer composed of four subunits and that there are two types of these subunits (16, 17). The subunits may combine in all possible combinations of four to yield five different tetramers of lactic dehydrogenase, the number commonly found in mammalian tissues. The recent demonstration of a sixth lactic dehydrogenase in human sperm (18) has not yet been related to the general hypothesis.

The present investigations were concerned with the changes during fetal development in the composition of lactic dehydrogenase in human and rat tissues. In addition, the multiple forms of malic dehydrogenase from the nonparticulate cell fraction of developing human, rat, and chick tissues were studied.

The nonparticulate fraction of all of these tissues contains no more than five proteins with lactic dehydrogenase activity and two proteins with malic dehydrogenase activity which are separable by starch grain electrophoresis. During development, quantitative differences are evident in the distribution of the total enzymatic activity among these different molecular forms.

EXPERIMENTAL PROCEDURE

Rats obtained from Charles River Laboratories were killed by a blow on the head and tissues were excised. In experiments with fetal and newborn rats, tissues from the entire litter of a particular mother were pooled to provide an adequate amount of tissue for analysis. Fertilized white Leghorn eggs were incubated in a dry heat incubator (Sears Roebuck model 213.57). Tissue from a number of embryos at the same stage of development (19) were pooled.

Human fetal tissues obtained at therapeutic interruptions of pregnancy were frozen immediately in Dry-Ice and stored at −10°C in sealed plastic containers. All experiments with human tissues were carried out with frozen tissues; this permitted analyses of several different organs from a single specimen. Flexner et al. (12) demonstrated that similar results are obtained with frozen and fresh tissues. In our own experiments, frozen rat tissues gave the same number of electrophoretic bands of lactic and malic dehydrogenase activity as fresh tissues, with approximately the same distribution of enzymatic activity among the different bands.

The nonparticulate fraction of all of these tissues contains no more than five proteins with lactic dehydrogenase activity and two proteins with malic dehydrogenase activity which are separable by starch grain electrophoresis. During development, quantitative differences are evident in the distribution of the total enzymatic activity among these different molecular forms.

Purified potato starch was obtained from Fisher Chemical Company, New York, or Kebo, Stockholm. Reagent grade sucrose was obtained from Merck and Company, and diethylbarbituric acid and sodium diethylbarbiturate from Merck or Kebo. Nicotinamide adenine dinucleotide, nicotinamide hypoxanthine dinucleotide, thio-dicocinamide adenine dinucleotide, and acetylpyridine adenine dinucleotide from Nutritional Biochemicals Corporation or Sigma Chemical Company were used in enzyme assays. D,L-Lactic acid (85%) was obtained from Malinekrodt Chemical Works, and L-malic acid from Nutritional Biochemicals Corporation.

In experiments with unfractionated enzymes, 10% homogenates of rat tissues were prepared in ice-cold 0.25 M sucrose by means of a glass homogenizer equipped with a motor-driven Teflon pestle. For the isolation of mitochondria, tissues were homogenized in ice-cold 0.88 M sucrose with an all-glass hand homogenizer (20). In making preparations to be used in electrophoretic experiments, 1 to 4 g of tissue were homogenized in an equal volume of barbital buffer (0.1 ionic strength, pH 8.6) in an all-glass hand homogenizer. In preparing the supernatant fraction of rat or chick tissues, homogenates were centrifuged at 600 x g for 10 minutes at 4°C in a Servall model RC2 centrifuge. The sediment was discarded, and the supernatant fluid was centrifuged in a Spinco model L preparative ultracentrifuge at 78,000 x g for 1 hour. The supernatant fraction of human fetal tissues obtained in Stockholm was prepared by means of a 1-hour centrifugation at 10,000 x g in the high speed attachment of a model 52 refrigerated International Centrifuge, since no model L ultracentrifuge was available. The same number of electrophoretic bands of lactic dehydrogenase and malic dehydrogenase activity was found under both sets of conditions. Mitochondria were isolated by centrifugation according to Hogeboom, Schneider, and Pallade (20).

Purified potato starch was mixed with distilled water in a ratio of 1:3, and the starch particles were allowed to settle for 30 minutes. The supernatant fluid containing the finest particles was poured off, and the procedure was repeated three more times to remove all of the fine particles. The starch was then washed once with water and once with barbital buffer (0.1 ionic strength, pH 8.6) on a large Buehner funnel. The washed starch was mixed with enough barbital buffer to give a barely pourable paste (21).

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† Predoctoral Fellow of the National Cancer Institute, National Institutes of Health.
A plastic mold, 33 × 7 × 1 cm, fitted with a hollow base for circulating ice water, was lined with thin plastic wrap, leaving about 6 inches of excess on either end and 3 to 4 inches of excess on either side. Cotton flannel wicks about 7 cm wide, sewn in double thickness, were placed at either end of the mold. The ends of the wicks were moistened with barbital buffer to allow them to lie flat against the ends of the mold. The starch paste was poured into the mold until a block 1 cm thick was formed. Excess liquid was removed from the starch by putting several double thickness, were placed at either end of the mold. The layers of paper toweling in contact with the flannel wicks. When a solid yet moist starch block was formed, the paper towels were removed and a segment 1 cm wide × 5.5 cm long was cut out of the starch block 11 cm from one end.

The block was cooled for at least 1 hour by circulating cold water (1°C) through the hollow base, and the tips of the flannel wicks were then dipped into barbital buffer in the electrode vessels. Each vessel was equipped with a platinum electrode which was connected to a voltage-regulated power supply (Research Specialties, model 1910). Each electrode vessel consisted of two compartments connected by filter paper wicks.

The enzyme sample, 4 ml or less, was mixed with completely dry washed starch to form a thick paste, which was poured into the excavation previously prepared. Excess moisture was withdrawn from the newly applied segment by gently pressing layers of filter paper on each side. The starch block was covered with the plastic wrap, a glass plate was placed over the mold, and electrophoresis was continued for 36 hours at 250 volts across the starch block. The power supply was regulated at 400 volts and 30 ma. Water at a temperature of 1°C was circulated in the plastic mold throughout electrophoresis.

After electrophoresis was complete, any excess moisture was removed from the starch block by blotting with filter paper. The block was cut into 1-cm segments, and each segment was eluted with 5 ml of ice-cold 0.15 M NaCl by means of suction on a sintered glass filter. The average recovery of the lactic dehydrogenase was 80% (range, 70 to 95%). Aliquots of the eluates were assayed spectrophotometrically for lactic and malic dehydrogenase activity.

Assay—Lactic dehydrogenase activity was measured spectrophotometrically by a method adapted from Neilands (22). Standard glass cuvettes of 1-cm light path contained 0.017 M sodium 2-l-lactate, 0.7 × 10⁻³ M NAD or NAD analogue, and either 0.02 ml of supernatant fluid or up to 1 ml of eluate from the starch block and 0.1 M glycine-NaOH buffer, pH 10, to a total volume of 3 ml. The reaction was initiated by adding the enzyme, and the absorbance at 340 mµ was measured in either a Beckman DU or a Zeiss PMG II spectrophotometer at 23 ± 1°C. Malic dehydrogenase activity was assayed by the method of Wolfe and Neilands (23). Standard glass cuvettes of 1-cm light path contained 0.1 M sodium L-malate, 0.7 × 10⁻³ M NAD or NAD analogue, 0.02 ml of supernatant or up to 1 ml of eluate from the starch block, and 0.1 M glycine-NaOH buffer, pH 10, to a total volume of 3 ml. The protein content of the eluates was determined by the method of Lowry et al. (24).

Antisera—Antisera prepared in rabbits against chick HHHH lactic dehydrogenase and against chick MMMM lactic dehydrogenase were the generous gifts of Dr. Nathan O. Kaplan, Graduate Department of Biochemistry, Brandeis University. In tests for the inhibition of human lactic dehydrogenase by these antisera, 1 unit of enzyme activity was arbitrarily defined as that amount of lactic dehydrogenase which gives a change in optical density of 0.02 per 30-second interval. For each such unit in a 1-ml sample, 0.01 ml of undiluted anti-M4 or anti-H4 was added. A corresponding amount of 0.9% NaCl solution was added to another milliliter of each enzyme sample to serve as a control. After the enzymes were incubated with the antisera or NaCl solution at 25°C for 1 hour, the solutions were tested for lactic dehydrogenase activity. The percentage inhibition by an antibody was calculated from a comparison between enzyme activity in the tubes to which antiserum was added and those to which NaCl solution was added.

Malic dehydrogenase was obtained from rat liver and human fetal liver mitochondria by subjecting them to ultrasonic vibration for 4 minutes in a Bronwill model CH 21 Bioacoustic apparatus. This was followed by centrifugation at 24,000 × g for 40 minutes to obtain a clear supernatant solution containing enzyme activity.

RESULTS

Five electrophoretically separable bands of lactic dehydrogenase activity were detected in adult rat heart, in agreement with the results of Wieland et al. (5). These bands of lactic dehydrogenase activity are numbered I through V in order from cathode to anode. Only Bands I and II were detected in rat liver and lung. The pattern of electrophoretic migration on starch grain of the lactic dehydrogenases of rat heart is shown in Fig. 1. The distribution of the total lactic dehydrogenase activity from rat heart, liver, and lung among the five bands is presented in Table I. Preparations of fetal rat tissues were tested for activity with nucleotide analogues. There was a slight increase with development in the ratio of the reactivity of the unfraccionated enzyme with NHD to that with NAD, but the ratio of activity with TNAD to activity with NAD remained approximately constant. Rat fetuses weighing 0.76 g had an NHD: NAD ratio of 0.55 for liver lactic dehydrogenase and 0.53 for heart lactic dehydrogenase. Fetuses weighing 4.01 g had ratios of 0.74 and 0.73 for liver and heart lactic dehydrogenases, respectively.

Five electrophoretically separable bands of lactic dehydrogenase activity were found in tissues from human fetuses and newborn (Fig. 2). The fraction of the total lactic dehydrogenase activity associated with each electrophoretically separable band in the several tissues is summarized in Table II. In a specimen of fetal kidney from a fetus of 21.5-cm crown-rump length, nearly

The abbreviations used are: NHD, nicotinamide hypoxanthine dinucleotide; TNAD, thionicotinamide adenine dinucleotide.

### Table I

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Stage</th>
<th>Lactic dehydrogenase activity in Band</th>
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</thead>
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<tr>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Heart</td>
<td>Adult</td>
<td>9.3</td>
</tr>
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<td>Newborn</td>
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<td>Adult</td>
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<td>Newborn</td>
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<td>Adult</td>
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*Average weight.
78% of total lactic dehydrogenase activity was concentrated in Bands IV and V, but all five bands were present.

Tests of the five bands of human lactic dehydrogenase for their activity with NHD revealed some increase in the ratio of reactivity with NHD relative to NAD of the bands in order I through V. The ratio for Band I was 0.47, and for Band V, 0.77.

An antibody prepared against chick M4 lactic dehydrogenase produced the greatest inhibition of human lactic dehydrogenase activity in Bands I and II and none at all with Band V (Table III).

Similar results were obtained with lactic dehydrogenases from fetal heart and from term placenta. In contrast, an antibody prepared against chick H4 lactic dehydrogenase did not inhibit any of the forms of human lactic dehydrogenase. In other experiments, half as much anti-chick M4 lactic dehydrogenase gave the same degree of inhibition as the full amount.

Two and only two electrophoretically separable bands of malic dehydrogenase activity were found in all rat, chick, and human tissues examined. Band I migrates toward the cathode and Band II migrates toward the anode during electrophoresis at pH 8.6. A typical electrophoretic pattern, that found in adult rat lung, is presented in Fig. 3. The fraction of total malic dehydrogenase activity associated with Band II decreased in heart and lung as development proceeded, but tended to remain constant in the liver.

The two bands of nonparticulate malic dehydrogenase activity from rat, chick, and human tissues were tested with the analogues AcPyAD and TNAD (Table VII). There is no significant difference in the relative rates of reaction of the rat, chick, and human malic dehydrogenase I with the given analogue. Malic dehydrogenase II is also very similar in the three species. However, the two malic dehydrogenases differ significantly from each other in their relative rates of reactivity with TNAD.

Malic dehydrogenase prepared from rat liver mitochondria and subjected to electrophoresis yielded a single band with the characteristics of the band in the nonparticulate fraction migrating toward the cathode (Fig. 6). The rates of reaction with analogues of NAD of the band of malic dehydrogenase migrating toward the cathode during electrophoresis are summarized in Table VIII. These figures emphasize the similarity in the rate...
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B. O. Wiggert and C. A. Villee

TABLE II
Percentage of total lactic dehydrogenase activity associated with each electrophoretically separable fraction from human tissues

<table>
<thead>
<tr>
<th>Age*</th>
<th>Lactic dehydrogenase activity in Band</th>
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<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
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<td>6.6 21.1 34.4 22.2 15.7</td>
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</tr>
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<tr>
<td>Heart</td>
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<td>0.7 28.2 31.8 31.8 7.5</td>
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<tr>
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<tr>
<td>16.0</td>
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<td>2.7 12.0 33.7 37.7 11.9</td>
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<tr>
<td>Lung</td>
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<tr>
<td>Whole brain</td>
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<tr>
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</tr>
<tr>
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<td>Brain stem</td>
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<tr>
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</tr>
<tr>
<td>19.5</td>
<td>2.8 6.2 38.6 39.2 16.5</td>
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<td></td>
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<tr>
<td>Cerebral cortex</td>
<td>Cerebral cortex</td>
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<tr>
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<td>4.8 29.6 57.0 3.4 5.2</td>
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<td></td>
<td></td>
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<tr>
<td>Term placenta</td>
<td>Term placenta</td>
<td></td>
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</tr>
</tbody>
</table>

* Crown-rump length in centimeters.

TABLE III
Percentage inhibition of each electrophoretically separable fraction of lactic dehydrogenase activity from human fetal heart and human term placenta by anti-chick M₄a lactic dehydrogenase

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Inhibition of lactic dehydrogenase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Fetal heart</td>
<td>%</td>
</tr>
<tr>
<td>Term placenta</td>
<td>72.2</td>
</tr>
<tr>
<td>Liver</td>
<td>59.0</td>
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</tbody>
</table>

* Average weight.

TABLE V
Percentage of total nonparticulate malic dehydrogenase activity associated with each electrophoretically separable fraction from chick tissues

<table>
<thead>
<tr>
<th>Organ</th>
<th>No. of days incubated</th>
<th>Malic dehydrogenase activity in Band</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal muscle</td>
<td>11</td>
<td>54.9</td>
</tr>
<tr>
<td>Whole brain</td>
<td>11</td>
<td>10.9</td>
</tr>
<tr>
<td>Heart</td>
<td>11</td>
<td>24.0</td>
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<tr>
<td>Heart</td>
<td>17</td>
<td>28.7</td>
</tr>
<tr>
<td>Heart</td>
<td>17</td>
<td>33.3</td>
</tr>
<tr>
<td>Liver</td>
<td>11</td>
<td>52.8</td>
</tr>
<tr>
<td>Liver</td>
<td>17</td>
<td>8.1</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Most of the differences in the lactic dehydrogenase composition of the developing tissues studied were of a quantitative rather than a qualitative nature. One specimen of human fetal lung lacked lactic dehydrogenase II, and several specimens of human fetal brain were found in which one or two bands of lactic dehydrogenase activity were missing, but in none of the human tissues was any of the five peaks absent in all the stages studied. For example, brain stem of a 13.5-cm (crown-rump) specimen lacked lactic dehydrogenase I, but Band I was detected in the brain stem of a 19.5-cm (crown-rump) specimen. Similarly, Band V was not detected in the cerebral cortex of the 19.5-cm fetus, but it was present in the cerebral cortex of the 13.5-cm fetus.

A comparison of fetal and newborn rat lung and liver with the homologous tissues from fetal and newborn human specimens reveals both qualitative and quantitative differences. First, human fetal and newborn liver and lung tissues, with one exception, possess all five bands of lactic dehydrogenase activity. In rat liver and lung tissues, only Bands I and II were detected, and most of the lactic dehydrogenase activity was present in Band I. In contrast, both fetal and term human liver had no more than 19% of the total lactic dehydrogenase activity in Band I, human fetal lung had no more than 3%, and term lung had no more than 37% of the total lactic dehydrogenase activity in Band I.

Perhaps these differences in lactic dehydrogenase composition reflect differences in metabolic requirements between fetal and newborn rat and human liver and between fetal and newborn rat and human lung. It has been pointed out (17) that the different
forms of lactic dehydrogenase appear to operate differently under varying metabolic conditions. Lactic dehydrogenase I operates at relatively high levels of pyruvate, whereas lactic dehydrogenase V is inhibited by high levels of pyruvate.

Kaplan and Ciotti (25), using analogues of NAD to study unfraccionated lactic dehydrogenases, found few differences between lactic dehydrogenases from newborn and adult rat liver. This is consistent with the finding in this study that in both newborn and adult rat liver most of the lactic dehydrogenase activity is concentrated in Band I. It is interesting to note that Wieland et al. (5) found that Band I, the MMMM form of lactic dehydrogenase, predominates in adult human liver and that the HHHH form, Band V, predominates in adult human heart. Pfleiderer and Wachsmuth (16) examined the lactic dehydrogenases in a number of adult human tissues and described three types of distribution of lactic dehydrogenase activity among the five bands. Heart, cerebrum, cerebellum, and kidney have more than 60% of the total lactic dehydrogenase activity in Bands IV and V. Smooth muscle, testis, ovary, cartilage, lung, spleen, thymus, prostate, and thyroid have most of the lactic dehydrogenase activity in Bands II, III, and IV. Skeletal muscle, liver, and epidermis have a third type of distribution with 70 to 95% of the total lactic dehydrogenase activity in Band I. Pfleiderer and Wachsmuth also examined the lactic dehydrogenases of heart and skeletal muscle from a 19.5-cm (crown-rump) human fetus, and found that in both tissues more of the total lactic dehydrogenase activity was in Bands II, III, and IV than in either Band V, which predominates in adult human heart, or Band I, which predominates in adult human skeletal muscle.

<table>
<thead>
<tr>
<th>Age (cm)</th>
<th>Malic dehydrogenase activity in Band</th>
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</thead>
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<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
</tr>
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<td>Heart</td>
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<td>10.0</td>
<td>27.4</td>
</tr>
<tr>
<td>11.0</td>
<td>51.5</td>
</tr>
<tr>
<td>14.5</td>
<td>47.2</td>
</tr>
<tr>
<td>16.0</td>
<td>52.5</td>
</tr>
<tr>
<td>Term anencephalic</td>
<td>59.8</td>
</tr>
<tr>
<td>Whole brain</td>
<td>8.0</td>
</tr>
<tr>
<td>Brain stem</td>
<td>10.0</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>13.5</td>
</tr>
<tr>
<td>19.5</td>
<td>79.0</td>
</tr>
<tr>
<td>Kidney</td>
<td>21.5</td>
</tr>
<tr>
<td>Term placenta</td>
<td>64.6</td>
</tr>
</tbody>
</table>

* Crown-rump length in centimeters
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The slight increase in the ratio of the reactivity of the human lactic dehydrogenase bands with NHD to that with NAD, going from Band I to Band V (that is from M-type to H-type lactic dehydrogenase), is consistent with the results obtained with the reduced forms of NHD and NAD and a lower concentration of pyruvate with NHDH than with NADH (17). The increase during development in the ratio of the reactivity of unfractonated fetal rat liver and heart lactic dehydrogenase may indicate an increase in H-type lactic dehydrogenase relative to M-type.

Table VII

<table>
<thead>
<tr>
<th>Species</th>
<th>Malic dehydrogenase I</th>
<th>Malic dehydrogenase II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>0.45 ± 0.10</td>
<td>0.20 ± 0.03</td>
</tr>
<tr>
<td>Chick</td>
<td>0.48 ± 0.06</td>
<td>0.24 ± 0.04</td>
</tr>
<tr>
<td>Human</td>
<td>0.44 ± 0.08</td>
<td>0.25 ± 0.06</td>
</tr>
</tbody>
</table>

* Millimicromoles of reduced NAD analogue formed per minute/millimicromoles of NADH formed per minute.

Table VIII

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cell fraction</th>
<th>AcPyAD:NAD*</th>
<th>TNAD:NAD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult rat liver</td>
<td>Nonparticulate</td>
<td>0.46</td>
<td>0.24</td>
</tr>
<tr>
<td>Adult rat liver</td>
<td>Nonparticulate</td>
<td>0.53</td>
<td>0.23</td>
</tr>
<tr>
<td>Adult rat liver</td>
<td>Mitochondrial</td>
<td>0.48</td>
<td>0.19</td>
</tr>
<tr>
<td>Adult rat liver</td>
<td>Nonparticulate +</td>
<td>0.59</td>
<td>0.25</td>
</tr>
<tr>
<td>Human fetal liver</td>
<td></td>
<td>0.45</td>
<td>0.25</td>
</tr>
<tr>
<td>Human fetal liver</td>
<td></td>
<td>0.38</td>
<td>0.24</td>
</tr>
<tr>
<td>Pig heart</td>
<td>Worthington, purified</td>
<td>0.48</td>
<td>0.27</td>
</tr>
</tbody>
</table>

* Millimicromoles of reduced NAD analogue formed per minute/millimicromoles of NADH formed per minute.
† From a fetus with crown-rump length of 24 cm.
The experiments with the antibody to chick M₄ lactic dehydrogenase revealed that, as would be predicted from the "hybrid" theory (17), there was a decreasing inhibition by this antibody going from human lactic dehydrogenase I to lactic dehydrogenase V. The exception to this was the identical inhibition of Bands I and II. Plagemann, Gregory, and Wróblewski (11, 13) also found that lactic dehydrogenase Bands I and II of rabbit tissues are identically inhibited by an antibody prepared against Band I. The failure of an antibody to chick H₄ lactic dehydrogenase to inhibit any of the five forms of human lactic dehydrogenase could mean that this antibody is less potent than the antibody to chick M₄ lactic dehydrogenase. Alternatively, the chick H-type subunit may be a different polypeptide from the human H-type subunit, whereas the M-type subunits of the two species may be similar polypeptides.

The pattern of "pure" and "hybrid" enzymes seen in the electrophoretic pattern of lactic dehydrogenases is not evident in the malic dehydrogenases of rat, chick, and human. There are only two electrophoretically separable forms of malic dehydrogenase in the nonparticulate cell fractions of all the rat, chick, and human tissues examined. Quantitative changes occur in the distribution of the total nonparticulate malic dehydrogenase activity between the two electrophoretic bands at different developmental stages of a particular tissue. These results with vertebrate species are quite different from those obtained by Moore and Vilic (27) with Arabidopsis, in which five electrophoretic bands of L-malic dehydrogenase activity were detected. Thorne, Grossman, and Kaplan (28) found that Bacillus subtilis has several bands of malic dehydrogenase activity.

From the positions occupied by the two forms of malic dehydrogenase from rat, chick, and human tissues during starch grain electrophoresis, together with their reactivities with AcPyAD and TNAD, it appears likely that the same two forms of malic dehydrogenase are present in the different tissues of a given species and that the rat, chick, and human tissues all possess the same two malic dehydrogenases. This is consistent with the findings of Kaplan and Ciotti (29) that there are no striking differences in reactivities with NAD analogues among the unfractonated malic dehydrogenases of various vertebrate species.

It is also evident from both electrophoretic and analogue data that the band of malic dehydrogenase activity in the supernatant fraction which migrates toward the cathode is probably the same protein as the malic dehydrogenase located in the mitochondrial fraction of adult rat liver and human fetal liver. These results are similar to those of Grinn and Doherty (30), who found, after homogenizing beef heart in isotonic sucrose, that a cathodic band of malic dehydrogenase activity was present in approximately equal amounts in the mitochondrial and supernatant fractions. They also found an anodic band of malic dehydrogenase activity present only in the supernatant fraction, in agreement with the results of this study with rat liver and human fetal liver. In the experiments reported here, the cathodic band of malic dehydrogenase was found in both fresh and frozen tissue, so that its presence was not the result of leakage from the mitochondrial fraction because of freezing. This malic dehydrogenase band was found in the same relative amounts when rat liver was homogenized in 0.88 M sucrose to preserve mitochondrial structure instead of in the barbital buffer used in most experiments.

The possibility remains that the malic dehydrogenase is loosely attached to the mitochondria and may leak into the nonparticulate fraction during even very short periods of homogenization. If this is true, then the malic dehydrogenase must be relatively loosely attached to the mitochondria, for in many of the tissues examined this cathodic malic dehydrogenase was present in the nonparticulate cell fraction in amounts equal to or greater than the anodic malic dehydrogenase. If the cathodic malic dehydrogenase present in the nonparticulate fraction were due entirely to leakage from the mitochondria, one might expect to find more malic dehydrogenase I relative to malic dehydrogenase II in the early fetal stages than in the later stages, assuming that the tissues are more fragile in the earlier stages. In several tissues—rat lung, human lung, human heart, and chick brain—however, there is actually a tendency for there to be less malic dehydrogenase I relative to malic dehydrogenase II in the earlier stages than in the later stages of development.

If malic dehydrogenase I of the nonparticulate fraction is really in this cell fraction in the intact cell, it may be that the fundamental genetic control of the two different malic dehydrogenases is similar to that for lactic dehydrogenase (that is, two genes, one for each kind of subunit) but that the malic dehydrogenase subunits do not form "hybrids" as do the lactic dehydrogenases.

**SUMMARY**

Horizontal starch grain electrophoresis revealed a total of five bands of lactic dehydrogenase activity in human fetal tissues. As gestation proceeds, quantitative differences in the distribution of the total lactic dehydrogenase activity among the five electrophoretic bands occur. Most of the lactic dehydrogenase activity is concentrated in the "hybrid" forms, Bands II, III, and IV, rather than in either of the "pure" forms, Bands I and V. Bands I and II were inhibited most, and Band V least, by an antibody prepared against chick M₄ type lactic dehydrogenase. An antibody prepared against chick H₄ lactic dehydrogenase did not inhibit any of the forms of human lactic dehydrogenase. Adult rat heart contained five electrophoretic bands of lactic dehydrogenase activity, but fetal, newborn, and adult rat liver and lung tissues contained only Bands I and II, with most of the lactic dehydrogenase II in the early stages, and in the later stages of development.

Two electrophoretic bands of malic dehydrogenase activity, one migrating toward the cathode and the other toward the anode, are present in the nonparticulate cell fractions of human, rat, and chick tissues. From their positions after electrophoresis and from their reactivities with acetylpyridine and thiocticin-
amido adenine dinucleotide analogues, it appears likely that the same two malic dehydrogenases occur in all three species. Some differences in the distribution of the total nonparticulate malic dehydrogenase between the two electrophoretic bands were observed at various developmental stages. Malic dehydrogenase obtained by ultrasonic disruption of mitochondria from rat liver and human fetal liver appears to be the same enzyme as the cathodic band of malic dehydrogenase from the nonparticulate fraction of these tissues. Although the presence of this malic dehydrogenase in the nonparticulate fraction was not due either to freezing the tissues or to homogenizing in barbital buffer, the possibility remains that it may leak into the nonparticulate fraction during even very short periods of homogenization.

REFERENCES

Multiple Molecular Forms of Malic and Lactic Dehydrogenases during Development

Barbara O. Wiggert and Claude A. Villee