**Induction of Malate Dehydrogenase Isoenzymes in Livers of Young and Old Rats**

*(cortisone/actinomycin D/adrenalectomy/hepatectomy/aging)*

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**ABSTRACT** The activity of cytoplasmic malate dehydrogenase (EC 1.1.1.27) of livers of young (9-10 weeks) and old (60-70 weeks) rats decreases after adrenalectomy and increases after administration of cortisone to adrenalectomized rats. These changes are significantly lower in old rats. The induction by cortisone is inhibited by actinomycin D. Adrenalectomy decreases and cortisone increases the activity of mitochondrial malate dehydrogenase of young rats, but not of old rats. Cortisone, however, induces both mitochondrial and cytoplasmic malate dehydrogenase of the regenerating liver at both the ages. Thus, impairment of the inducibility of mitochondrial malate dehydrogenase that occurs in old age is repaired in dividing cells. Immunological studies on purified malate dehydrogenase from young and old rats show that the molecular species of the enzyme is apparently the same at both ages. The changes in activities of the enzymes seen in old age may be due to changes in template activity of the corresponding genes, which apparently do not change with age.

The activities of several enzymes decrease, and of several others increase, as a function of age of organisms (1). A possible reason for such changes may be a decrease and an increase, respectively, of the template activity of the corresponding genes. We have studied the two isoenzymes of malate dehydrogenase (EC 1.1.1.27), cytoplasmic and mitochondrial, as a model system to get some insight into this problem.

Finch et al. (2) have shown that tyrosine aminotransferase is induced in livers of both young and old rats after they are exposed to cold temperature for a brief period, but the lag period for induction is longer in old rats. However, once induced, the rate of increase in rats of both ages is the same. Such age-dependent impairment of induction has also been shown for glucokinase (3).

Alterations in the isoenzyme pattern of lactate dehydrogenase of various tissues as a function of age of a rat have been reported from this laboratory (4, 5). The induction of the isoenzymes of malate dehydrogenase has not been reported. Both the cytoplasmic and the mitochondrial enzymes are NAD+ dependent (6). The malate dehydrogenase from fish has two types of subunits, A and B, that are under the control of two separate genes (7). The cytoplasmic and mitochondrial malate dehydrogenase of birds are similar in molecular weight, but are different in aminoacid composition, fingerprint, and immunological properties (8). Hence, their syntheses may depend on two separate genes. The cytoplasmic enzyme is important for gluconeogenesis in the cytoplasm; it converts malate to oxalacetate, which is then converted to phosphoenolpyruvate (9). The mitochondrial enzyme is located in mitochondria for catalysis of the Krebs cycle. Thus, the two forms of malate dehydrogenase are located in two separate compartments and perform two distinct metabolic functions, so it is possible that they may be subjected to different control mechanisms that may change with age. The present study was undertaken to find out (a) if the two isoenzymes of malate dehydrogenase of rat liver are induced by cortisone, (b) if there is any difference in their induction as a function of age, and (c) if the nature of their induction is different in regenerating livers of young and old rats.

**MATERIALS AND METHODS**

*Animals.* Young (9-10 weeks) and old (60-70 weeks) female albino rats of Wistar strain, kept at 24 ± 2°C, were used for the studies on induction. 6- and 107-week-old male rats were used for the purification of malate dehydrogenase from their limb muscles for immunological studies. They were fed standard Anidiet ‘A’ (Chelsea Chemical Laboratory, Poona), gram (*Cicer arietinum*), and a diet containing powdered milk and flour (1:4) prepared daily.

*Assay of Isoenzymes.* The liver was excised, homogenized in 0.25 M sucrose, and centrifuged at 700 × g in an MSE high-speed refrigerated centrifuge for 15 min. The supernatant was centrifuged at 14,000 × g for 30 min. The supernatant obtained was used for the assay of cytoplasmic malate dehydrogenase. The pellet was suspended in 0.25 M sucrose and was used for assay of the mitochondrial enzyme. Both the isoenzymes were assayed spectrophotometrically (10). The protein was estimated (11), and the enzyme activity was expressed as specific activity (units/mg of protein). The data were collected from 4 or 5 rats of the same age and were statistically analyzed. 5% or lower values of *P* were taken as significant.

*Effects of Cortisone on Isoenzymes.* Two sets of experiments were done as follows:

(a) **Effect of cortisone on adrenalectomized rats.** Young and old rats were divided into four groups, each having 4-5 rats. Rats in group I were injected with 0.9% of NaCl intraperitoneally. Rats in groups II, III, and IV were bilaterally adrenalectomized and maintained for 10 days on 0.9% NaCl instead of water (12). They were fed the usual diet. On the eleventh day, rats in group II were given 0.9% NaCl. Rats in group III were given cortisone (3 mg/100 g body weight in 1.0 ml of 0.9% NaCl) at 24-hr intervals for 3 days. Rats in group IV were given actinomycin D (10 μg/100 g body weight in 1.0 ml of
TABLE 1. Effects of adrenalectomy (Ad), cortisone (C), and actinomycin D (A) on the specific activities* of cytoplasmic and mitochondrial malate dehydrogenase of livers of 9- and 70-week-old female rats

<table>
<thead>
<tr>
<th></th>
<th>9 Weeks</th>
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<th>70 Weeks</th>
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<tbody>
<tr>
<td></td>
<td>Mean† SD</td>
<td>P</td>
<td>Mean† SD</td>
<td>P</td>
</tr>
<tr>
<td>Cytoplasmic malate dehydrogenase</td>
<td></td>
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<tr>
<td>Normal</td>
<td>152.15 ± 8.18</td>
<td>&lt;0.01</td>
<td>444.60 ± 24.33</td>
<td>&lt;0.01</td>
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<tr>
<td>Ad + salt</td>
<td>97.63 ± 1.32</td>
<td>(-35%)</td>
<td>326.00 ± 43.61</td>
<td>(-27%)</td>
</tr>
<tr>
<td>Ad + C</td>
<td>172.83 ± 2.94</td>
<td>&lt;0.01</td>
<td>408.85 ± 38.84</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Ad + A + C</td>
<td>151.72 ± 12.11</td>
<td>(-25.6%)</td>
<td>323.26 ± 11.06</td>
<td>(-15%)</td>
</tr>
<tr>
<td>Mitochondrial malate dehydrogenase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>52.10 ± 7.11</td>
<td>&lt;0.05</td>
<td>139.16 ± 21.23</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>Ad + salt</td>
<td>41.97 ± 4.06</td>
<td>(-21%)</td>
<td>132.12 ± 14.58</td>
<td>(NE)</td>
</tr>
<tr>
<td>Ad + C</td>
<td>66.41 ± 7.20</td>
<td>&lt;0.05</td>
<td>124.68 ± 27.81</td>
<td>(NE)</td>
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<tr>
<td>Ad + A + C</td>
<td>55.64 ± 4.35</td>
<td>(-27%)</td>
<td>133.55 ± 18.80</td>
<td>(NE)</td>
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</table>

* Specific activity = units/mg protein.
† +, stimulation; -, inhibition; NE, no effect.

0.9% NaCl 1 hr before administration of cortisone for 3 days. The rats were killed on the fourth day for assay of the isoenzymes. The rats in group II served as controls.

(b) Effect of cortisone on isoenzymes of regenerating liver. Young and old rats were hepatectomized by excision of the left lateral and caudate lobes. The tissue removed was used for determination of the normal activity of the isoenzymes. The hepatectomized rats were divided into three groups. Rats in group I were given 0.9% NaCl, rats in group II were given cortisone, and rats in group III were administered actinomycin D and cortisone intraperitoneally for 3 days as mentioned earlier. The rats were killed on the fourth day and used for the assay of cytoplasmic and mitochondrial malate dehydrogenase.

Purification of Malate Dehydrogenase. The enzyme was purified from skeletal muscle of young (6 week) and old (107 week) rats (13) for immunological studies. The tissue from 6-8 rats of each age was pooled. A 75-fold purification of the enzyme was achieved for both ages. No attempt was made to purify the isoenzymes.

Immunological Studies. Purified muscle malate dehydrogenase (1.0 mg) from young and old rats was separately emulsified with Freund's complete adjuvant and injected intramuscularly into both thighs of separate female rabbits. A booster dose was given after 15 days. 10 Days later, blood was taken from the ear, and serum was separated for immunodiffusion and immunoelectrophoresis in 1% agar-agar gel prepared in sodium barbiturate buffer (0.05 M, pH 8.6). Immunoelectrophoresis was done at 150 V for 3 hr with 36 mM sodium citrate buffer (pH 7.0).

RESULTS AND DISCUSSION

One of the well-known changes that occurs in the tissues of aging animals is the alteration in activities of several enzymes (1). Our results show that the specific activities of the two isoenzymes of malate dehydrogenase, cytoplasmic and mitochondrial, of liver are about 3-fold higher in older rats as compared to young rats (Table 1). The amount of glucose in rat blood increases during old age (14). It is possible, therefore, that the higher activity of cytoplasmic malate dehydrogenase in old rats may be a contributory factor to this physiological change, since it aids gluconeogenesis. The increase in the activity of mitochondrial malate dehydrogenase, on the other hand, may make the liver more aerobic in old age. This is in agreement with the earlier finding from this laboratory (4, 5) that the M4-lactate dehydrogenase of brain, heart, skeletal muscle, and liver of rats decreases in old age, which may make these tissues more aerobic.

An increase in the activity of an enzyme after the administration of a hormone may be due to (a) an increase in the transcription or translation of the messenger RNA of the enzyme, resulting in an increase in its concentration, (b) a decrease in degradation of the enzyme, or (c) the synthesis of a protein molecule that modifies the conformation of the enzyme in such a way that its catalytic activity increases without any change in the net synthesis or degradation of the apoenzyme (15).
Table 2. Effects of hepectomy (L), cortisone (C), and actinomycin D (A) on the specific activity of cytoplasmic and mitochondrial malate dehydrogenase of regenerating livers of 10- and 60-week-old female rats

<table>
<thead>
<tr>
<th></th>
<th>10 Weeks</th>
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<th>60 Weeks</th>
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<tbody>
<tr>
<td></td>
<td>Mean*</td>
<td>SD</td>
<td>P</td>
<td>Mean*</td>
<td>SD</td>
<td>P</td>
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<tr>
<td>Cytoplasmic malate dehydrogenase</td>
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<tr>
<td>Normal</td>
<td>208.77 ± 17.38</td>
<td>&gt;0.10</td>
<td></td>
<td>338.32 ± 15.00</td>
<td>&lt;0.05</td>
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<tr>
<td>L + salt</td>
<td>217.38 ± 13.26 (NE)</td>
<td>&lt;0.02</td>
<td></td>
<td>282.06 ± 0.80 (-16.5%)</td>
<td>&lt;0.01</td>
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<tr>
<td>L + C</td>
<td>280.93 ± 37.27 (+34%)</td>
<td>&lt;0.05</td>
<td></td>
<td>362.88 ± 3.25 (+28.5%)</td>
<td>&lt;0.01</td>
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<tr>
<td>L + A + C</td>
<td>226.00 ± 0.55 (-17%)</td>
<td></td>
<td></td>
<td>199.56 ± 2.43 (-45%)</td>
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<tr>
<td>Mitochondrial malate dehydrogenase</td>
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<tr>
<td>Normal</td>
<td>67.44 ± 12.53</td>
<td>&gt;0.10</td>
<td></td>
<td>109.87 ± 4.87</td>
<td>&gt;0.10</td>
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<tr>
<td>L + salt</td>
<td>69.72 ± 19.65 (NE)</td>
<td>&lt;0.05</td>
<td></td>
<td>126.77 ± 6.27 (NE)</td>
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<tr>
<td>L + C</td>
<td>91.00 ± 3.91 (+30%)</td>
<td>&lt;0.05</td>
<td></td>
<td>181.00 ± 9.95 (+43.6%)</td>
<td>&lt;0.05</td>
<td></td>
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<tr>
<td>L + A + C</td>
<td>68.85 ± 0.40 (-20%)</td>
<td></td>
<td></td>
<td>138.75 ± 1.25 (-23%)</td>
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</table>

* +, stimulation; -, inhibition; NE, no effect.

The decrease in activity of cytoplasmic malate dehydrogenase in young and old rats, and of the mitochondrial enzyme in young rats after adrenalectomy, and their increase after the administration of cortisone also show that the levels of these isoenzymes are regulated, at least partially, by the adrenal corticoids in normal rats. It may be of interest to study the effect of other corticoids on the induction of cytoplasmic and mitochondrial malate dehydrogenase as a function of age of the rat.

Table 2 shows that the activity of the two isoenzymes of malate dehydrogenase in regenerating livers of young and old rats 72 hr after hepectomy is the same as that of the normal, except for the cytoplasmic enzyme of old rats. Cortisone stimulates the activity of cytoplasmic malate dehydrogenase of regenerating livers to the same extent in both young and old rats. It is also seen that after hepectomy, the inducibility of the cytoplasmic enzyme in young rats is reduced to the level found in older rats both before and after hepectomy. However, it is of much significance that the hormone also stimulates the activity of the mitochondrial enzyme of regenerating livers of young as well as old rats, whereas in normal rats it stimulates the activity of the mitochondrial enzyme in young, but not in old, rats. Thus, regenerating livers of old rats behave like normal livers of young rats with respect to the induction of mitochondrial dehydrogenase by cortisone. Thus, the responsiveness of the gene for the mitochondrial enzyme to cortisone impaired in the normal liver of old rats is repaired in dividing cells of regenerating liver. It is possible, therefore, that this gene becomes more accessible for direct binding with the inducer (16) in the proliferative stage of cells.

Studies on immunodiffusion (Fig. 1) and immunoelectro-
Similar results were obtained when purified malate dehydrogenase from old rats was used. Fig. 2 shows that the precipitin lines formed are similar for purified malate dehydrogenase of both ages. Thus, it appears that the malate dehydrogenase of young rats is similar to that of old rats, and the genes for cytoplasmic and mitochondrial dehydrogenase apparently do not undergo structural changes in old age.

The above observations are consistent with the gene regulation theory of aging (1), according to which the changes in the levels of enzymes seen during aging may be due to alterations in the template activity of corresponding genes that may be brought about by various factors or modulators produced during growth. In our studies, the gene for cytoplasmic malate dehydrogenase and also the gene for the mitochondrial enzyme appear to be structurally the same in both young and old rats, as there is no apparent difference in the malate dehydrogenase of young and old rats. However, whereas the inducibility of the cytoplasmic enzyme is reduced in old age, that of the mitochondrial enzyme is totally impaired (Table 1). The impairment of induction of mitochondrial malate dehydrogenase is repaired if the cells are made to divide (Table 2). Structural changes in the chromatin that may occur in old age due to factors produced during growth may contribute to the impairment of the inducibility of this gene. Another possibility may be that the gene is located in the mitochondrial DNA, which may account for the difference in its response to the inducer as compared to that of the gene for cytoplasmic malate dehydrogenase.

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**Fig. 1.** Immunodiffusion test between purified malate dehydrogenase of (a) young rats (A) and antisera prepared against malate dehydrogenase of young (x) and old (y) rats; (b) old rats (B) and antisera prepared against malate dehydrogenase of young (x) and old (y) rats.

**Fig. 2.** Immunoelectrophoresis showing the precipitin lines formed between antisera prepared against purified malate dehydrogenase of young (x) and old (y) rats, and malate dehydrogenase from (a) young (A) rats; (b) old (B) rats.