Spectrophotometric Analysis of Cytochromes in Morris Hepatomas

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SUMMARY

The contents of mitochondrial and microsomal cytochromes in slowly growing Morris hepatomas have been determined spectrophotometrically and compared with those in the livers of the host, normal adult, and embryo and in regenerating livers. As compared to the normal adult livers, the hepatomas are characterized by (a) the low content of cytochromes \( a + a_3 \), \( b \), and \( c \), and nearly equal levels of cytochrome \( c \) in mitochondria, giving a low \( a + a_3/c \) ratio, and (b) low contents of cytochromes \( b \) and \( P-450 \) in microsomes. Analysis in whole homogenates of hepatomas showed more pronounced decrease of the amount of cytochrome \( a + a_3 \), presumably due to the decrease of the number of mitochondria in hepatoma cells. The livers of the tumor-bearing rats are also characterized by the similar, although less significant, cytochrome pattern observed in tumors. A low \( a + a_3/c \) ratio was also observed in embryonic livers, whereas regenerating liver showed no significant difference in the content of cytochromes as compared to that of the normal adult liver.

INTRODUCTION

In the preceding paper (15) we have described the results of spectrophotometric analyses of the cytochrome contents in several ascites hepatomas and some other fast-growing tumor cells. The remarkable feature of cytochrome constitution of the respiratory system in these tumors is the low level of cytochromes \( a + a_3 \), \( b \), and \( c \), and the nearly equal level of cytochrome \( c \), resulting in a low cytochrome \( a + a_3/c \) ratio as compared to that in normal liver mitochondria. Another characteristic of these fast-growing hepatomas is the deficiency of microsomal hemoproteins, cytochrome \( b \), and \( P-450 \), which are the usual constituents in the normal livers.

In the present investigation, we extended the analytical study of cytochromes to the minimal-deviation Morris hepatomas which are well differentiated by histological criteria and retain a number of enzymic and metabolic characteristics of the normal livers (10, 11). The cytochrome constitution of these tumors was compared with those of the livers of the normal and the tumor-bearing rats as well as those in embryonic and regenerating states. Generally speaking, these slowly growing hepatomas have characteristics similar to those found in rapidly growing ascites hepatomas (1), although the deviation from the normal tissues is less significant than those observed in rapidly growing hepatomas.

MATERIALS AND METHODS

Tumors. The Morris hepatomas 7794A, 7316B, and 7793 (9) were transplanted by trocar i.m. in both hind legs of Buffalo rats. When the transplanted hepatomas grew to appropriate size (150 to 180 days for 7794A and 80 to 120 days for 7316B), the animals after a fast of 24 hr were killed by decapitation. The tumors were carefully separated from necrotic and hemorrhagic materials, rinsed with cold isotonic sucrose medium containing 0.1 mM EDTA and 10 mM phosphate (pH 7.4) (sucrose/EDTA/phosphate), and minced with scissors.

Livers. The livers of normal and tumor-bearing rats were obtained from Buffalo rats weighing 180 to 240 g. The regenerating livers were obtained 26 and 48 hr after the removal of about two-thirds of the liver of Donryu rats. (There is no significant difference in the cytochrome level between these 2 strains) weighing about 200 g. The embryonic livers were collected from embryos of pregnant rats of the same strain at about 2 days prior to the expected delivery.

The normal and regenerating livers were thoroughly perfused via portal vein with cold 1.15% KCl. The embryonic livers were rinsed with the cold sucrose/EDTA/phosphate medium described above. All livers were minced with scissors before homogenization.

Preparation of Homogenate, Mitochondria, and Microsomes. The minced tumors or livers were thoroughly homogenized with 9 volumes (9 ml/g of wet tissue) of the sucrose/EDTA/phosphate in a Potter-Elvehjem glass homogenizer with a Teflon pestle. The 10% homogenates were filtered with double-folded gauze to remove large debris. The amount of the debris was small and its cytochrome contents were neglected. A part (10 to 20%) of the homogenate was stock for the determination of the contents of cytochromes. The remaining homogenate was centrifuged at 600 \( \times g \) for 5 min, and the supernatant was further centrifuged at 8,000 \( \times g \) for 8 min. The resulting mitochondrial pellet was washed by suspension in 4 volumes of the sucrose/EDTA/phosphate followed by centrifugation at 12,000 \( \times g \) for 5 min and suspended in the same medium at a concentration of 1.5 to 2.5 mg protein per ml.

To obtain microsomes, the supernatant of the centrifugation at 8,000 \( \times g \) for 8 min was further centrifuged at 15,000
x g for 15 min to remove light mitochondria and then spun again at 105,000 x g for 60 min. The resulting pellet (microsomes) was suspended in isotonic KCl to a concentration of 1.5 to 2.5 mg protein per ml.

**Determination of Cytochrome Contents.** For comparison of cytochrome contents in the hepatomas and various types of livers, the following was determined: (a) contents of the respiratory cytochromes \( a + a_3, b \) [mainly \( b_K \) (2, 16)], \( c, \) and \( c_1 \) in the mitochondria; (b) contents of the above cytochromes in the homogenates; (c) contents of the microsomal cytochromes \( b_s \) and P-450 in the microsomes and in the homogenates. The values obtained for mitochondria and microsomes were accurate since almost no additional interfering pigments other than the above-mentioned cytochromes are present there. The contents of cytochromes in the homogenates represent those in the whole tissues; however, the determination in the homogenate is rather difficult due to the presence of pigments other than the cytochromes. Especially, the contamination of hemoglobin in tumors and even in theperfused livers remarkably disturbed the assay of most cytochromes. However, a few cytochromes, such as cytochrome \( c \) (or \( c + c_1 \)), were measured accurately by the CO treatment which largely eliminates the interference of hemoglobin in the reduced minus oxidized spectrum. Using these accurately measured values and the ratio of each cytochrome in isolated mitochondria and microsomes, the contents of all cytochromes in tissues were also calculated (Table 2).

The determination of the contents of cytochromes \( a + a_3, b, \) and \( c + c_1 \) in the suspension of isolated mitochondria was carried out by the difference spectra between the reduced and oxidized states at room temperatures in a way similar to that described in the previous paper (15). The reduced state was brought by anaerobiosis by the mitochondrial respiration with succinate (5 mM) as substrate and the oxidized state was attained by aeration, both in the presence of rotenone (4 \( \mu \)M). No correction for intensification of the interference of cytochrome oxidase and hemoglobin in the reduced form difference spectrum was taken in order to avoid interference of cytochrome oxidase and hemoglobin in the reduced minus oxidized spectrum. Using these accurately measured values and the ratio of each cytochrome in isolated mitochondria and microsomes, the contents of all cytochromes in tissues were also calculated (Table 2).

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In order to know the value for cytochrome \( c \) and \( c_1 \) separately, the ratio of the content of cytochrome \( c \) to \( c_1 \) was calculated from the following equation using the difference spectrum measured at the liquid nitrogen temperature:

\[
\text{Cytochrome } c = \frac{(E_{548.5} - E_{535}) - 0.9 (E_{554} - E_{535})}{(E_{548.5} - E_{535}) - 0.3 (E_{559} - E_{570})}
\]

\[
\text{Cytochrome } c_1 = \frac{(E_{548.5} - E_{535}) - 0.3 (E_{559} - E_{570})}{(E_{548.5} - E_{535}) - 0.6 (E_{554} - E_{535})}
\]

This equation is determined from the low-temperature spectrum of each purified cytochrome as reported by Hagihara and lizuka (6). \( E_{548.5}, E_{535}, \) and \( E_{554} \) are the extinction of cytochromes \( c, c_1, \) and \( b \) (\( b_K \)), respectively; \( E_{559} \) is the extinction at the reference wavelength for cytochrome \( c \) and \( c_1 \), and \( E_{570} \) is that for cytochrome \( b \); thus 0.3 \((E_{559} - E_{570})\) in the numerator or is the correction factor for cytochrome \( c \) contents due to the contribution of cytochrome \( b \) to the cytochrome \( c_1 \) peak, and 0.9 \((E_{548.5} - E_{554}) - 0.3 (E_{559} - E_{570})\) in the denominator is the correction factor for cytochrome \( c \) content due to the contribution of cytochrome \( c_1 \) to the cytochrome \( c \) peak; no correction of cytochrome \( c_1 \) concentration is necessary due to the contribution of cytochrome \( c \) absorption. The latter simplified expression can be used for average mitochondria where the ratios of cytochromes \( c_1 \) and \( b \) are similar.

The respiratory cytochromes in the homogenates of livers and tumors were measured by the reduced and oxidized spectra with the following CO treatment to remove the disturbance of hemoglobin. A mixed gas composed of 2% CO and 98% \( O_2 \) was passed through the homogenate in the reference, and that of 2% CO and 98% \( N_2 \) was passed through the measure cuvet. The above concentration of CO gas converted most of the hemoglobin to carboxyhemoglobin in both aerobic and anaerobic homogenate. Cytochrome \( a_3 \) did not react with this concentration of CO in the aerobic state but combined partly with CO in the anaerobic state. Therefore, the amount of cytochrome \( a + a_3 \) was somewhat underestimated. This was about 10%, as checked by the same treatment on the isolated mitochondria free from hemoglobin. Other cytochromes were not or lightly affected by the present gas treatment. However, the determination of cytochrome \( b \) seemed to be rather more inaccurate in homogenate than in mitochondria by the presence of many other pigments that show maxima around \( b \) region.

For the determination of cytochrome \( b_T \) (\( b_{559} \)) (2, 16, 23), which has recently been found to exist in all kinds of mitochondria, and of cytochrome \( b_{559} \) (5, 14, 19, 21), which is present in mitochondria of liver and some other animal tissues, conditions for the detection of hemoproteins 565 and 559 (14) were used; i.e., difference spectra were taken between the presence and absence of dithionite in the anaerobic suspension of mitochondria or homogenate. The amounts of cytochrome \( b_T \) and \( b_{559} \) were not calculated in the present study but were considered only qualitatively.

Cytochrome \( b_s \) was determined by the following difference spectrum; homogenate or microsomal suspension was put into the measure and reference cuvets, NADH (final concentration, 100 \( \mu \)M) was added to the measure cuvet, and then the difference spectrum was recorded (12). In the case of the homogenates rotenone (4 \( \mu \)M) was added in advance of the homogenate rotenone (4 \( \mu \)M) was added in advance, and finally a small amount of sodium dithionite was added only to remove the effects of rotenone-sensitive, NADH-reducing system. Other cytochromes were not or lightly affected by the present gas treatment. However, the determination of cytochrome \( b \) seemed to be rather more inaccurate in homogenate than in mitochondria by the presence of many other pigments that show maxima around \( b \) region.

Cytochrome P-450 in microsomes was determined by the following difference spectrum: homogenate or microsomal suspension was put into the measure and reference cuvets, NADH (final concentration, 100 \( \mu \)M) was added to the measure cuvet, and then the difference spectrum was recorded (12). The use of CO gas converted most of the hemoglobin to carboxyhemoglobin in both aerobic and anaerobic homogenate. Cytochrome \( a_3 \) did not react with this concentration of CO in the aerobic state but combined partly with CO in the anaerobic state. Therefore, the amount of cytochrome \( a + a_3 \) was somewhat underestimated. This was about 10%, as checked by the same treatment on the isolated mitochondria free from hemoglobin. Other cytochromes were not or lightly affected by the present gas treatment. However, the determination of cytochrome \( b \) seemed to be rather more inaccurate in homogenate than in mitochondria by the presence of many other pigments that show maxima around \( b \) region.

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to the measure cuvet to reduce cytochrome P-450. In both cuvets treated as described above, hemoglobin and cytochrome oxidase were in CO complex form, and all other respiratory cytochromes and cytochrome b$_5$ were in reduced form. However, cytochrome P-450 was in CO complex form in the measure cuvet and in oxidized form in the reference cuvet, since only the reduced form of cytochrome P-450 combines with CO (13). The extinction coefficients used for the calculation of cytochromes a + a$_3$, b, c, and c$_1$ were previously described (15). That of cytochrome b$_5$ was $E_{555-570} = 24.0 \text{ mM}^{-1} \text{ cm}^{-1}$ (8), and that for cytochrome P-450 was $E_{450-490} = 91.0 \text{ mM}^{-1} \text{ cm}^{-1}$ (13).

RESULTS

Contents of Respiratory Cytochromes in Mitochondria.

Chart 1 illustrates the typical examples of the difference spectra between the anaerobic and aerobic state of the suspension of mitochondria isolated from the normal adult liver, livers of 7794A and 7316B hepatoma-bearing rats, hepatoma 7794A, and hepatoma 7316B. In the presence of rotenone all respiratory cytochromes are fully oxidized in the aerobic state (1), while they are nearly fully reduced, except cytochrome b$_T$ (b$_{560}$) (16, 21) and b$_{559}$ (5, 21), in the anaerobic state brought by succinate respiration. In each spectrum, peaks shown by arrows at 605, 562, and 551 nm are due to the $\alpha$-absorption maxima of reduced cytochrome a + a$_3$, b and c + c$_1$ (mixture of c and c$_1$ which cannot be distinguished at room temperature), respectively; those at 444, 430, and 418 nm are due to the $\gamma_{\text{max}}$ of the above cytochromes, respectively. Comparison of these peaks in each spectrum shows that cytochrome a content in the mitochondria of the livers from the tumor-bearing rats is considerably low and that of the hepatoma is extremely low as compared to that in the normal liver, while the contents of cytochrome c + c$_1$ are nearly equal in all cases. Especially, differences of the shape of these spectra in the $\gamma$ region are remarkable.

Chart 2 shows typical examples of the low-temperature (77°K) difference spectra of the suspensions of mitochondria isolated from normal liver, the liver of embryonic rats, the livers of 7316B hepatoma-bearing rats, and 7316B hepatoma. Chart 2A is "anaerobic minus aerobic" difference spectra, and the peaks in each spectrum at 601, 559, 554, and 548 nm are due to the $\alpha$-peaks of reduced cytochromes a + a$_3$, b$_T$ (16), c$_1$, and c$_T$, respectively. The characteristics of cytochromes in mitochondria shown by these spectra are as follows: (a) relatively low content of cytochrome a + a$_3$, as compared to that of cytochrome c$_1$ in mitochondria of embryonic livers; (b) relatively low contents of cytochrome a + a$_3$, b and c$_1$ in mitochondria of the tumor-bearing rat liver; and (c) extremely low content of cytochrome a + a$_3$ and relatively low contents of cytochromes b and c$_1$ in the hepatoma.

Chart 2B shows the difference spectra of the above 4 kinds of mitochondria between the presence and absence of dithionite in the anaerobic state. The absorption peaks at 563 nm are due to $\alpha_1$ peak of cytochrome b$_T$ (b$_{560}$) and that at 555 nm is considered to be composed of the $\alpha$ peak of cytochrome b$_{559}$ and the $\alpha_2$ peak of cytochrome b$_T$. It was noticed from these spectra that no remarkable difference was present in the contents of cytochrome b$_T$ and b$_{559}$ among mitochondria from the normal liver, the tumor-bearing...
Chart 2. Two types of low-temperature (77°K) difference spectra of mitochondria from various livers and a hepatoma. A, difference spectra between the anaerobic and aerobic states; B, difference spectra between the presence and absence of sodium dithionite in the anaerobic state. I and B are the spectra (from the top) of normal adult liver (1.8 mg protein per ml); livers of embryonic rats (3.9 mg protein per ml); liver of hepatoma 73I6B-bearing rat (2.0 mg protein per ml); hepatoma 73I6B (1.9 mg protein per ml) mitochondria. In the case of spectra in A, mitochondrial suspensions were treated as in Chart 1 and then frozen to the temperature of liquid nitrogen. In the case of spectra in B, mitochondrial suspensions in both the reference and measure cuvet were brought to the anaerobic state by respiration with succinate (5 mM) in the presence of NADH (0.5 mM) and dithionite (4 /¿M); then dithionite was added only in the measure cuvet. The optical path was 3 mm in both cases. A, 0.01 A; B, 0.005 A.

Table 1
Contents of respiratory cytochromes in mitochondria from various states of livers and Morris hepatomas

<table>
<thead>
<tr>
<th>Materials</th>
<th>Concentration of cytochromes* (nmoles/mg protein)</th>
<th>Relative contents*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$a + a_3$</td>
<td>$b$</td>
</tr>
<tr>
<td>Normal rat liver (9)</td>
<td>0.24 ± 0.01</td>
<td>0.14 ± 0.01</td>
</tr>
<tr>
<td>Embryonic rat liver (2)</td>
<td>0.30</td>
<td>0.09</td>
</tr>
<tr>
<td>Regenerating liver (2)</td>
<td>0.28</td>
<td>0.14</td>
</tr>
<tr>
<td>7794A-bearing rat liver (7)</td>
<td>0.17 ± 0.02</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>7793-bearing rat liver (2)</td>
<td>0.20</td>
<td>0.15</td>
</tr>
<tr>
<td>7316B-bearing rat liver (4)</td>
<td>0.18 ± 0.02</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>Hepatoma 7794A (7)</td>
<td>0.15 ± 0.01</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>Hepatoma 7793 (2)</td>
<td>0.18</td>
<td>0.12</td>
</tr>
<tr>
<td>Hepatoma 7316B (4)</td>
<td>0.17 ± 0.01</td>
<td>0.13 ± 0.02</td>
</tr>
</tbody>
</table>

* Contents of cytochromes $a + a_3$, $b$, and $c + c_1$ were obtained from the difference spectra at room temperature and those of $c$ and $c_1$ were calculated using the $c/c_1$ ratio obtained from those at 77°K.
* Relative contents are expressed by taking cytochrome $c$ content as 1.
* Number of experiments are given in parentheses.
* Mean ± S.E.

From the spectra as shown in Charts 1 and 2A, the content and the ratio of each component of the respiratory cytochromes were calculated; mean values are shown in Table 1. For hepatomas 7794A, 7316B, and 7793, the content of cytochrome $a + a_3$ decreases to about 60 to 70% of the normal level. The contents of cytochromes $b$ and $c_1$ are also considerably decreased in the above hepatomas while the content of cytochrome $c$ is similar to or even higher than that of normal liver. When the molar ratios of cytochromes $a + a_3$, $b$, and $c_1$ to $c$ are calculated, the $a + a_3/c$ ratio is remarkably low and the $b/c$ and $c_1/c$ ratio are somewhat lower in tumors than in normal liver.

In the liver mitochondria of tumor-bearing rats, the content of cytochrome $a + a_3$ is also decreased as compared to the normal liver; while that of cytochrome $c$ is similar, resulting in a low $a + a_3/c$ value which is not as remarkable as those of tumors.

In embryonic liver mitochondria, the contents of respiratory cytochromes were found to be considerably lower than those of the normal liver (Table 1). In the regenerating liver, no remarkable differences were observed in the contents of mitochondrial cytochromes as compared to normal liver.

Contents of the Respiratory Cytochromes in Homogenates. In order to know the absolute values in tissues, analysis of the homogenates is carried out and the results are shown in Table 2.

As described in “Materials and Methods,” the contents of cytochromes $b$ and $a + a_3$ were difficult to measure in the homogenates while cytochrome $c + c_1$ could be determined accurately. The measured and the calculated values of cytochromes $a + a_3$, $b$, $c_1$, and $c$ are presented in Table 2. These calculated values are thought to be more exact than the measured values since these cytochromes are present only in mitochondria.
Cytochromes in Morris Hepatomas

Table 2

Contents of respiratory cytochromes in homogenates of various states of livers and hepatomas

<table>
<thead>
<tr>
<th>Materials</th>
<th>Cytochrome contents (nmoles/mg protein)</th>
<th>Measured</th>
<th>Calculateda</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a + a3</td>
<td>b</td>
<td>c + c1</td>
</tr>
<tr>
<td>Normal rat livers (6)</td>
<td>0.068 ± 0.007</td>
<td>0.045 ± 0.008</td>
<td>0.091 ± 0.009</td>
</tr>
<tr>
<td>Embryonic rat livers (2)</td>
<td>0.028</td>
<td>0.040</td>
<td>0.045</td>
</tr>
<tr>
<td>Regenerating livers (2)</td>
<td>0.074</td>
<td>0.054</td>
<td>0.104</td>
</tr>
<tr>
<td>7794A-bearing rat livers (6)</td>
<td>0.042 ± 0.005</td>
<td>0.042 ± 0.006</td>
<td>0.087 ± 0.011</td>
</tr>
<tr>
<td>7316B-bearing rat livers (6)</td>
<td>0.038 ± 0.006</td>
<td>0.055 ± 0.004</td>
<td>0.089 ± 0.012</td>
</tr>
<tr>
<td>Hepatoma 7794A (6)</td>
<td>0.036 ± 0.006</td>
<td>0.030 ± 0.006</td>
<td>0.070 ± 0.008</td>
</tr>
<tr>
<td>Hepatoma 7316B (3)</td>
<td>0.034 ± 0.003</td>
<td>0.047 ± 0.004</td>
<td>0.083 ± 0.012</td>
</tr>
</tbody>
</table>

*a Concentrations of cytochromes calculated directly from the "anaerobe-aerobe" difference spectra of homogenate treated with 2% CO (see "Materials and Methods" and Charts 1 and 2). Only cytochrome c + c1 is measured accurately.

It is noticed from Table 2 that the contents of cytochromes a + a3 in the tumor homogenates are much lower than that in the normal one, and these decrements are more remarkable than the case of mitochondria shown in Table 1 (see "Discussion").

Contents of Cytochromes b5 and P-450. Typical examples of the difference spectra that were used for the determination of the concentration of cytochromes P-450 and b5 in microsomes are shown in Chart 3. The contents of cytochromes b5 and P-450 in microsomes and in homogenates of normal adult, embryonic, tumor-bearing, and regenerating livers and of the hepatomas are presented in Table 3. Cytochrome b5, which was not detected in ascites hepatomas (15), is still detectable in the present minimal-deviation hepatomas, although its content is considerably low. Cytochrome P-450 which was not detected in ascites hepatomas (15) is also present in these Morris hepatomas. The low content of cytochrome b5 is found not only in the hepatomas but also in the livers of hepatoma-bearing rats, although the decrements are not as significant as in the hepatomas. On the contrary, the contents of cytochrome P-450 are almost normal in tumor-bearing rat livers.

In embryonic liver the contents of cytochromes b5 and P-450 are extremely low. In regenerating livers the contents of cytochromes b5 and P-450 are similar to those of the normal cases.

DISCUSSION

It appears that there is a possible correlation between rapid growth rate of tumors and the low a + a3/c ratio where the ratios in most of rapidly growing hepatomas were less than one-half of the normal liver (15), while in slowly growing Morris hepatomas it was about 60%. In the case of Morris hepatoma 7316B, the more transplantations there are from generation to generation, the faster becomes the growth rate of the tumor. During the 22nd to 38th generation, the a + a3/c ratio decreases from 0.65 to 0.55.
Table 3

Contents of cytochromes P-450 and b₅ in microsomes and homogenates from various states of livers and hepatomas

<table>
<thead>
<tr>
<th>Materials</th>
<th>P-450</th>
<th>b₅</th>
<th>b₅/P-450</th>
<th>P-450</th>
<th>b₅</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rat liver (8)</td>
<td>0.86 ± 0.06*</td>
<td>0.39 ± 0.03</td>
<td>0.45</td>
<td>0.35 ± 0.05</td>
<td>0.19 ± 0.02</td>
</tr>
<tr>
<td>Embryonic rat liver (2)</td>
<td>0.08</td>
<td>0.09</td>
<td>0.88</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Regenerating liver (2)</td>
<td>0.80</td>
<td>0.32</td>
<td>0.40</td>
<td>0.30</td>
<td>0.16</td>
</tr>
<tr>
<td>7794A-bearing rat liver (6)</td>
<td>0.78 ± 0.06</td>
<td>0.29 ± 0.02</td>
<td>0.37</td>
<td>0.32 ± 0.02</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>7793-bearing rat liver (3)</td>
<td>0.83 ± 0.05</td>
<td>0.29 ± 0.03</td>
<td>0.35</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>7316B-bearing rat liver (4)</td>
<td>0.88 ± 0.04</td>
<td>0.35 ± 0.02</td>
<td>0.40</td>
<td>0.41 ± 0.06</td>
<td>0.14 ± 0.01</td>
</tr>
<tr>
<td>Hepatoma 7794A (6)</td>
<td>0.15 ± 0.06</td>
<td>0.11 ± 0.03</td>
<td>0.73</td>
<td>0.06 ± 0.02</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>Hepatoma 7793 (3)</td>
<td>0.19 ± 0.04</td>
<td>0.20 ± 0.04</td>
<td>1.05</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Hepatoma 7316B (4)</td>
<td>0.26 ± 0.06</td>
<td>0.28 ± 0.02</td>
<td>1.08</td>
<td>0.08 ± 0.04</td>
<td>0.10 ± 0.03</td>
</tr>
</tbody>
</table>

* Mean ± S.E.

(N. Sato and B. Hagihara, unpublished results). The a + a₅/c, b/c, and c/c ratios in hepatoma-bearing rats were lower than the normal level which suggests that the cytochrome composition of the tumor-bearing rats undergoes some intermediate pattern between the normal liver and the hepatomas. In embryonic livers that show rapid growth as tumors, the a + a₅/c ratio was also lower but the b/c and c/c ratios were the same as or higher than those of normal adult liver. In regenerating livers, the growth of the tissues exceeds even that of tumors, but the cytochrome composition was similar to the normal liver (Table 4).

It is noticed from the comparison of Tables 1 and 2 that the decreased cytochrome a + a₅ content in the present hepatomas as compared to the normal liver is mostly due to the decreased concentration of this cytochrome in mitochondria and partly due to the decreased number of mitochondria in the tissues.

The protein contents of the normal adult liver, 7794A hepatoma, and 7316B hepatoma were 28, 18, and 16 mg nitrogen per g, wet weight of the tissues, respectively. Such low protein content in these hepatoma cells is comparable with the observation of other workers (20) on the other types of Morris hepatomas. Considering such low content of protein in the hepatoma, decrement of the contents of cytochromes per wet weight of the tissues, respectively. Such a decrease in the microsomal cytochromes between the Morris hepatomas and rapidly growing tumors is comparable to the difference in the contents of various enzymes reported in the above 2 kinds of hepatomas.

The protein content of the tumor-bearing rats under rapid growth as tumors, the a + a₅/c ratio was also lower but the b/c and c/c ratios were the same as or higher than those of normal hepatoma. The difference in the microsomal cytochromes between the Morris hepatomas and rapidly growing tumors is comparable to the difference in the contents of various enzymes reported in the above 2 kinds of hepatomas.

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Cytochromes in Morris Hepatomas


Spectrophotometric Analysis of Cytochromes in Morris Hepatomas

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