130. Cytochrome in Tumor Tissues.

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Since the work of Warburg1) on the metabolism of tumor cells the ability of these cells to survive under anaerobic conditions has attracted much attention. According to the experiments of Okamoto2), Fischer3), Wind4), etc., however, different types of tumors show different susceptibility to a lowered oxygen tension. In this connection it may be significant that the Rous chicken sarcoma, capable of anaerobic proliferation, contains practically no glutathione, while the Flexner-Jobling rat carcinoma, a much less "anaerobic" tumor, is rich in this important respiratory substance (Yaoi and Nakahara5). Anaerobic bacteria are notably deficient in glutathione (Callow and Robinson6)).

From the standpoint of the above consideration, it is of interest to refer to Keilin's7) observation that cytochrome, a respiratory pigment, is abundantly present in aerobic microorganisms but is absent in anaerobic ones. Yaoi and Tamiya8) recently confirmed and elaborated upon this interesting parallelism between the cytochrome contents and oxygen requirement of bacteria, and showed that obligate aerobes, facultative anaerobes and obligate anaerobes contain a large amount, medium amount, and no demonstrable amount, respectively, of this respiratory pigment. For obvious reasons, these observations lead us to the examination of the cytochrome contents of malignant tumors.

Experiments.

Cytochrome as an oxygen carrier or respiratory catalyst in plants and animal cells has been established by Keilin. This pigment can be easily demonstrated spectroscopically, and it gives four characteristic

absorption bands, consisting of band c (550), which is usually the strongest, a (605), b (565), and d (521), which is faint. A lower concentration of cytochrome is indicated by the reduced intensity and consequently also by the invisibility of fainter bands.

Our observations were carried out with the aid of Abbe’s microspectroscope, using a 500 Watt electric lamp for illumination. Tissues were removed from animals freshly bled to death, were cut into slices of about 3 mm. thick, and were pressed between a slide and cover glass for examination. The results are shown in the following table, where a, b, c, and d refer to the four characteristic absorption bands of reduced cytochrome according to the usage in Keilin’s original papers. The presence of all four bands is taken to mean a high concentration, and a reduction in the number of the bands a correspondingly lower concentration of the pigment.

Table I.

<table>
<thead>
<tr>
<th>Tumors</th>
<th>Absorption bands of cytochrome</th>
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<tbody>
<tr>
<td>Rous chicken sarcoma No. 1 (9 determinations)</td>
<td>None or c only.</td>
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<tr>
<td>Flexner-Jobling rat carcinoma (4 determinations)</td>
<td>b and c, also a in 1 case</td>
</tr>
<tr>
<td>Bashford mouse carcinoma (2 determinations)</td>
<td>b and c, also a in 1 case</td>
</tr>
<tr>
<td>Fujinawa rat sarcoma (3 determinations)</td>
<td>a, b, c, and d.</td>
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In all these examinations, special care was taken to use only the active layer of tumor tissue, excluding necrotic parts and peritumoral normal tissues. Necrotic tissue shows no cytochrome. In the case of Rous sarcoma, because of its infiltrative growth, it was sometimes difficult to obtain sarcoma tissue free from muscle fibres, and this might account for the occasional appearance of one band (c), which is usually absent in most of the samples. These negative findings were checked up by the re-examination of the tissue after treatment with Na$_2$S$_2$O$_4$. Tumors used were taken from 9 days to 3 weeks after implantation, but no difference in the cytochrome contents was noted according to the age.

For the sake of comparison, we also examined several normal tissues. In the chicken as well as in the rat, heart muscle and mature testicle showed four bands; skeletal muscle, liver, brain, spinal cord and immature testicle usually three bands; while in the spleen and
lung no band was definitely demonstrable. These results agree well with those reported by Bierich, Rosenbohm and Kolle\textsuperscript{1}).

\textit{Discussion.}

In view of the varying degrees of anaerobic capacities among different tumors, as referred to in the first part of this paper, the small or negative cytochrome content of the Rous sarcoma may be of special interest. This tumor contains but little glutathione, and is capable of proliferation to some extent under anaerobic conditions (Wind). From analogy, it may be speculated that such tumors as the Fujinawa rat sarcoma, containing much glutathione as well as cytochrome, probably require as much oxygen as many normal tissues do. It must not be forgotten, however, that there are important differences between the so-called anaerobiosis of the tumor cells and the case of true anaerobic microorganisms.

It is now recognized that the chicken sarcoma is a true tumor, not a peculiar disease caused by a filterable virus. On the other hand, the cells of this tumor seem to be peculiar in many ways. These cells are resistant to desiccation, glycerination, etc. (Nakahara\textsuperscript{2}), are deficient in glutathione, and are unable to reduce methylene blue (Yaoi and Nakahara\textsuperscript{3}). We now add another interesting fact, that this sarcoma is deficient in the respiratory pigment, cytochrome.

\textit{Summary.}

We examined four types of transplantable tumors for the occurrence of cytochrome, an intracellular respiratory pigment recently discovered by Keilin, and found that Fujinawa rat sarcoma contains a large amount, Flexner-Jobling rat carcinoma and Bashford mouse carcinoma a medium amount, and Rous chicken sarcoma either small or no demonstrable amount of this respiratory pigment. It is probable that these facts can be correlated with the difference in the ability of different types of tumors to withstand the deprivation of oxygen.

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