**Difference of the Inhibitor of DNA Synthesis in Liver Extract from Liver Arginase**

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**Summary.** The presence of a protein-like inhibitor of DNA synthesis in liver extract has been reported earlier. This inhibitor is different from liver arginase, which is known as a potent growth inhibitor of tissue culture cells. Some evidence is also presented that the inhibition is not due to the destruction of essential nutrients in the medium but to the inhibitor’s direct effect on the cells.

**Introduction.** An extract of liver was previously found to inhibit DNA synthesis of AH-414 ascites hepatoma cells after a short incubation period of 1–2 hours (11). Other investigators had demonstrated, earlier, that similar liver extracts inhibited the growth of tissue culture cells; subsequent studies revealed that this inhibition of growth could be ascribed to the presence of arginase which hydrolyzes arginine, an essential amino acid for cell growth (1, 3, 7–9). This finding led to the immediate question as to whether the inhibitor of DNA synthesis, mentioned above, could also be due to arginase in the liver extract; indeed, Holley (6) obtained inhibition of DNA synthesis by incubating cultured cells with purified liver arginase for relatively prolonged periods of time.

The purpose of this communication is to show that the inhibition of DNA synthesis in malignant cells produced by short-term incubation with liver extracts is not due to the presence of arginase.

**Materials and Methods.** Beef liver was homogenized in two volumes of 0.05 M Tris-HCl buffer (pH 7.6) in a Waring blender and then centrifuged at 70,000 X g for two hours in a Spinco L ultracentrifuge. The supernatant was dialyzed against 10–4 M manganese sulfate and used as the liver extract.

Arginase was extracted and purified from acetone powder of beef liver according to the method of Bach and Killips (2) with a slight modification in the final step. After the isopropanol treatment (step G of Bach and Killips), diethylaminoethyl cellulose column chromatography was used for further purification. Arginase was eluted from the column with 0.01 M Tris-HCl buffer (pH 7.6) without any retention by diethylaminoethyl cellulose; the solution eluted can be kept at -20°C for several weeks without significant loss of arginase activity. It was also dialyzed against 10–4 M manganese sulfate.

Arginase was assayed by the method of Nadai (10). The enzyme unit was defined as that amount of enzyme which liberated 1 μmole of urea from a 0.21 M L-arginine solution in 10 minutes at 30°C at pH 9.2.

Sarcoma 180 ascites cells were inoculated intraperitoneally into mice (about 25 million cells/animal). After 4 to 6 days, cells were washed from the peritoneal cavity with Hanks’ salt solution containing 50 μg/ml of streptomycin and 40 units/ml of penicillin; the cells were collected by centrifugation. The pellet of cells was resuspended in the same solution and washed twice by centrifugation. Finally, the packed cells were suspended in two volumes of Hanks’ salt solution containing streptomycin, penicillin, and horse serum (10% v/v).

Aliquots of 0.1 ml of the above Sarcoma 180 suspension were then diluted with 1.9 ml of Hanks’ solution (containing streptomycin, penicillin, and horse serum), and 0.5 ml of liver extract was added. The concentration of ascites cells in this mixture was about 107 cells/ml.

The mixture was incubated at 37°C in a shaking water bath. After one hour, then thymidine-2-14C2 (0.025 μc/0.05 ml) was added to the mixture and incubation was continued for 30 more minutes. At the end of the incubation period, 1 ml of the mixture was withdrawn and centrifuged. To the pellet, 3 ml of cold trichloroacetic acid (TCA) was added, mixed well, and kept at 0°C for 30 minutes. The cold TCA-insoluble precipitates were collected on a Millipore filter (pore size 0.45 μ). After drying with an infrared light, the filters were transferred into scintillation vials filled with 15 ml of toluene-based scintillation juice containing 0.5% of 2,5-diphenyloxazole and 0.05% of p-bis(5-phenyloxazoyl)-benzene in toluene, and the radioactivity was counted in a Tri-Carb liquid scintillation spectrometer.

**Results and Discussion.** The effect of dialyzed arginase on DNA synthesis was compared with that of crude liver extract, which was also dialyzed against the same buffer-containing manganese sulfate. As shown in Table 1, arginase did not inhibit DNA synthesis, whereas the crude liver extract, under comparable conditions, showed a marked inhibitory effect.

The inhibitor of DNA synthesis in liver extract is apparently similar to arginase with respect to its heat stability, molecular weight, and behavior on zone electrophoresis (13). Both are

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2Thymidine-2-14C (specific activity 24.4 μc/mmole) was purchased from Calbiochem, Los Angeles, California.
Table 1

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<tr>
<th>Arginase units in assay</th>
<th>cpm</th>
<th>Inhibition (%)</th>
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<tbody>
<tr>
<td>Control</td>
<td>4602</td>
<td></td>
</tr>
<tr>
<td>Liver extract</td>
<td>1250</td>
<td>56</td>
</tr>
<tr>
<td>Arginase</td>
<td>3750</td>
<td>4683</td>
</tr>
</tbody>
</table>

Effect of arginase and crude liver extract on DNA synthesis. Control contained 0.5 ml of 0.01 M Tris-HCl buffer pH 7.6 containing $10^{-4}$ M manganese sulfate.

also present in liver but absent in many hepatomas (5, 12). As already mentioned, arginase inhibits DNA synthesis of tissue culture cells (6). Hence, it was assumed that the inhibitor and arginase are identical.

The present results, however, show clearly that arginase purified from beef liver does not inhibit DNA synthesis during a short-term incubation period, whereas crude liver extract does. Furthermore, birds, like all uricotelic animals, do not have arginase in their livers (4), yet liver extract from chickens was previously found to inhibit DNA synthesis as markedly as did the extract of rat livers (12). These findings strongly support the conclusion that the arginase present in crude liver extract is not responsible for the inhibition of DNA synthesis.

On considering the site of action of this inhibitor, the possibility was raised that it might not, in fact, act on the cell directly, but rather by enzymic destruction of a nutrient in the medium essential for the synthesis of DNA. Since the medium used in this assay consists of Hanks' salt solution supplemented with horse serum, any essential factor which may be required for DNA synthesis would be derived from the horse serum. Hence, an experiment was done without the presence of horse serum. As shown in Table 2, removal of horse serum from the medium effects DNA synthesis only slightly. This result indicates that cells can continue DNA synthesis for at least a few hours in Hanks' salt solution without any supply of serum. Therefore, the inhibition of DNA synthesis by liver extract is not due to the destruction of an essential organic factor in the medium.

Table 2

<table>
<thead>
<tr>
<th>cpm</th>
<th>Inhibition (%)</th>
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<tbody>
<tr>
<td>Control (with horse serum)</td>
<td>5293</td>
</tr>
<tr>
<td>Control (without horse serum)</td>
<td>3061</td>
</tr>
<tr>
<td>Liver extract (with horse serum)</td>
<td>117</td>
</tr>
<tr>
<td>Liver extract (without horse serum)</td>
<td>59</td>
</tr>
</tbody>
</table>

Effect of liver extract on DNA synthesis of Sarcoma 180 cells in the medium with or without horse serum.

Furthermore, the liver extract also inhibits the synthesis of DNA in the serum-free medium (in Table 2), thus ruling out the possibility that the liver extract in the original experiment reacted with serum to produce an inhibitory factor.

The above findings strongly support the conclusion that the inhibitor of DNA synthesis present in liver extract is acting directly on the cell and not on the medium. Further investigations are required on the site and mechanism of the action.

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References

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