IN VITRO SYNTHESIS OF RAT SERUM LIPOPROTEINS AND PROTEINS BY MORRIS HEPATOMA 7777

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Received 31 July 1972

1. Introduction

We previously reported a large increase in the concentration of serum lipids and lipoproteins in Buffalo strain rats bearing transplanted Morris hepatoma 7777 [1]. This elevation in serum lipoproteins could have been contributed by the host liver, by the growing tumor or by both tissues. In an attempt to answer this question, we have investigated the incorporation of \( [U^{14}C] \)L-leucine into five classes of serum lipoproteins by slices of normal liver, host liver and hepatoma 7777. There is very little information in the literature concerning the synthesis of serum lipoproteins by neoplastic tissues [2–4], and this investigation has demonstrated an increased capacity for serum lipoprotein and protein synthesis by Morris hepatoma 7777 as compared to both host liver and normal liver.

2. Methods

Control Buffalo-strain rats (female, ave. wt. 170 g) as well as rats bearing transplanted Morris hepatoma 7777 (generation 62) were supplied by Dr. H.P. Morris. Hepatoma 7777 is a rapidly growing, poorly differentiated tumor [5]. Five weeks after the injection of the tumor, blood was obtained through the abdominal aorta of normal and tumor-bearing rats under Nembutal anes-

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**North-Holland Publishing Company – Amsterdam**
Incorporation of [U-¹⁴C]leucine into serum lipoproteins, serum proteins and tissue proteins by slices of Morris hepatoma 7777, host liver and control liver.

<table>
<thead>
<tr>
<th>Description of tissue</th>
<th>Lipoprotein or protein</th>
<th>Total activity&lt;sup&gt;a&lt;/sup&gt; (cpm/5 ml medium)</th>
<th>Specific activity (cpm/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal liver</td>
<td>VLDL&lt;sup&gt;b&lt;/sup&gt; (d &lt; 1.019)</td>
<td>302 ± 70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>600 ± 126</td>
</tr>
<tr>
<td>Host liver</td>
<td>VLDL&lt;sup&gt;b&lt;/sup&gt; (d &lt; 1.019)</td>
<td>440 ± 158</td>
<td>791 ± 247</td>
</tr>
<tr>
<td>Tumor</td>
<td>VLDL&lt;sup&gt;b&lt;/sup&gt; (d &lt; 1.019)</td>
<td>1779 ± 366 (P &lt; 0.001&lt;sup&gt;c&lt;/sup&gt;, P &lt; 0.001&lt;sup&gt;d&lt;/sup&gt;)</td>
<td>3113 ± 703 (P &lt; 0.005, P &lt; 0.001)</td>
</tr>
<tr>
<td>Normal liver</td>
<td>LDL&lt;sup&gt;b&lt;/sup&gt; (1.019 &lt; d &lt; 1.050)</td>
<td>94 ± 23</td>
<td>134 ± 30</td>
</tr>
<tr>
<td>Host liver</td>
<td>LDL&lt;sup&gt;b&lt;/sup&gt; (1.019 &lt; d &lt; 1.050)</td>
<td>172 ± 48</td>
<td>232 ± 56</td>
</tr>
<tr>
<td>Tumor</td>
<td>LDL&lt;sup&gt;b&lt;/sup&gt; (1.019 &lt; d &lt; 1.050)</td>
<td>635 ± 189 (P &lt; 0.005, P &lt; 0.01)</td>
<td>914 ± 217 (P &lt; 0.005, P &lt; 0.005)</td>
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<tr>
<td>Normal liver</td>
<td>HDL&lt;sub&gt;1&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt; (1.050 &lt; d &lt; 1.063)</td>
<td>174 ± 23</td>
<td>218 ± 27</td>
</tr>
<tr>
<td>Host liver</td>
<td>HDL&lt;sub&gt;1&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt; (1.050 &lt; d &lt; 1.063)</td>
<td>257 ± 63 (P &lt; 0.025)</td>
<td>322 ± 75 (P &lt; 0.025)</td>
</tr>
<tr>
<td>Tumor</td>
<td>HDL&lt;sub&gt;1&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt; (1.050 &lt; d &lt; 1.063)</td>
<td>987 ± 161 (P &lt; 0.001, P &lt; 0.001)</td>
<td>1136 ± 190 (P &lt; 0.001, P &lt; 0.001)</td>
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<tr>
<td>Normal liver</td>
<td>HDL&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt; (1.063 &lt; d &lt; 1.125)</td>
<td>382 ± 80</td>
<td>130 ± 24</td>
</tr>
<tr>
<td>Host liver</td>
<td>HDL&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt; (1.063 &lt; d &lt; 1.125)</td>
<td>887 ± 127 (P &lt; 0.001)</td>
<td>300 ± 51 (P &lt; 0.001)</td>
</tr>
<tr>
<td>Tumor</td>
<td>HDL&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt; (1.063 &lt; d &lt; 1.125)</td>
<td>3022 ± 840 (P &lt; 0.005, P &lt; 0.005)</td>
<td>1090 ± 306 (P &lt; 0.005, P &lt; 0.005)</td>
</tr>
<tr>
<td>Normal liver</td>
<td>HDL&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;e&lt;/sup&gt; (1.125 &lt; d &lt; 1.21)</td>
<td>28 ± 29</td>
<td>29 ± 27</td>
</tr>
<tr>
<td>Host liver</td>
<td>HDL&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;e&lt;/sup&gt; (1.125 &lt; d &lt; 1.21)</td>
<td>158 ± 67 (P &lt; 0.005)</td>
<td>156 ± 64 (P &lt; 0.005)</td>
</tr>
<tr>
<td>Tumor</td>
<td>HDL&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;e&lt;/sup&gt; (1.125 &lt; d &lt; 1.21)</td>
<td>565 ± 207 (P &lt; 0.005, P &lt; 0.005)</td>
<td>596 ± 202 (P &lt; 0.005, P &lt; 0.005, P &lt; 0.01)</td>
</tr>
<tr>
<td>Normal liver</td>
<td>BFP&lt;sup&gt;f&lt;/sup&gt; (d &gt; 1.21)</td>
<td>3846 ± 790</td>
<td>12 ± 3</td>
</tr>
<tr>
<td>Host liver</td>
<td>BFP&lt;sup&gt;f&lt;/sup&gt; (d &gt; 1.21)</td>
<td>18337 ± 8806 (P &lt; 0.025)</td>
<td>56 ± 25 (P &lt; 0.025)</td>
</tr>
<tr>
<td>Tumor</td>
<td>BFP&lt;sup&gt;f&lt;/sup&gt; (d &gt; 1.21)</td>
<td>34217 ± 7408 (P &lt; 0.001, P &lt; 0.025)</td>
<td>110 ± 23 (P &lt; 0.001, P &lt; 0.01)</td>
</tr>
<tr>
<td>Normal liver</td>
<td>Tissue protein</td>
<td>3408 ± 341</td>
<td>3408 ± 341</td>
</tr>
<tr>
<td>Host liver</td>
<td>Tissue protein</td>
<td>5670 ± 1514 (P &lt; 0.05)</td>
<td>27215 ± 4549 (P &lt; 0.001, P &lt; 0.001)</td>
</tr>
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</table>

<sup>a</sup> The total activity given is the radioactivity released into 5 ml of medium in 4 hr.

<sup>b</sup> Mean ± S.D. of five separate determinations from liver or tumor from individual rats.

<sup>c</sup> Compared against control liver.

<sup>d</sup> Compared against host liver.

<sup>e</sup> The HDL<sub>3</sub> fractions were purified by a second ultracentrifugation at density 1.21.

<sup>f</sup> Bottom fraction proteins, i.e., total serum proteins devoid of most lipoproteins.
lier [8]. In brief, after removal of cellular debris by sedimentation at 28,620 g for 20 min, five classes of lipoproteins (very low density lipoproteins, VLDL; low density lipoproteins, LDL; high density lipoproteins, HDL1, HDL2 and HDL3) were ultracentrifugally isolated at 10° using solution densities (1.019, 1.050, 1.063, 1.125 and 1.21 g/ml) at 114,480 g according to the technique of Havel et al. [9]. The lipoproteins and proteins were dialyzed exhaustively against physiological saline containing 0.05% unlabelled leucine, 0.01% merthiolate and 0.005% versene in order to remove free radioactive leucine. Aliquots of the dialyzed fractions were solubilized in NCS reagent and counted at approx. 80% efficiency in a liquid scintillation counter. By measuring the radioactivity of the lipid extracts of the various fractions, it was established that labeled leucine was almost entirely incorporated (between 90 to 95%) into the protein moiety of the lipoproteins. Protein was determined according to the procedure of Lowry et al. [10]. The results have been expressed both as total activity (cpm/5 ml medium) as well as specific activity (cpm/mg protein).

Immediately after incubation, liver and tumor slices were rinsed quickly in ice cold saline, homogenized in cold saline and aliquots were solubilized and counted as described above. Portions of the homogenates were also rapidly treated with hot 80% ethanol in order to extract the free amino acids in the tissues. Aliquots of these extracts were also counted in a liquid scintillation spectrometer.

3. Results and discussion

The total incorporation of radioactive leucine into serum lipoproteins and total serum proteins was many fold greater with tumor slices as compared with normal liver slices. The host liver incorporated labeled leucine into serum proteins and most serum lipoproteins to a much greater extent than control liver but to a lesser degree than the tumor (table 1).

While the total incorporation of the label into VLDL by the host liver was not significantly different from that observed with normal liver, it was several fold increased with Morris hepatoma 7777 (P<0.001). The host liver and tumor slices, respectively, incorporated about 2 and 6 times as much of the label into LDL as compared with normal liver slices. In both cases, the difference between control liver and tumor or host liver was highly significant (P<0.005, P<0.01, respectively). In spite of the increased incorporation into LDL by the host liver, the tumor slice was significantly more active (P<0.01). With the lipoprotein of intermediate electrophoretic motility, HDL1, the incorporation was also significantly greater with the tumor as compared with the control and host liver (P<0.001).

In the case of the principal lipoprotein (HDL2) of rat serum, the total incorporation by the tumor was about 8 times of that incorporated by the normal liver (P<0.005). The host liver was also considerably active (P<0.001, host vs. control) but it incorporated only a third as much as the tumor (P<0.005). In view of the low counts observed with the HDL3 faction from normal liver and also due to high variability of the results in this case, the absolute value for total incorporation may be subject to considerable error. However, it is noteworthy that the ratio of incorporation into this lipoprotein by host liver to that by the tumor paralleled the behavior observed with HDL2.

Disc electrophoresis was performed on several samples from each lipoprotein class isolated from the medium (serum) that was used for the three systems (control liver, host liver and tumor). The amino black patterns were substantially similar to that reported earlier for lipoprotein fractions isolated from normal rat serum [11] and established the absence of any contaminating serum proteins in the various lipoproteins that were used in this study.

The protein synthetic capacity of both the tumor and the host liver was clearly reflected by the enormous increase (4–8 fold) in the total incorporation in total serum proteins (BFP) by these tissues as compared with normal liver (P<0.001, P<0.025, respectively). While the specific incorporation of the label into host liver was only about twice as much as into normal liver protein, it was increased 7 times in the case of the tumor proteins and appeared to reflect the rapid growth rate of hepatoma 7777.

The present results have demonstrated an enormous increase in the total incorporation of labeled leucine into serum lipoproteins and total serum proteins by hepatoma 7777 as compared with normal liver. These results confirm the data obtained in a previous in vitro investigation using hepatoma 7777, its host liver and normal liver conducted under the same con-
In the present experiments, immediately after the in-

puts were not added to the medium. While the radioac-
tivity was greater in all fractions, the trend in the re-
sults, particularly the ratios of incorporation between
tumor and control were similar though not identical.
In the present experiments, immediately after the in-
cubation, the radioactive slices were rapidly homoge-
nized (after quick rinses in cold saline), and treated
with hot 80% alcohol to extract the free amino acids,
which were then separated by chromatography. The
specific radioactivity of free leucine thus isolated was
approximately the same for the three tissues studied
and suggested that the observed increase in radioactiv-
ity of the lipoprotein and proteins with tumor slices
appeared to reflect true synthesis under defined optimum conditions and not merely elevated incorpora-
tion obtained fortuitously.

The present findings are also in agreement with the
increased total incorporation reported earlier with a
primary rat hepatoma induced by N-2-fluorenylacet-
amide [12]. As compared with the corresponding nor-
mal liver, the increased incorporation into LDL, HDL
and BFP was several-fold greater with hepatoma 7777
than with the primary hepatoma, under the same ex-
perimental conditions. Since hepatoma 7777 is a well
"characterized" tumor, it is possible that the increas-
ed incorporation may be related to the absence of nor-
mal and preneoplastic cells that may be expected to
be present in primary hepatomas. On the other hand,
the results may also be explained as due to the differ-
ences in characteristics between hepatoma 7777 and
the primary hepatoma. While the primary hepatoma
was histologically characterized as a well differenti-
ed tumor of the trabecular type [13], hepatoma 7777
is a rapidly growing, poorly differentiated tumor [5].
Obviously, it would be of interest to investigate the in-
corporation of amino acids into serum lipoproteins
and proteins using other Morris hepatomas, especially
those which are medium or well differentiated and
those which have a slower growth rate than hepatoma
7777. Although studies have been conducted on the
incorporation of labeled amino acids into tumor pro-
teins [14], surprisingly there is little information on
the in vitro synthesis of serum proteins by Morris he-
patomas of different growth rates. A correlation be-
tween the growth rate of the tumor and its ability to incor-
porate labeled amino acids into tissues proteins was
reported by Wagle et al. [14]. It, however, remains to
be established whether a similar relationship exists be-
tween the growth rate of tumors and the extent of
synthesis of serum proteins and lipoproteins.

This study clearly showed increased incorporation
into the protein moieties of LDL and HDL and was
in contrast to that reported using cholesterol-fatty liv-
ers [7, 15]. In the latter case, the synthesis of the pro-
tein moiety was unaffected, whereas the secretion of
cholesterol into the medium by fatty liver was increas-
ed as compared with normal liver. Majerus et al. [16]
have reported that, under certain conditions, hepa-
toma 7777 synthesizes fatty acids 7 times as fast as
the host liver. Therefore, it appears likely that the lip-
id moiety as well as the protein moiety of serum lipo-
proteins are synthesized in quantity by hepatoma
7777.

Our earlier work has established that the presence
of a large-sized hepatoma 7777 elicited a substantial
increase in host serum LDL and HDL [1]. From the
increased incorporation into several lipoprotein classes
observed using host liver, it can be stated that the
host liver contributed significantly to the increased
level of lipoproteins in rats bearing hepatoma 7777.
Whether the lipoproteins and proteins synthesized in
vivo by the transplanted tumor were also released into
circulation or were directly utilized by the tumor it-
self cannot be stated at this time.

The greatly elevated synthesis of serum lipoproteins
and proteins by the tumor in vitro may represent a
shunt mechanism because of the obvious lack of cel-
lular growth per se under these conditions. Arguments
have been previously advanced concerning the possi-
ble utilization of serum high density lipoproteins by
dividing cells, perhaps in the assemblage of cellular
membranes [11]. The greatly increased incorporation
into BFP and the predominant lipoprotein of rat se-
rum, namely HDL₂, may suggest utilization of these
proteins in some manner by the tumor. In this con-
text, it may be pointed out that Busch et al. [3, 17]
have demonstrated that labeled albumin was incorpo-
rated to a much greater extent into tumor homoge-
nates, mitochondria and microsomes than into the cor-
responding normal fractions. These and other studies
have led to the suggestion that tumors make use of
plasma proteins, particularly albumin, in the process
of biosynthesis of their tissue proteins [3]. In what
way the tumor is able to elicit a specific response in
the host liver with respect to serum lipoprotein syn-
thesis and what changes within the host liver are responsible for the large increase in serum protein synthesis are fundamental questions that may have a bearing on the control of cancer.

4. Conclusions

The incorporation of [U-14C] L-leucine into rat serum lipoproteins and proteins by rapidly growing Morris hepatoma 7777, its host liver and normal liver was investigated under in vitro conditions. The total incorporation into tissue proteins, serum proteins and lipoproteins was many fold greater with tumor slices as compared with normal liver slices and demonstrated that hepatoma 7777 has the ability to synthesize serum lipoproteins and proteins. The host liver incorporated radioactive leucine into serum proteins and most lipoproteins to a much greater extent than control liver but to a lesser degree than the tumor. The greatly increased incorporation, especially into serum proteins and the high density lipoproteins (HDL2) by both host liver and tumor may suggest direct utilization of these proteins in some manner by the tumor.

Acknowledgements

This investigation was supported by research grants CA-01932 and CA-10727 and a research career development award, SK3-CA-31,063 to K.A.N. from USPHS. Appreciation is due to Mrs. Linda Williams and Mrs. Linda Maynes for their valuable technical assistance.

References