Serum Lipoproteins in Rats with Tumors Induced by 9,10-Dimethyl-1,2-benzanthracene and with Transplanted Walker Carcinosarcoma 256

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SUMMARY

The tumor chemically induced by 9,10-dimethyl-1,2-benzanthracene had 2 pronounced effects upon serum lipoproteins in rats. In the low-density class of lipoproteins floating in a sodium chloride density gradient of 1.063 gm/ml, the flotation (or sedimentation) class 0-10 (S/0-10), usually a single component, was altered such that 2 components appeared after tumors became palpable. In no experiments with normal rats or with rats bearing the transplanted Walker carcinosarcoma 256 was this alteration in S/0-10 observed. The presence of the chemically induced tumor resulted in a significant decrease in the quantity of the high-density lipoprotein which floats in a salt gradient of 1.125 gm/ml.

The transplanted tumor, Walker carcinosarcoma 256, also resulted in a significant decrease in the quantity of the high-density lipoprotein (D < 1.125 gm/ml). In addition, this tumor caused a consistent increase in one of the very low-density lipoproteins in the S/0-20 class (D < 1.006 gm/ml). The relative amounts of phosphatidylcholine and sphingomyelin were increased in lipids extracted from the very low-density lipoproteins of serum from rats with the Walker tumor. There were relatively more triglycerides in lipids in the low-density lipoproteins when rats had this tumor.

Few studies were concerned with the effects of cancer upon serum lipoproteins in man or in the rat. The earliest paper, in 1955 (6), reported that low-density lipoproteins (β-lipoproteins) were increased and high-density lipoproteins (α-lipoproteins) were decreased in serum of patients with cancer. These observations were elaborated by subsequent experiments (5,7), and several confirmatory studies from other laboratories followed (18,20,30). As for individual lipids, Mueller and Watkin (19) observed that plasma unesterified fatty acid concentrations increased in patients with cancer.

INTRODUCTION

Certain definite relationships between lipoproteins, their constituent lipids, and cancer are becoming more evident. Early studies (16), reviewed by Haven and Bloor (15) and by Begg (8), emphasized the pronounced hyperlipemia and accumulation of lipids in tissues from rats bearing the Walker carcinosarcoma 256. Neither the individual classes of lipoproteins nor the individual lipid components were completely characterized in these early experiments.

MATERIALS AND METHODS

Induced Tumor Study on Serum from Rats Fed the Hydrocarbon DMBA

In 2 sequential experiments, I-D and II-D, performed in the same manner, 25 normal and 25 carcinogen-treated female Sprague-Dawley rats were used in each. The first experiment, I-D, extended from May to October, and the second, II-D, from October to April, with blood samples removed every 8 weeks. For induction of tumors, 50-day-old rats were given a single oral dose of 15 mg of DMBA in 1.0 ml of sesame oil [procedure of Huggins and co-workers (17)]. Control rats were fed 1.0 ml of sesame oil at the same time.

All animals were examined 3 times weekly. The number and size of tumors, food consumptions, and body weights were recorded from the 60th to the 160th day after carcinogen or sesame
oil feeding. From each rat approximately 1 ml of blood was withdrawn from the retroorbital venous plexus after a 12-hour fast at the following times: 1 day before administration of DMBA or sesame oil (0 day); then after 60, 100, and 160 days, by which latter times all carcinogen-fed rats had developed tumors. At each period, blood samples from the 25 carcinogen-treated rats were pooled, serum was obtained (about 10–15 ml), and the lipoproteins were separated therefrom. With the same procedures, blood samples were removed from the rats which received only sesame oil, and the serum samples analyzed.

Transplanted Tumor Study on Serum from Rats in Which the Walker Carcinosarcoma 256 Had Been Growing for 14 Days

Four separate experiments were performed in the same manner: I-W in early March; II-W in April; III-W in July; and IV-W in January. In each, 50 CFN Wistar male rats 50 days old were used. In each experiment, 25 rats were implanted by trocar with 0.25 cu cm of standardized fragments of the W256 tumor (28), and 25 rats were reserved as control animals. Fourteen days after implantation the rats were deprived of food for 12 hours, anesthetized with Nembutal, and exsanguinated by cardiac puncture. Samples of 58 ml of pooled serum from control rats and 45 ml of pooled serum from W256 tumor-bearing rats were obtained for analyses in each of the 4 experiments.

Procedures for the Determination of Serum Lipoproteins in Both Induced and Transplanted Tumor Studies

The various fractions and classes of lipoproteins which are present in serum were separated by taking advantage of their different density properties, as the authors described in detail elsewhere (4). Briefly, this involves centrifuging unaltered serum (D = 1.006 gm/ml) in the preparative ultracentrifuge (Spinco model L; 40.3 rotor at 114,000 × g (average)) for 24 hours. The top layer of material is removed quantitatively and comprises the VLDL fraction. The infranatant solution is adjusted to a density of 1.063 gm/ml, with the volume increased to 6.0 ml and the preparative centrifugation repeated. The lipoprotein fraction which floats under these conditions comprises the other low-density group and may be easily removed quantitatively because of its distinctive coloring. The infranatant solution from this separation is increased to 6.0 ml with a salt solution, producing a final density of 1.125 gm/ml, and the preparative centrifugation is repeated. This separates HDL2, the less dense of the 2 high-density lipoprotein fractions. The infranatant from this separation is adjusted to 1.21 gm/ml, again in 6.0 ml, and after centrifugation the high-density lipoprotein, HDL4, floats to the surface of the tube.

As the top fractions are obtained, they are centrifuged in the analytic ultracentrifuge (Spinco model E) and the quantities of lipoproteins determined. Each fraction floating in unaltered serum (VLDL) and LDL can be further divided into classes which have different flotation (or sedimentation) properties, designated as Sf. The VLDL are routinely characterized as Sf 0–20 of VLDL does not contain Sf 10–20 of LDL. In these experiments, the 2 fractions of high-density lipoproteins, HDL2 and HDL4, are separate classes not assigned Sf or −S numbers.

Determination of the Lipid Components in the Lipoprotein Fractions of Serum from Rats Bearing the W256 Tumor in the Transplanted Tumor Study

Each fraction of lipoproteins was dialyzed against distilled water at 4°C to remove excess salt. The dialyzed fractions were immediately extracted with 20 volumes of cold methanol:chloroform (1:2), filtered, and extracted again with the solvent mixture at 24°C. The lipid solutions were evaporated to dryness under nitrogen and then dissolved in toluene. Phospholipids and neutral lipids were separated with thin-layer chromatography by the procedures developed in this laboratory (24–26).

RESULTS

Induced Tumor Study

The usual parameters for observing the effects of a single feeding of DMBA to young rats were seen in the 2 sequential experiments, I-D and II-D. Before the 60th day, the tumor-bearing rats began to consume less food, and by the 100th day the differences in weight between the control and tumor-bearing rats were more pronounced.

With time, the number of tumors arising per rat increased gradually along with a decrease in the number of tumors with diameters < 0.5 cu cm. The average tumor diameter increased. By the 100th day after ingesting the DMBA, the rats had tumors.

Effects of DMBA-induced Tumors upon Serum Lipoproteins. This tumor had no consistent effect upon the quantities of the different classes of the VLDL, the values for which are given in Table 1. Statistical evaluation of the data in both experiments showed that values from rats fed DMBA were not significantly different from those obtained from control rats at any time period.

There was no difference in the total quantities of the LDL in either experiment nor at any time period. However, there was an effect which caused a change in the behavior of the gradient or schlieren boundary observed in the LDL in serum from rats with the DMBA-induced tumor. The Sf 0–10 class as it appears in serum from normal animals can be seen as the rather well-defined single boundary on the photograph in Fig. 1A–II. After tumors developed in these rats, the Sf 0–10 class appeared in the ultracentrifuge as 2 separate boundaries (Fig. 1B–I and B–II). At the 60th day these 2 components appeared only in animals with tumors, and both were within the Sf 0–10 range; that is, the 32- and 23-mg values in Table 1 both represent B-I boundaries on Fig. 1 and have Sf range 6–10. However, by the 100th and 160th days, the B-I boundaries have moved upward more rapidly and are now within the Sf 6–15 range.

The DMBA-induced tumor caused a consistent decrease in the amount of the HDL2 (isolated at D = 1.125 gm/ml and thus separated from the other high-density classes). The values on Table 1 are from the 2 sequential experiments, I-D and II-D, conducted in the induced tumor study. After the 100th day the differences between the mean values from the control and ex-
TABLE 1
Induced Tumor Study: Lipoprotein Values in Serum from Control Rats and Those Fed DMBA* in 2 Separate Sequential Experiments, I-D and II-D

<table>
<thead>
<tr>
<th>Lipoproteins</th>
<th>Treatment</th>
<th>Day 0</th>
<th>Day 60</th>
<th>Day 100</th>
<th>Day 160</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I-D</td>
<td>II-D</td>
<td>I-D</td>
<td>II-D</td>
</tr>
<tr>
<td>VLDL (D &lt; 1.006 gm/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S&lt;sub&gt;f&lt;/sub&gt; 100-400</td>
<td>Control</td>
<td>21</td>
<td>2</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Fed DMBA</td>
<td>17</td>
<td>12</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>S&lt;sub&gt;f&lt;/sub&gt; 20-100</td>
<td>Control</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>Fed DMBA</td>
<td>21</td>
<td>21</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>S&lt;sub&gt;f&lt;/sub&gt; 0-20</td>
<td>Control</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Fed DMBA</td>
<td>14</td>
<td>9</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>LDL (D &lt; 1.063 gm/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S&lt;sub&gt;f&lt;/sub&gt; 0-10</td>
<td>Control</td>
<td>29</td>
<td>36</td>
<td>65</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>Fed DMBA</td>
<td>20</td>
<td>34</td>
<td>23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14</td>
</tr>
<tr>
<td>HDL&lt;sub&gt;a&lt;/sub&gt; (D &lt; 1.125 gm/ml)</td>
<td>Control</td>
<td>85</td>
<td>129</td>
<td>168</td>
<td>152</td>
</tr>
<tr>
<td></td>
<td>Fed DMBA</td>
<td>101</td>
<td>127</td>
<td>157</td>
<td>119</td>
</tr>
<tr>
<td>HDL&lt;sub&gt;b&lt;/sub&gt; (D &lt; 1.21 gm/ml)</td>
<td>Control</td>
<td>90</td>
<td>115</td>
<td>124</td>
<td>157</td>
</tr>
<tr>
<td></td>
<td>Fed DMBA</td>
<td>100</td>
<td>100</td>
<td>121</td>
<td>140</td>
</tr>
</tbody>
</table>

* DMBA, 9,10-dimethyl-1,2-benz(a)anthracene; VLDL, very low-density lipoproteins with density as indicated; LDL, low-density lipoproteins; HDL, high-density lipoproteins; S<sub>f</sub>, flotation (or –sedimentation).

Values are mg/100 ml serum.

<sup>c</sup> These components at Day 60 still remain within S<sub>f</sub> 6-10.

Experimental animals were significant at the 5% level. Although the values of the other ma in high-density fraction studied, HDL<sub>a</sub>, were usually decreased, the differences were not statistically significant.

Transplanted Tumor Study

Effects of W256 upon Serum Lipoproteins. Table 2 shows the quantities of the different lipoproteins determined in sera from normal rats and those with the W256 tumor. The S<sub>f</sub> 0-20 class of the VLDL was the only class to be increased in every experiment, but the total increase in serum from the rats with the W256 was not statistically significant (P < 0.20). Similar results were obtained with the S<sub>f</sub> 0-10 class of the LDL.

The only significant change in the lipoproteins when rats had the W256 tumor occurred in the HDL<sub>a</sub>. In every experiment there was a decreased amount of this component, with significance at the 5% level.

Effects of W256 upon the Relative Amounts of the Different Lipids in the Lipoprotein Fractions. Fig. 2<sup>f</sup> illustrates the relative compositions of phospholipids present in lipids extracted from the different lipoprotein fractions, shown in Table 2, of normal rat serum. These fractions are designated Columns A through E for the lipoproteins with increasingly higher densities. In lipoprotein fractions from normal serum there were greater quantities of phosphatidylcholine, sphingomyelin, and phosphatidylinositol per 1500 µg of lipid applied in all lanes, as the lipoprotein densities increased from VLDL (Column A) to HDL<sub>a</sub> (Column C). HDL<sub>b</sub> (Column D) had very low amounts of these phospholipids.

Lysophosphatidylcholine was present in increasing amounts from VLDL to lipoproteins with D > 1.21 gm/ml (Column E).

Fig. 3<sup>f</sup> illustrates the relative amounts of the neutral lipids present in the 200-µg samples of total lipids applied to the chromatogram for the lipoprotein fractions, as listed on Table 2, and shows the results from normal rat serum. LDL (Column B) and HDL<sub>a</sub> (Column C) contained the greatest relative quantities of cholesterol esters and cholesterol. The other high-density lipoproteins (Columns D, E) also contained cholesterol esters and cholesterol, but in lower amounts.

Hydrocarbons and free fatty acids were present in relatively greater amounts from the LDL to HDL<sub>a</sub> and those with D > 1.21 gm/ml (Columns A to E, Fig. 3<sup>i</sup>). Triglycerides were carried largely by the VLDL (Column A), but smaller amounts were present even in the fraction with D > 1.21 gm/ml (Column E).

Table 2 shows that serum from rats bearing the W256 contained greater amounts of the VLDL (S<sub>f</sub> 0-20 class) than serum from normal rats. This VLDL fraction (Column A, Fig. 2<sup>i</sup>) from rats with the W256 also contained relatively greater amounts of phosphatidylcholine and sphingomyelin than the complementary lipoproteins from normal rats (Fig. 2<sup>i</sup>). The same amounts of lipid, 1500 µg, were applied in both I and II. Fig. 4<sup>i</sup> (Columns 103 AP and 103 AP (tumor)) illustrates a thin-layer chromatogram on which 400 µg of lipids from serum fractions of both control and tumor-bearing rats are chromatographed together. Note the larger areas for phosphatidylcholine and sphingomyelin in Column 103 AP (tumor).

The relative amounts of neutral lipid components observed in all the lipoprotein fractions from rats with tumors are illus-
### TABLE 2

**Transplanted Tumor Study: Effects of the Walker Carcinosarcoma 256 on Serum Lipoproteins**

<table>
<thead>
<tr>
<th>Lipoproteins</th>
<th>Experiments and dates</th>
<th>I-W (March 29)</th>
<th>II-W (April 21)</th>
<th>III-W (June 22)</th>
<th>IV-W (Jan. 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>W256&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Control</td>
<td>W256</td>
<td>Control</td>
</tr>
<tr>
<td>VLDL&lt;sup&gt;a&lt;/sup&gt; (D &lt; 1.006 gm/ml)</td>
<td>(A)</td>
<td>Classes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S&lt;sub&gt;r&lt;/sub&gt; 100–400</td>
<td></td>
<td>13</td>
<td>2</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>S&lt;sub&gt;r&lt;/sub&gt; 20–100</td>
<td></td>
<td>52</td>
<td>25</td>
<td>98</td>
<td>93</td>
</tr>
<tr>
<td>S&lt;sub&gt;r&lt;/sub&gt; 0–20</td>
<td></td>
<td>18</td>
<td>32</td>
<td>39</td>
<td>52</td>
</tr>
<tr>
<td>LDL (D &lt; 1.063 gm/ml) (B)</td>
<td>Class</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S&lt;sub&gt;s&lt;/sub&gt; 0–10</td>
<td></td>
<td>58</td>
<td>100</td>
<td>61</td>
<td>76</td>
</tr>
<tr>
<td>HDL&lt;sub&gt;s&lt;/sub&gt; (D &lt; 1.125 gm/ml) (C)</td>
<td></td>
<td>72</td>
<td>52</td>
<td>83</td>
<td>63</td>
</tr>
<tr>
<td>HDL&lt;sub&gt;s&lt;/sub&gt; (D &lt; 1.210 gm/ml) (D)</td>
<td></td>
<td>61</td>
<td>42</td>
<td>61</td>
<td>83</td>
</tr>
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</table>

<sup>a</sup> Values are mg/100 ml serum.

<sup>b</sup> W256, Walker carcinosarcoma 256; VLDL, very low-density lipoproteins with density as indicated; LDL, low-density lipoproteins; HDL, high-density lipoproteins; S<sub>r</sub>, flotation (or sedimentation).

<sup>c</sup> See Fig. 27.

**TABLE 3**

**Effect of Sex upon the Serum Lipoproteins in Normal 50-day-old Rats**

<table>
<thead>
<tr>
<th>Lipoproteins</th>
<th>Females (Sprague-Dawley)</th>
<th>Males (CFN Wistar)</th>
<th>P value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experiments&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>Ia</td>
<td>II</td>
</tr>
<tr>
<td>VLDL&lt;sup&gt;c&lt;/sup&gt; (D &lt; 1.006 gm/ml)</td>
<td>Classes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S&lt;sub&gt;r&lt;/sub&gt; 100–400</td>
<td></td>
<td>21</td>
<td>17</td>
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<tr>
<td>S&lt;sub&gt;r&lt;/sub&gt; 20–100</td>
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<tr>
<td>S&lt;sub&gt;r&lt;/sub&gt; 0–20</td>
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<td>14</td>
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<tr>
<td>LDL (D &lt; 1.063 gm/ml)</td>
<td>Class</td>
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<td></td>
</tr>
<tr>
<td>S&lt;sub&gt;s&lt;/sub&gt; 0–10</td>
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<td>29</td>
<td>20</td>
</tr>
<tr>
<td>HDL&lt;sub&gt;s&lt;/sub&gt; (D &lt; 1.125 gm/ml)</td>
<td></td>
<td>85</td>
<td>101</td>
</tr>
<tr>
<td>HDL&lt;sub&gt;s&lt;/sub&gt; (D &lt; 1.210 gm/ml)</td>
<td></td>
<td>93</td>
<td>100</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values are mg/100 ml serum.

<sup>b</sup> When the P value is 0.05 or less the difference is usually considered to be significant, i.e., not due to chance alone.

<sup>c</sup> VLDL, very low-density lipoproteins with density (D) as indicated; LDL, low-density lipoproteins; HDL, high-density lipoproteins; S<sub>r</sub>, flotation (or sedimentation).

**TABLE 4**

**Effect of Sex upon the Serum Lipoproteins in Normal 50-day-old Rats**

<table>
<thead>
<tr>
<th>Lipoproteins</th>
<th>Females (Sprague-Dawley)</th>
<th>Males (CFN Wistar)</th>
<th>P value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experiments&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>Ia</td>
<td>II</td>
</tr>
<tr>
<td>VLDL&lt;sup&gt;c&lt;/sup&gt; (D &lt; 1.006 gm/ml)</td>
<td>Classes</td>
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<td></td>
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<tr>
<td>S&lt;sub&gt;r&lt;/sub&gt; 100–400</td>
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<td>14</td>
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<tr>
<td>LDL (D &lt; 1.063 gm/ml)</td>
<td>Class</td>
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<td></td>
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<tr>
<td>S&lt;sub&gt;s&lt;/sub&gt; 0–10</td>
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<td>20</td>
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<tr>
<td>HDL&lt;sub&gt;s&lt;/sub&gt; (D &lt; 1.125 gm/ml)</td>
<td></td>
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<td>101</td>
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<tr>
<td>HDL&lt;sub&gt;s&lt;/sub&gt; (D &lt; 1.210 gm/ml)</td>
<td></td>
<td>93</td>
<td>100</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values are mg/100 ml serum.

<sup>b</sup> When the P value is 0.05 or less the difference is usually considered to be significant, i.e., not due to chance alone.

<sup>c</sup> VLDL, very low-density lipoproteins with density (D) as indicated; LDL, low-density lipoproteins; HDL, high-density lipoproteins; S<sub>r</sub>, flotation (or sedimentation).

The lipid extracted from serum VLDL and LDL (Columns A and B, respectively) of rats with the W256 had relatively more neutral lipid, especially triglycerides, than seen in normal serum (Fig. 3I), from the same quantity of lipid applied, 200 µg. Fig. 4II shows 2 VLDL and LDL fractions from normal serum, 3 AN and 3 BN (200 µg of lipid), chromatographed simultaneously with 108 AN (tumor) and 108 BN (tumor) (200 µg of lipid) and illustrates the differences more clearly. The amounts of cholesterol were the same. Whereas the amounts of phospholipids and neutral lipids appeared to be the same for normal rats and rats with the W256 in the fraction containing HDL<sub>s</sub> (Columns C, Figs. 2, 3), a consistent difference was observed between phospholipids in HDL<sub>s</sub>. Total lipid extracted from HDL<sub>s</sub> of serum from rats with tu-
mors, Fig. 2II (Column D), had somewhat larger areas for phosphatidylcholine and sphingomyelin than observed from normal rats (Fig. 2I, Column D), each from 1500 µg of lipid applied. Compared with serum from tumor-bearing rats, the phospholipids from lipoproteins of normal rat serum were more clearly separated when chromatographed. The difference was particularly striking in the fraction HDL₂ (Column C). The areas for free fatty acids were somewhat smaller when rats had tumors (Fig. 3II, Columns D, E), each from 200 µg of lipid applied.

Effects of Aging and Sex upon the Serum Lipoprotein Levels in Rats. Since one purpose of the studies was to compare the effects of 2 different rat tumors upon serum lipoproteins, certain prevailing conditions known to cause changes in serum lipoprotein levels in normal animals had to be considered (3). In the induced tumor study, Experiments I-D and II-D, serial samples from rats were taken periodically during a 6-month time. Most lipoprotein components in serum from both control and treated rats gradually increased as the rats aged, from Day 0, when they were only 50 days old, to when they were 210 days old. Even though there was a tendency for the quantities of all lipoproteins to increase, the values for HDL₂ were lower when rats had tumors.

The effects of sex are readily visible on Table 3. Serum from male rats had significantly greater quantities of 2 classes of the VLDL, S₀ 20–100 and S₀ 0–20. Male rats also had very high values for S₀ 0–10 of the LDL. In these male rats the presence of the tumor resulted in increased values for 2 of these lipoproteins which are very high in normal male rats. In female rats, cancer had no elevating effect upon either VLDL or LDL, both of which are significantly lower in normal female rats than in normal male rats.

Although the values for serum HDL₂ are significantly different between males and females (Table 3), in both sexes cancer caused a significant decrease in HDL₂ in serum.

DISCUSSION

It is now quite evident that the presence of cancer in an animal has some effect upon the metabolism of serum lipoproteins and lipids. There may be an increase in certain low-density lipoproteins [more pronounced in man (7)], but there is a substantial decrease in HDL₂ in all species studied.

The DMBA-induced tumor is, so far, unique in that an alteration in the physical characteristics of a serum lipoprotein occurs when several of these tumors have become palpable. This represents a qualitative difference in a serum lipoprotein component resulting from the presence of DMBA-induced cancer in the rat. The lipoprotein class affected is included in and comprises the greatest quantity of those frequently termed “β-lipoproteins.” Since other conventional classes of density less than 1.063 gm/ml (β-lipoproteins) are absent in the rat, the S₀ 0–10 class affected is essentially the entire “β-lipoprotein” in serum from rats. Blumberg (9) has suggested the possible relationship between cancer antigens and β-lipoproteins which may be altered as a consequence of the disease. There is some indication of such an alteration (in physical and possibly chemical properties) from the division of the S₀ 0–10 class after numerous large tumors are growing in the rat. The alteration may be in either the lipid or the protein moiety and is probably of metabolic origin rather than a result of in vitro degradation of the lipoproteins (22).

One of the low-density classes which is appreciably increased in serum from patients with breast cancer is the S₀ 10–20 of LDL (7). In serum samples from normal women, this class is usually present, but greater quantities occur when the subjects have cancer. This component is absent from normal rat serum. At the 100th and 160th days after feeding DMBA the faster moving portion, or boundary of the S₀ 0–10 class (LDL), attained flotation characteristics S₀ 6–15, which would cause this class to approach (but not entirely coincide with) the S₀ 10–20 class. This could indicate that the rat with advanced DMBA-induced tumors tends to have a serum lipoprotein class which is not observed in normal rats.

The most consistent effect of both types of cancer in rats and cancer of the breast in women (7) is that HDL₂ is significantly decreased. In this fraction the relative amounts of the individual known lipids are not altered to any extent even though the total amount of lipoproteins is decreased by cancer.

The presence of the Walker tumor results in a relative increase in the phospholipids in HDL₂ even though the total lipoprotein fraction does not increase. This may suggest that the lipid moieties in HDL₂ are changed, but alterations in both lipid and protein moieties of these high-density lipoproteins cannot be excluded.

Although the individual lipids in the transplant tumor study are presented on a comparative basis only, studies in which absolute quantities of the phospholipids and neutral lipids are determined on human serum lipoproteins indicate that the general pattern of lipid compositions of serum from rats and humans is essentially similar in all lipoprotein fractions (23). There is a tendency for the phospholipids (phosphatidylcholine, lysophosphatidylcholine, phosphatidylcholine, and sphingomyelin) and certain neutral lipids (hydrocarbons and free fatty acids) to increase from the lowest density lipoprotein fraction to the high-density fractions. Some lipids, such as triglycerides, cholesterol, and cholesterol esters, decrease. Any alterations resulting from cancer in the rat are also somewhat similar to those observed in quantitative thin-layer chromatographic analyses of lipids extracted from serum of patients with cancer (unpublished observations).

In addition to any changes in serum lipoproteins resulting from the presence of cancer, 3 other possible factors which could affect lipoprotein levels were involved: seasonal variations, aging, and sex. Thorpe (29) has suggested that certain serum lipids (notably cholesterol, which is present in substantial quantities in LDL and HDL₂) are lower in August, September, and February and higher in November-December, March, and April. Fluctuations in the lipoprotein levels should be considered with this in mind, since some of the highest values for LDL in both studies are in December and April, with lower values for HDL₂ in August and February (induced tumor study).

Increasing age results in elevated levels of serum lipoproteins in man (1), and there is a tendency for this to occur in rats. Since aging is a common denominator in the rats in the induced tumor study, the differences observed in their serum lipoproteins do not result from aging. Even though the HDL₂ tends to increase with age, it decreases when cancer is present.

The relationship between sex and the effects of cancer in rats is rather intriguing. In male rats cancer causes an increase in serum lipoproteins, which are usually high when the rat has no
In female rats cancer does not cause an increase in these same lipoproteins, which, in normal female rats, are much lower than in serum from males.

HDL<sub>2</sub> was significantly lower in both studies; therefore it appears that when animals [including man (7)] have cancer, this high-density lipoprotein is either not synthesized or is rapidly metabolized so that the values in serum remain low. Other factors, such as age and sex, which also influence the serum levels of this high-density lipoprotein, do not offset the effects of cancer.

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**REFERENCES**


**Serum Lipoproteins in Rats with Tumors**

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FIG. 1. Photograph of ultracentrifugation of $S_r$ 0-10 (density < 1.063 gm/ml) lipoprotein class shown in $A$ from serum of normal rats. $B$ illustrates presence of 2 classes in serum from rats with the dimethylbenzanthracene (DMBA)-induced tumor, 100 days after ingestion of 15 mg of DMBA. The class on the left, $I$, has an $S_r$ 6-15, while that on the right, $II$, has an $S_r$ 0-6. The amounts of these may be seen in Table 1. The samples were centrifuged simultaneously under the same preparative and analytic ultracentrifugal conditions.
Serum Lipoproteins in Rats with Tumors

Fig. 2. Chromatograms of phospholipids in different fractions of lipoproteins from serum: I, Normal rat serum. II, Serum from rats bearing the Walker carcinosarcoma 256. F, Solvent front containing all neutral lipids, some acidic phospholipids, i.e., cardiolipin, phosphatidic acid, and cerebrosides, if present. Phospholipids: PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PC, phosphatidylcholine (lecithin); Sp, sphingomyelin; LPC, lysophosphatidylcholine; O, origins. Lipoprotein fractions: A, very low-density lipoproteins (VLDL), D < 1.006 gm/ml; B, low-density lipoproteins (LDL), D < 1.063 gm/ml; C, high-density lipoproteins (HDLa), D < 1.21 gm/ml; D, HDLb, D < 1.21 gm/ml; E, D > 1.21 gm/ml. Amounts applied in all fractions, both I and II: 1500 µg. S, standards, 400 µg of rat liver lipids used as a mixture of standards.
FIG. 3. Chromatograms of neutral lipids in different fractions of lipoproteins from serum: I, normal rat serum. II, serum from rats bearing the Walker carcinosarcoma 256. Neutral lipids: HC, hydrocarbons; CE, cholesterol esters; TG, triglycerides; FFA, free fatty acids; DG, diglyceride; CHOL, free cholesterol, MG, monoglyceride; O, origins. Un-1, -2, -3 are unknown compounds. Lipoprotein fractions: A, very low-density (D) lipoproteins (VLDL), D < 1.006 gm/ml; B, low-density lipoproteins (LDL), D < 1.063 gm/ml; C, high-density lipoproteins (HDL), D < 1.21 gm/ml; D, HDL4, D < 1.21 gm/ml; E, D > 1.21 gm/ml. Amounts applied in all fractions, both I and II: 200 µg. S, Standards, 3-10 µg.
Fig. 4. I, Phospholipids of very low-density (D) lipoproteins (VLDL), D < 1.006 gm/ml from sera of normal rats, 5AP; and from sera of rats bearing the Walker carcinosarcoma 256 (W256), 103 AP (tumor). F, see legend for Fig. 2. Phospholipids: PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PC, phosphatidylethanolamine (lecithin); Sp, sphingomyelin; LPC, lyso-phosphatidylcholine; 400 μg of lipid were applied in all lanes. S, standard compounds. II, neutral lipids of 2 low-density lipoprotein fractions, VLDL, D < 1.006 gm/ml, 3AN, and LDL, D < 1.063 gm/ml, 3BN, from sera of normal rats; and from sera of rats bearing the W256, 103AN (tumor) and 103BN (tumor). Neutral lipids: HC, hydrocarbons; CE, cholesterol esters; TG, triglycerides; FFA, free fatty acids; CHOL, free cholesterol; MG, monoglyceride; 200 μg of lipid were applied in all lanes. S, standard compounds.
Serum Lipoproteins in Rats with Tumors Induced by 9,10-Dimethyl-1,2-benzanthracene and with Transplanted Walker Carcinosarcoma 256

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