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 ($S_f$ 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 $S_f$ 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 $S_f$ 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 ($\beta$-lipoproteins) were increased and high-density lipoproteins ($\alpha$-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.

Since 1960 numerous reports on the effects of cancer upon lipids in rats and mice have been published (2, 10-14, 21, 27), the majority of which indicate that the presence of cancer definitely affects lipid metabolism in these animals.

The main purpose of this report is to present information about the effects of 2 different tumors upon lipoproteins and lipids in serum of rats in order to relate the effects with those observed in serum of patients with cancer (7).

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 withdraw
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 lipopro-
tein fractions 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 man-
ner: 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 car-
diae 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 x 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, pro-
ducing a final density of 1.125 gm/ml, and the preparative cen-
trifugation is repeated. This separates HDLs, 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, HDLs, 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
100–400, Sf 20–100, and Sf 0–20 classes. The LDL are charac-
terized as Sf 20–100 and Sf 0–10, at that particular density. Ob-
viously, since these 2 types of lipoproteins have quite different
overall density properties, the Sf classes do not coincide; i.e.,
Sf 0–20 of VLDL does not contain Sf 10–20 of LDL. In these
experiments, the 2 fractions of high-density lipoproteins, HDLs
and HDLs, 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:chlo-
roform (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 cm. The average tumor diameter increased.
By the 100th day after ingesting the DMBA, the rats had tui-
mors.

Effects of DMBA-induced Tumors upon Serum Lipoproteins. This tumor had no consistent effect upon the quanti-
ties 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 ap-
pears 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
components in LDLs. At the 100th day these 2 components ap-
ppeared 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 rep-
resent B-I boundaries on Fig. 1 and have Sf range 6–10. How-
ever, 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 HDLs (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-

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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 100</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) Classes</td>
<td>Control</td>
<td>21</td>
<td>2</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>Sf 100-400</td>
<td>Fed DMBA</td>
<td>17</td>
<td>12</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>Sf 20-100</td>
<td>Control</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td>Sf 0-20</td>
<td>Fed DMBA</td>
<td>21</td>
<td>21</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>LDL (D &lt; 1.063 gm/ml) Class Sf 0-10</td>
<td>Control</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>LDL (D &lt; 1.063 gm/ml) Class Sf 0-10</td>
<td>Fed DMBA</td>
<td>14</td>
<td>9</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>HDL (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>HDL (D &lt; 1.125 gm/ml)</td>
<td>Fed DMBA</td>
<td>101</td>
<td>127</td>
<td>157</td>
<td>119</td>
</tr>
<tr>
<td>HDL (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>HDL (D &lt; 1.21 gm/ml)</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; Sf, flotation (or sedimentation).

Values are mg/100 ml serum.

These components at Day 60 still remain within Sf 6-10.

Experimental animals were significant at the 5% level. Although the values of the other ma in high-density fraction studied, HDLs 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 Sf 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 Sf 0-10 class of the LDL.

The only significant change in the lipoproteins when rats had the W256 tumor occurred in the HDLa. 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. 2f 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 HDLs (Column C). HDLs (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. 3f 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 HDLs (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 HDLs and those with D > 1.21 gm/ml (Columns A to E, Fig. 3f). 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 (Sf 0-20 class) than serum from normal rats. This VLDL fraction (Column A, Fig. 2f) from rats with the W256 also contained relatively greater amounts of phosphatidylcholine and sphingomyelin than the complementary lipoproteins from normal rats (Fig. 2f). The same amounts of lipid, 1500 µg, were applied in both I and II. Fig. 4f (Columns 8 AP and 108 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 108 AP (tumor).

The relative amounts of neutral lipid components observed in all the lipoprotein fractions from rats with tumors are illus-
### TABLE 1

**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>W256b</td>
<td>Control</td>
<td>W256</td>
<td>Control</td>
</tr>
<tr>
<td>VLDL (D &lt; 1.006 gm/ml) (A)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Classes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sf 100-400</td>
<td>13</td>
<td>2</td>
<td>16</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Sf 20-100</td>
<td>52</td>
<td>25</td>
<td>98</td>
<td>93</td>
<td>46</td>
</tr>
<tr>
<td>Sf 0-20</td>
<td>18</td>
<td>32</td>
<td>39</td>
<td>52</td>
<td>28</td>
</tr>
<tr>
<td>LDL (D &lt; 1.063 gm/ml) (B) Class</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sf 0-10</td>
<td>58</td>
<td>100</td>
<td>61</td>
<td>76</td>
<td>52</td>
</tr>
<tr>
<td>HDL (D &lt; 1.125 gm/ml) (C)</td>
<td>72</td>
<td>52</td>
<td>83</td>
<td>63</td>
<td>56</td>
</tr>
<tr>
<td>HDL (D &lt; 1.21 gm/ml) (D)</td>
<td>61</td>
<td>42</td>
<td>61</td>
<td>83</td>
<td>45</td>
</tr>
</tbody>
</table>

*a Values are mg/100 ml serum.

*b W256, Walker carcinosarcoma 256; VLDL, very low-density lipoproteins with density as indicated; LDL, low-density lipoproteins; HDL, high-density lipoproteins; Sf, flotation (or sedimentation).

### TABLE 2

**Effect of Sex upon the Serum Lipoproteins in Normal 60-day-old Rats**

<table>
<thead>
<tr>
<th>Lipoproteins</th>
<th>Females (Sprague-Dawley)</th>
<th>Males (CFN Wistar)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experiments&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Experiments&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>VLDL (D &lt; 1.006 gm/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Classes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sf 100-400</td>
<td>21</td>
<td>17</td>
</tr>
<tr>
<td>Sf 20-100</td>
<td>29</td>
<td>21</td>
</tr>
<tr>
<td>Sf 0-20</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>LDL (D &lt; 1.063 gm/ml) Class</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sf 0-10</td>
<td>29</td>
<td>60</td>
</tr>
<tr>
<td>HDL (D &lt; 1.125 gm/ml)</td>
<td>85</td>
<td>101</td>
</tr>
<tr>
<td>HDL (D &lt; 1.21 gm/ml)</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; Sf, 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. 3). From the same quantity of lipid applied, 200 µg. Fig. 4 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>2</sub> (Columns C, Figs. 2, 3), a consistent difference was observed between phospholipids in HDL<sub>2</sub>. Total lipid extracted from HDL<sub>2</sub> of serum from rats with tu-
mors, Fig. 2f (Column D), had somewhat larger areas for phosphatidylethanolamine and sphingomyelin than observed from normal rats (Fig. 2l, 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. 3j, Column 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, Sf 20–100 and Sf 0–20. Male rats also had very high values for Sf 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 g/ml (β-lipoproteins) are absent in the rat, the Sf 0-10 class affected is essentially the "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 Sf 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 Sf 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 Sf 0-10 class (LDL), attained flotation characteristics Sf 6-15, which would cause this class to approach (but not entirely coincide with) the Sf 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 transplanted 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 (phosphatidylethanolamine, lysophosphatidylethanolamine, lysophosphatidylinositol, 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
cancer. 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.

HDL2 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 fac-
tors, such as age and sex, which also influence the serum levels
of this high-density lipoprotein, do not offset the effects of can-
cer.

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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.
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, phosphatidylerine; PI, phosphatidylinositol; PC, phosphatidylcholine (lecithin); Sp, sphingomyelin; LPC, lyso phosphatidylcholine; O, origins. Lipoprotein fractions: A, very low-density lipoproteins (VLDL), density (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, HDLa, 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 < 1.125 gm/ml; D, HDL3a, 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.
Serum Lipoproteins in Rats with Tumors

Fig. 4. I, Phospholipids of very low-density (D) lipoproteins (VLDL), D < 1.006 gm/ml from sera of normal rats, 3AP; and from sera of rats bearing the Walker carcinosarcoma 256 (W256), 103 AP (tumor). F, see legend for Fig. 2. Phospholipids: PE, phosphatidylethanolamine; PS, phosphatidylycerine; PI, phosphatidylinositol; PC, phosphatidylcholine (lecithin); Sp, sphingomyelin; LPC, lysophosphatidylcholine; 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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