The Interaction between Carcinogenic Hydrocarbons and Serum Lipoproteins

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It has been established that water-insoluble carcinogens and their metabolic products are transported in the blood following their administration to animals (4). Settleri and Ermala found that hydrocarbons, when fed, enter the circulation by a route similar to that of dietary fats (9). These investigators observed fluorescence in chylomicrons in lymph and serum obtained from animals after ingestion of 3,4-benzpyrene.

It has been previously shown that hydrocarbons can be suspended or solubilized in vitro in human or animal serum. Andervont and Lorenz (1) have suspended 1,2,5,6-dibenzanthracene in serum by treatment of the latter with an ethereal solution of the hydrocarbon and subsequent evaporation of the solvent. Under these conditions, however, the low-density lipoproteins must have lost most of the neutral lipides and changed their composition (3). Falk, Miller, and Kotin (6) demonstrated that human plasma will extract polycyclic hydrocarbons from soot. Wunderly and Pezold (11) carried out electrophoresis of serum on paper previously saturated with carcinogenic hydrocarbons and found that the compounds moved in association with albumin and a and β globulins. Chalmers (5) has shown that benzpyrene and other hydrocarbons dissolved in serum in vitro are mainly associated with β-lipoprotein. In a previous study (3) whole serum and isolated serum lipoproteins were found to combine in vitro with cholesterol and other sterols and steroids. Using a similar method we have incorporated several carcinogens into serum. Some studies on the nature and properties of such solutions are reported in this paper.

MATERIALS AND METHODS

2-Nitrofluorene-9-C14 (2-NF), 1.6 × 10^{-2} μc/mg; 2-aminofluorene-9-C14 (2-AF), 7.2 × 10^{-1} μc/mg; and 2-acetylamino fluorene-9-C14 (2-AAF), 9.0 × 10^{-1} μc/mg, were radiochemically pure, as established by paper chromatography with several solvent systems. 1,2-Benzanthracene-9-C14 (1,2-BA), 25 μc/mg, was purchased from Nuclear Chicago Corporation. 1,2,5,6-Dibenzanthracene-9,10-C14 (1,2,5,6-DBA), 1.8 μc/mg; 1,2,3,4-dibenanzthracene-9,10-C14 (1,2,3,4-DBA), 42 μc/mg and 20-methylcholanthrene-11-C14 (20-MCA), 8.0 × 10^{-1} μc/mg, were chromatographically purified and recrystallized from a suitable solvent mixture.

The method employed for incorporation of these materials into serum in vitro was the same as that used previously with cholesterol and other sterols (3). It consisted essentially of incubating, with shaking, serum at 37°C under nitrogen for 16–18 hours with Celite-545, on which the substance to be dissolved was adsorbed. The stimulation of the incorporation rate through the use of Celite as carrier was particularly pronounced with the nonpolar substances. At the end of the incubation the serum was passed through a Scilt microfilter to give a clear and stable solution. Centrifugal fractionation of serum was carried out according to Havel, Eder, and Bragdon (7). A lower-density fraction floating at solvent density 1.063, a high-density fraction floating at solvent densities between 1.063 and 1.21, and material sedimenting at density 1.21 will be subsequently designated as 1.063 T, 1.21 T, and 1.21 B, respectively. Liver cell suspensions were prepared by passing the organ through a tissue press, suspending it in saline, and filtering it through a fine nylon mesh. To remove the subcellular structures the cells were washed several times with saline and sedimented by low-speed centrifugation. They were finally suspended in a bicarbonate buffer pH 7.4, 0.154 M, and aliquots were pipetted for incubation. Radioactivity in serum and tissue samples was determined by treatment with hot alcohol-acetone mixture, 1:1, evaporation of the solvents, and counting of the residues in the Pack-
ard Tri-Carb Scintillation Spectrometer. Quenching corrections were made where necessary.

RESULTS

The amounts of the compounds dissolved as a result of the incubation procedure are given in Table 1. The values were calculated on the basis of radioactivities. Control samples with buffer instead of serum were also included. The hydrocarbons which had insignificant solubility in buffer dissolved to a certain extent in serum. Similarly, 2-AF and 2-AAF, having a limited solubility in buffer, were manyfold more soluble in serum. The concentrations in solutions achieved by in vitro incubation did not depend on the excess present in the solid phase. It was observed that hypercholesterolemic human sera were generally better solvents than normal ones. The results in Table 1 were obtained with normal sera.

Rat sera labeled in vitro with radioactive carcinogens as well as sera obtained from animals previously fed with trace amounts of some of the compounds were fractionated ultracentrifugally (Table 2). The hydrocarbons 1,2,5,6-DBA and 20-MCA were associated mainly with the lipoprotein fractions and not with the bulk of the infranatant proteins; the opposite was true for the more polar 2-AAF.

Rat sera containing radioactive carcinogens incorporated in vitro were administered intravenously to recipient rats, and the disappearance of the label from the circulation was followed with time. The results (Chart 1) show that the compounds employed disappear very rapidly from the circulation and that the 2-AAF is removed at a faster rate than the two dibenzanthracenes.

The availability of the carcinogen to tissue cells was also studied in vitro by following the uptake by liver cells of 1,2,5,6-DBA and 2-AF from a serum solution in the course of an incubation. Under aerobic conditions irregular results were obtained and, to avoid biological oxidation and gradual change of solubility properties of the labeled materials, anaerobic conditions were used.

TABLE 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>Bicarbonate buffer, pH 7.4</th>
<th>Human serum</th>
<th>Rat serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(µg/ml)</td>
<td>(µg/ml)</td>
<td>(µg/ml)</td>
</tr>
<tr>
<td>2-NF</td>
<td>19</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>2-AP</td>
<td>14</td>
<td>229</td>
<td></td>
</tr>
<tr>
<td>2-AAF</td>
<td>9.2</td>
<td>289</td>
<td>200</td>
</tr>
<tr>
<td>1,2-BA</td>
<td>0.1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>1,2,3,4-DBA</td>
<td>0.0</td>
<td>76</td>
<td>19</td>
</tr>
<tr>
<td>1,2,5,6-DBA</td>
<td>0.3</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td>20-MCA</td>
<td>0.1</td>
<td>190</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>Method of incorporation</th>
<th>Centrifugal fraction*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>in vitro</td>
<td>1.063T</td>
</tr>
<tr>
<td>1,2,5,6-DBA</td>
<td>in vitro</td>
<td>1.21T</td>
</tr>
<tr>
<td>20-MCA</td>
<td>in vitro</td>
<td>1.21B</td>
</tr>
<tr>
<td>2-AAF</td>
<td>in vitro</td>
<td>1.063</td>
</tr>
</tbody>
</table>

- 1.063T, a low-density fraction floating at solvent density 1.063.
- 1.21T, a high-density fraction floating at solvent densities between 1.063 and 1.21.
- 1.21B, material sedimenting at density 1.21.

CHART 1.—Disappearance rate of carcinogens from rat sera after intravenous administrations of solutions prepared in vitro. Amounts administered: 1,2,3,4-DBA, 32 µg.; 1,2,5,6-DBA, 18.9 µg.; AAF, 400 µg. The ordinates are expressed as concentrations (µg/cc) per 100 µg. of material injected. First determinations made 2 minutes after administration.
subsequently applied. At the end of the incubation period the cells were washed twice with a large volume of saline, the lipides were extracted twice with hot alcohol-acetone (1:1), and the radioactivity was determined in the extract. Nearly all the absorbed radioactivity was extractable by this method. It was found that the liver cells absorbed, within less than 10 minutes, a certain maximum amount of labeled material, which did not increase on further incubation. The dependence of the uptake by liver cells of the two carcinogens on their concentration in serum is presented in Chart 2. In both cases very nearly straight lines were obtained, revealing that the proportion between the amount combined with the cells and that remaining in solution was constant regardless of concentration.

DISCUSSION

The findings that serum lipoproteins bind carcinogenic hydrocarbons, whereas the more polar derivatives are carried mostly by other serum components, probably albumin, are consistent with the findings of Chalmers (4) and with similar observations with various sterols and steroids (3). Serum obtained from animals fed with 1,2,5,6-DBA or 2-AAF contained the label distributed among the centrifugal fractions in a proportion similar to that found with in vitro preparations. It may be assumed that, apart from the possible chemical changes in part of the circulating carcinogen due to metabolic processes, the two types of preparations are analogous in their physical and biochemical properties. The rapid disappearance of carcinogens observed after injection of preparations made in vitro is compatible with observations that have been made on the rapid metabolism of these compounds when fed (8, 10). No qualitative differences in disappearance rate from circulation were observed between the slightly carcinogenic 1,2,3,4-DBA and the highly active 1,2,5,6-DBA. The 2-AAF, however, was removed more rapidly than the other two compounds. The uptake of carcinogen by tissue cells also takes place in the course of an in vitro incubation, and a rapid equilibrium is established between the carcinogen taken up by the cells and that in the incubating medium. The proportion between the amount of compound combined with the cells and that remaining in solution is constant irrespective of the absolute concentration of the carcinogen. This equilibrium does not depend on respiratory processes of the cells, and it may be the result of competition between the binding sites of serum and tissue. The transport mechanism of carcinogens and the relative affinities of the tissue cells for the various compounds could be conveniently studied with the aid of the soluble preparations described here.

SUMMARY

1. A method is described by which water-insoluble carcinogens are incorporated into serum in a soluble form in vitro.
2. The nonpolar carcinogens are associated mainly with the serum lipoproteins.
3. Carcinogens incorporated into serum by the present method disappear very rapidly from circulation when administered intravenously.

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REFERENCES


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