Carcinogen-Protein Complexes in Hamster Colon and Glandular Stomach during Long-Term Administration of 3-Methylcholanthrene

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ABSTRACT

Intragastric administration of 3-methylcholanthrene (MCA) has been reported by others to cause cancers of the glandular stomach and large intestines in an inbred strain of Syrian golden hamsters. In search of the molecular basis of this process, this study examined the diversity of the macromolecules and carcinogen-protein complexes in colon and glandular stomach cytosols during the long-term administration of MCA to the susceptible hamsters. Male hamsters of the inbred strain BIO 87.20 were gastrically intubated with MCA three times weekly for 0, 1, 3, 6, and 9 weeks or with corn oil (vehicle) for 10 weeks. Thereafter, 24 hr following an i.p. injection of [3H]MCA, the cytosols were prepared, and their constituents were resolved extensively according to molecular size by columns of Sephadex G-200 gel.

The carcinogen-protein complexes of the colon and glandular stomach cytosols were of similar and unchanging molecular size distributions during the 9 weeks of administration of MCA. The principal carcinogen-protein complex in both organ cytosols had a molecular weight of 80,000 to 90,000. The formation of the complex appears to require metabolism of the carcinogen, inasmuch as only relatively small amounts of complex of that size were generated in vitro by incubation of the carcinogen with the organ cytosols at 1–4°C. The major amount of the complex formed in vivo apparently derived from the two organs per se, rather than from the blood therein, which also contained complex of this same molecular size. The MCA-colon and MCA-glandular stomach systems are among a variety of carcinogen-organ systems at present 6 in number, in which the principal carcinogen-protein complex has been found in this laboratory to have a similar molecular size.

INTRODUCTION

Cancers of the colon and stomach are ranked among the most prevalent cancers in humans in many parts of the world. The origin of these cancers is suspected to be nutrition related (8). Despite the considerable magnitude of the public health problem and the evident need for relevant basic research, there is a paucity of animal model systems in which to study the process of oncogenesis in these organs. The rodent glandular stomach, which is structurally related to the human stomach, is not generally susceptible to chemical carcinogens (19, 20). As a consequence, little is known at the macromolecular level concerning oncogenesis in these organs by chemicals (1, 12). In contrast, there is a body of information on the interactions of various carcinogens with the proteins of liver (7, 9, 10, 15, 18), skin (13), and mammary gland (2, 3).

The long-term administration of the polycyclic aromatic hydrocarbon, MCA,3 to male Syrian golden hamsters of an inbred strain (BIO 87.20) has been reported to cause cancer of the colon and glandular stomach in relatively high incidence (5, 6). The present study therefore undertook to examine the types and amounts of carcinogen-protein complexes present in these organs during the course of this process.

MATERIALS AND METHODS

Long-Term Gastric Intubation of MCA. Male Syrian hamsters (86 to 92 days old) of the inbred strain BIO 87.20 (Trenton Experimental Laboratory Animal Co., Bar Harbor, Maine) were fed ad libitum a stock pellet diet (Wayne Lab-Blox; Allied Mills, Inc., Chicago, III.). Starting at the fifth to sixth day of the regimen, each hamster received i.g. a suspension of 4.0 mg of MCA in 0.15 ml of corn oil (Mazola; Best Foods, Englewood Cliffs, N. J.) 3 times weekly by means of a 4.5 cm No. 16 stainless steel needle with a bulb tip (5, 6). The MCA was commercially obtained (Eastman Kodak Co., Rochester, N. Y.) and further purified in near darkness by infiltration and crystallization from a 10% solution in hot benzene. The MCA (m.p. 178–179°) was suspended in the corn oil in amounts sufficient for 2 to 3 weeks and was stored in darkness at 1–4°C between uses. Groups of 6 to 9 hamsters were thus gastrically intubated for 0, 1, 3, 6, and 9 weeks with MCA. A control group of 6 hamsters was similarly given the corn oil without carcinogen for 10 weeks.

Single Injection of [3H]MCA. On the third day after the final i.g. dose, each hamster (102 to 134 g, 0 to 3 weeks; 125 to 166 g, 6 to 9 weeks; 131 to 180 g, 10 weeks) received a single i.p. injection of 100 μCi of generally labeled [3H]MCA (Amersham/Searle Corp., Arlington Heights, III.) in 0.2 ml of dimethyl sulfoxide (spectral grade; Fisher Scientific Co., Pittsburgh, Pa.). The specific activity was indicated by the vendor to be 7.9 Ci/mmol and 95 to 98% pure within 6 months prior to use. Our analyses by thin-layer chromatography on silica gel in n-hexane showed that the purity of 3 different lots of [3H]MCA was 94 to 97% after 1 to 3 months. One lot, assayed by us to be 96% pure 1 week and 1 month before use, yielded carcinogen-protein...
complexes identical with those of the other lots. The animals were then housed in a ventilated chemical hood, fed the above stock diet, and sacrificed 24 hr later. Subsequent operations were carried out at 1-4°.

**Preparation of Colon Cytosol.** In order to obtain the high concentrations of protein that are desirable in extensive molecular sieving (below), in most experiments fresh colon cytosols from a number of animals were used as the medium in which to homogenize additional colons. The suitability of the procedure of serial homogenizations of colon (and glandular stomach, below) was indicated by the general similarity of the molecular size distributions of the macromolecules of the cytosols resulting from 3 serial homogenizations, as compared to diluted cytosols resulting from 1 homogenization. Subsequent experiments were carried out according to the following 2-stage homogenization method.

Three to 4 colons were excised at their small intestinal and anal margins, rinsed free of their contents with 0.25 M sucrose, and slit open. The organs were then washed well 3 additional times with this medium, lightly blotted on clean filter paper, weighed (4 to 7 g), and finely minced. The minces were homogenized in a 30-ml glass-Teflon Potter-Elvehjem apparatus (Size B; A. H. Thomas, Philadelphia, Pa.) in 0.25 M sucrose (1:0.75, w/v) with at least 12 stroke cycles. After centrifugation at 105,000 × g (average) for 20 to 30 min, the supernatant fluids were used as the medium in which to homogenize additional freshly prepared colons (3 to 5) according to the same procedure. The final centrifugation at 105,000 × g (average) for 120 min yielded 4 to 8.5 ml of clear yellow cytosols that contained 22 to 36 mg protein per ml (method of Lowry et al.).

**Preparation of Glandular Stomach Cytosol.** Glandular stomachs were also homogenized in the fresh cytosols of that organ. Three to 4 glandular stomachs were excised at their margins with forestomach and pylorus, opened, and cleaned. The organs were washed well 3 times with 0.25 M sucrose, blotted on filter paper, weighed (1.2 to 2.1 g), and finely minced. The minces were homogenized in a 10-ml glass-Teflon Potter-Elvehjem apparatus (Size A; A. H. Thomas) in 0.25 M sucrose (1:1, w/v) with at least 8 stroke cycles. After centrifugation at 105,000 × g (average) for 20 to 30 min, the supernatant fluids were used as the medium in which to homogenize additional freshly prepared glands (3 to 5) according to the same procedure. The final centrifugation at 105,000 × g (average) for 120 min yielded 4 to 8.5 ml of clear yellow cytosols that contained 22 to 36 mg protein per ml (method of Lowry et al.).

**Preparation of Blood Cytosol.** In 2 experiments (1, 2, 3, the other 9), 3 or 4 of the inbred hamsters, that had not previously received MCA, were given single i.p. injections of [3H]MCA (as above). At sacrifice 24 hr later, blood from jugular veins was collected in the container of a Potter-Elvehjem apparatus (Size C; A. H. Thomas), clotted, and homogenized at 1-4°. Centrifugation at 105,000 × g (average) for 120 min yielded 1.0 to 2.0 ml of supernatant fluid at protein concentrations of 68 to 71 mg/ml (method of Lowry et al.).

**In Vitro Incubation of Organ Cytosols with [3H]MCA.** The colon and glandular stomach cytosols of hamsters treated with MCA for 6 weeks were prepared by the 2-stage homogenization method. Seven ml of the colon cytosol (31 mg/ml) and 1.7 ml of the glandular stomach cytosol (45 mg/ml) were separately incubated with [3H]MCA (added in dimethyl sulfoxide) at a final concentration of 1 µCi/ml (2.4 × 10^-7 M) for 90 min at 1-4° with stirring and in darkness. The cytosols were then directly resolved by molecular sieving in Sephadex G-200 gel, as described below.

**Molecular Sieving of Cytosols.** Filtrations of tissue and blood cytosols through Sephadex G-200 gel were carried out at 1-4° in 0.5 M Tris-Cl buffer, pH 7.5, containing 0.2 M NaCl, as previously described (16). Colon cytosols (above volumes) were applied to columns (175 × 2.5 cm, inside diameter), and were eluted as molecular size profiles at 12 to 15 ml/hr over 3 to 4 days. Protein concentration was measured spectrophotometrically at 280 nm at near maximum sensitivity (2.0 to 2.5-fold) with an automatic recording analyzer (Uvicord II; LKB Produkter AB, Bromma, Sweden). Six-ml fractions of the colon cytosol were usually collected, and radioactivity therein was counted in Aquasol 2 (New England Nuclear, Boston, Mass.) by β-liquid scintillation. The concentration of hemoglobin in the profiles was measured spectrophotometrically at 415 nm.

Glandular stomach cytosols (above volumes) were applied to columns (145 × 1.2 cm, inside diameter) of Sephadex G-200 gel, usually eluted as 3.5-ml fractions and processed as above.

The hamster blood cytosols (1.0 to 2.0 ml) were directly applied to columns (150 × 1.2 cm, inside diameter) of Sephadex G-200 gel, usually eluted as 3.5-ml fractions and processed as above.

**Molecular Weight Determinations.** Molecular weights at the modes of the macromolecular classes in the molecular size profiles of the colon and glandular stomach cytosols were determined by sieving in Sephadex G-200 gel calibrated by use of reference macromolecules. The procedure used was that previously applied to studies of rat liver cytosol (16). The reference macromolecules and their molecular weights were: Blue Dextran 2000 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) at 2 mg/ml (M.W. 2.0 × 10^6); bovine plasma γ-globulin (Fraction II, Lot 43109; Calbiochem, La Jolla, Calif.) at 15 mg/ml (M.W. 160,000); bovine plasma albumin, crystalline (Lot G4802; Armour Laboratory, Chicago, III.) at 15 mg/ml (M.W. 67,000); chicken ovalbumin, crystalline, lyophilized, and salt free (Grade V, Lot 117B-8050; Sigma Chemical Co., St. Louis, Mo.) at 15 mg/ml (M.W. 45,000); horse heart cytochrome c, (NH4)2SO4 and NaCl free (type III, Lot SJC-7515; Sigma) at 8 mg/ml (M.W. 12,384). One or 2 ml of a mixture of the 5 reference substances were applied to the gel column immediately after the elution of each organ cytosol and processed as above. Calculation of molecular weight was based on retardation ratio, i.e., the ratio of the elution volume (V) at the mode of each retarded macromolecular component, relative to the elution volume (V₀) at the mode of the fully excluded macromolecules (Component I) (16).

**RESULTS**

The cytosols of colon and glandular stomach contained an average of 34 and 43% of the protein of their homoge-
nates, respectively.

**Size Classes of Macromolecules.** The colon and glandular stomach cytosols contained a minimum of 5 molecular-size classes of macromolecules (I to V), and at least 2 size classes of small molecules (VI, VII). Representative molecular size profiles and hypothetical Gaussian macromolecular components (11) therein, obtained by molecular sieving in Sephadex G-200 gel, are demonstrated in Charts 1 and 2. Shown are the profiles of colon and glandular stomach cytosols of the inbred hamsters that were not treated with unlabeled MCA (0 week) and those given the unlabeled MCA i.g. for 6 weeks. The protein profiles of the colon and glandular stomach cytosols were generally similar to each other. They were generally unchanged by either the administration of MCA through 9 weeks or corn oil without carcinogen for 10 weeks.

The molecular weights of the macromolecules that were retarded in the gel sieving process are presented in Table 1. Listed are the values at the modes of Components II through V. The molecular weight at the mode of Component I (reference mode) is >250,000. The values were calculated from their corresponding retardation ratios, \( V/V_0 \), with the use of a linear relationship involving the logarithm of the molecular weights of the standard macromolecules (see "Materials and Methods"). The following average retardation ratios and standard deviations were obtained from 10 gel filtrations of the mixture of 5 reference macromolecules: Blue Dextran 2000, 1.0 (reference); \( \gamma \)-globulin, 1.25 ± 0.02 (S.E.); plasma albumin, 1.52 ± 0.03; ovalbumin, 1.67 ± 0.04; and cytochrome c, 2.02 ± 0.04. The decrease in the retardation ratio was linear in a semilogarithmic relationship to an extent reflected by a correlation coefficient of 0.991. The equation of the linear regression was \( Y = -0.689x + 4.85 \), where \( x = \log_{10} \) molecular weight, and \( Y = \) retardation ratio.

**Carcinogen-Protein Complexes Formed in Organ Cytosols in Vivo.** The colons and glandular stomachs of the inbred hamsters formed specific carcinogen-protein complexes in vivo in both organ cytosols 24 hr after i.p. injection of \( [\text{H}] \) MCA. These complexes were detectable from the start through 9 weeks of MCA administration. Charts 1 and 2 demonstrate the representative profiles of the complexes at the start (0 week) and after 6 weeks of intubation of MCA (A and B). Table 2 lists the relative amounts of the carcinogen-protein complexes among the size families of cytosolic proteins, as measured by planimetry of the extrapolated components (11).

One principal size class of carcinogen-protein complex was found in the cytosols of colon and glandular stomach throughout the 9-week interval. This class was at Component II of the molecular size profiles of the constituents and carcinogen-protein complexes in colon cytosols of inbred hamsters. Colon cytosols of the inbred strain BIO 87.20 were resolved by sieving through Sephadex G-200 gel. Absorption as % transmission (%T) at 280 nm [and constituent hypothetical Gaussian components (— — — —)] measured mainly protein in the macromolecular region of the profile, while radioactivity measured the content of carcinogen derivatives associated with the particular molecular sizes. Details are provided in the text. A. Hamsters, not previously treated with MCA (0 week), were given i.p. injections of \( [\text{H}] \) MCA, and sacrificed 24 hr later. B. Hamsters were treated i.g. with unlabeled MCA for 6 weeks and otherwise were treated as in A. C. Unlabeled MCA was gastrically intubated for 6 weeks. Unlabeled cytosol was then incubated in vitro with \( [\text{H}] \) MCA (1 \( \mu \)Ci/ml; \( 2.4 \times 10^{-7} \) M) at 1-4° for 90 min and processed as in A and B. The volume of the eluant fractions differed from those in A and B.

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**Chart 1. Molecular size profiles of the constituents and carcinogen-protein complexes in colon cytosols of inbred hamsters.** Colon cytosols of the inbred strain BIO 87.20 were resolved by sieving through Sephadex G-200 gel. Absorption as % transmission (%T) at 280 nm [and constituent hypothetical Gaussian components (— — — —)] measured mainly protein in the macromolecular region of the profile, while radioactivity measured the content of carcinogen derivatives associated with the particular molecular sizes. Details are provided in the text. A. Hamsters, not previously treated with MCA (0 week), were given i.p. injections of \( [\text{H}] \) MCA, and sacrificed 24 hr later. B. Hamsters were treated i.g. with unlabeled MCA for 6 weeks and otherwise were treated as in A. C. Unlabeled MCA was gastrically intubated for 6 weeks. Unlabeled cytosol was then incubated in vitro with \( [\text{H}] \) MCA (1 \( \mu \)Ci/ml; \( 2.4 \times 10^{-7} \) M) at 1-4° for 90 min and processed as in A and B. The volume of the eluant fractions differed from those in A and B.
Carcinogen-Protein Complexes in Colon and Stomach

Component III. It contained at least one-half of all the in vivo-formed macromolecular complexes in both organ cytosols. The average molecular weight of this principal species of complex was 90,000 in colon and 82,000 in glandular stomach (Table 1). In an attempt to quantitate the overall size distribution of the cytosolic complexes, the portions of Component III complexes that overlapped into adjacent components were arbitrarily assigned to the neighboring Components II and IV. Consequently, Components II and IV seemingly have secondary levels of complexes, i.e., 11 to 16% of the total (Table 2). However, Charts 1 and 2, A and B, show that these complexes are probably due to spillover from those of Component III. In that case, approximately three-fourths of the cytosolic carcinogen-protein complexes are those of Component III and with molecular weights of 80,000 to 90,000 in the 2 organ cytosols throughout the 9-week interval. Most of the remainder of the cytosolic complexes in both organs belonged to the break-

Chart 2. Molecular size profiles of the constituents and carcinogen-protein complexes in glandular stomach cytosols of the same inbred hamsters as in Chart 1. Distributions in A to C refer to experiments with glandular stomach cytosols and match those of Chart 1.
through Component I (M.W. > 250,000). Component VI contained nonmacromolecular labeled carcinogen and its derivatives, while the nonmacromolecular Component VII had essentially none.

**Carcinogen-Protein Complexes Produced in Organ Cytosols in Vitro.** In order to determine whether any of the carcinogen-protein complexes stemmed from simple hydrophobic interactions of unmetabolized hydrocarbon, we allowed [3H]MCA at 1–4° to interact directly in vitro with the colon and glandular stomach cytosols of the inbred hamsters that had been given MCA for 6 weeks. The molecular size profiles of the 2 organ cytosols and the carcinogen-protein complexes therein are shown in Charts 1C and 2C. The relative distributions of the complexes among the molecular size components are presented in Table 2.

The distributions of the carcinogen-protein complexes formed in vitro in colon and glandular stomach cytosols were markedly different from those produced in vivo. Most of the in vitro-formed complexes of [3H]MCA belonged to Components I (breakthrough) and II, which had molecular sizes of >250,000 and ~150,000, respectively. Little difference was noted in the 2 organ cytosols in this regard. Only 1 and 4% of the macromolecular bound [3H]MCA were associated with Component III in the glandular stomach and colon cytosols, respectively (Table 2). It thus appears reasonable to suggest that the in vivo-formed principal complexes with molecular weights of 80,000 to 90,000 may be the products of an interaction between protein and hydrocarbon metabolite(s).

**Carcinogen-Protein Complexes Formed in Blood Cytosol in Vivo.** Because the colon and glandular stomachs of the inbred hamsters were unperfused, it was necessary to examine whether the principal in vivo-formed complexes observed in the organ cytosols were intrinsically from the organs per se or from the blood therein. We therefore examined the carcinogen-protein complexes in 2 cytosol-like preparations of whole blood. Two molecular size families of complexes were present among the blood macromolecules (Chart 3). The minor complex exhibited a retardation ratio of 1.24 in the profile shown (male hamsters), and 1.00 in the other experiment, indicative of molecular weights of 170,000 and >250,000, respectively. In addition, the principal species of complex in the blood cytosols eluted with a retardation ratio of 1.47 in both experiments, characteristic of a molecular weight of 80,000, i.e., a size like that of the principal complex in the cytosols of the 2 organs. The question therefore arose concerning the extent to which the principal complex in the 2 organ cytosols was that of the blood in the unperfused organs. Approximation of the amount of the blood complex in organ cytosols (0 week), based on the content of hemoglobin, indicated that more than 97% of the colon and glandular stomach complexes with molecular weights of 80,000 to 90,000 were of the organs per se, rather than of the blood therein.

### Table 1

**Molecular weights at modes of size classes of cytosolic macromolecules of hamster colon and glandular stomach**

<table>
<thead>
<tr>
<th>MCA (wk)</th>
<th>V/V</th>
<th>M.W.</th>
<th>V/V</th>
<th>M.W.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Component II</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0°</td>
<td>1.15</td>
<td>230,000</td>
<td>1.27</td>
<td>155,000</td>
</tr>
<tr>
<td>6</td>
<td>1.14</td>
<td>240,000</td>
<td>1.28</td>
<td>150,000</td>
</tr>
<tr>
<td>9</td>
<td>1.12</td>
<td>255,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Av.</td>
<td></td>
<td>242,000</td>
<td></td>
<td>153,000</td>
</tr>
<tr>
<td><strong>Component III</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0°</td>
<td>1.43</td>
<td>91,000</td>
<td>1.45</td>
<td>85,000</td>
</tr>
<tr>
<td>6</td>
<td>1.43</td>
<td>91,000</td>
<td>1.46</td>
<td>83,000</td>
</tr>
<tr>
<td>9</td>
<td>1.46</td>
<td>82,000</td>
<td>1.48</td>
<td>77,000</td>
</tr>
<tr>
<td>10°</td>
<td>1.42</td>
<td>94,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Av.</td>
<td></td>
<td>90,000</td>
<td></td>
<td>82,000</td>
</tr>
<tr>
<td><strong>Component IV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0°</td>
<td>1.72</td>
<td>34,500</td>
<td>1.58</td>
<td>55,000</td>
</tr>
<tr>
<td>6</td>
<td>1.71</td>
<td>36,000</td>
<td>1.66</td>
<td>42,000</td>
</tr>
<tr>
<td>9</td>
<td>1.74</td>
<td>32,000</td>
<td>1.61</td>
<td>50,000</td>
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<tr>
<td>10°</td>
<td>1.70</td>
<td>37,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Av.</td>
<td></td>
<td>35,000</td>
<td></td>
<td>49,000</td>
</tr>
<tr>
<td><strong>Component V</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1.94</td>
<td>16,500</td>
<td>1.95</td>
<td>16,000</td>
</tr>
<tr>
<td>9</td>
<td>2.00</td>
<td>13,500</td>
<td>1.86</td>
<td>22,000</td>
</tr>
<tr>
<td>10°</td>
<td>1.96</td>
<td>15,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Av.</td>
<td></td>
<td>15,000</td>
<td></td>
<td>19,000</td>
</tr>
</tbody>
</table>

* The 0-week MCA experiment involved 1-stage homogenization. All other experiments involved 2-stage homogenizations.

**Table 2

**Bound [3H]MCA and its metabolites among cytosolic macromolecules of hamster colon and glandular stomach**

<table>
<thead>
<tr>
<th>MCA (wk)</th>
<th>[3H]MCA</th>
<th>Colon components</th>
<th>Stomach components</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I    II   III   IV   V</td>
<td>I    II   III   IV   V</td>
</tr>
<tr>
<td>0°</td>
<td>In vivo</td>
<td>15   12   58   12  3</td>
<td>19  9   50   15  7</td>
</tr>
<tr>
<td>1</td>
<td>In vivo</td>
<td>11   9    57   16  7</td>
<td>21  13  47   14  4</td>
</tr>
<tr>
<td>3°</td>
<td>In vivo</td>
<td>7    13   54   22  5</td>
<td>21  10  52   16  1</td>
</tr>
<tr>
<td>6</td>
<td>In vivo</td>
<td>15   11   58   14  2</td>
<td>14  13  58  12  4</td>
</tr>
<tr>
<td>9</td>
<td>In vivo</td>
<td>22   53   10   10  3</td>
<td>32  18  37  10  3</td>
</tr>
<tr>
<td>10 (oil)*</td>
<td>In vivo</td>
<td>17   11   45   21  6</td>
<td>21  9   50  14  6</td>
</tr>
<tr>
<td>Av. ± S.D.</td>
<td></td>
<td>13 ± 4 11 ± 2 57 ± 7 16 ± 5 4 ± 2</td>
<td>21 ± 6 12 ± 3 49 ± 7 14 ± 2 4 ± 2</td>
</tr>
</tbody>
</table>

* Experiments involved 3-stage homogenizations. All others involved 2-stage homogenizations.

Corn oil without MCA was gastrically intubated for 10 weeks (see text).
**DISCUSSION**

This report appears to be the initial description of the kinds and amounts of carcinogen-protein complexes that are present in colon and glandular stomach during carcinogenesis in those organs by any chemical. Colon and glandular stomach, which are reported to be susceptible to the carcinogenic action of MCA in inbred hamsters BIO 87.20 (5, 6), contain generally similar molecular size distributions of cytosolic carcinogen-protein complexes. Both cytosols have a major family of complexes of essentially the same molecular size, 90,000 in colon and 82,000 in glandular stomach. The formation of these complexes appears to require the metabolism of the carcinogen, inasmuch as only relatively small amounts of complex of that size were generated in vitro by incubation of the carcinogen with the organ cytosols at 1-4°C.

The molecular size distributions of the carcinogen-protein complexes of colon and glandular stomach exhibited a notable lack of change during the course of the long-term administration of MCA. Of the carcinogen-organ systems thus far studied, only that of N-2-fluorenylacacetamide in liver changed markedly from initially a great diversity of complexes to a remarkably high degree of specificity at 5 weeks of carcinogen feeding (18). All other distributions have not exhibited major changes during the course of carcinogenesis.

The carcinogen-organ systems thus far studied generate carcinogen-protein complexes of two general orders of diversity. At one end are the highly specific distributions of complexes encountered in liver, both with the aminoazo dyes (9, 15, 16) and also after long-term feeding of N-2-fluorenylacacetamide (18). The distributions of carcinogen-protein complexes formed by MCA in colon and glandular stomach (this report) and by the polycyclic aromatic hydrocarbons in mouse mammary glands (2, 3), with their single dominant species of complexes, are somewhat less specific. Additional specific distributions of complexes in other carcinogen-organ systems are cited below. At the other end are the relatively nonspecific distributions of complexes formed in rat liver by aflatoxin B1 (10), ethionine (17), and by N-2-fluorenylacacetamide at the start of carcinogen feeding (18).

This laboratory has accumulated evidence for the apparent presence of a molecular size family of principal target proteins of chemical carcinogens in 6 carcinogen-organ systems during carcinogenesis (4, 14). The cases span 4 types of chemical carcinogens and 4 different organs in 3 species, in the following 6 systems: the principal carcinogen-protein complex formed by the aminoazo dyes in rat liver (15, 16); the principal carcinogen-protein complexes resulting from MCA, 7,12-dimethylbenz[a]anthracene, and benzo(a)pyrene in mammary glands of virgin BALB/c mice (2, 3); and the principal carcinogen-protein complexes formed by MCA in colon and glandular stomach in Syrian hamsters of inbred strain BIO 87.20 (this report). In each system, the complex contains the major portion of the macromolecular-bound carcinogen in the cytosol. The possibility exists that the major target proteins are either identical or are of a family of proteins with similar properties (4, 14). In the case of the principal carcinogen-protein complex formed by the aminoazo dyes, this laboratory recently reported evidence in support of a model in which the principal target protein of the carcinogenic aminoazo dyes acts as a cytoplasmic receptor protein containing activated carcinogen. The complex of protein and carcinogen electrophile is presumed to protect the reactive carcinogen in an intramolecular hydrophobic environment isolated from cellular nucleophiles and to deliver the activated carcinogen to cell nuclei for covalent interaction with critical macromolecule(s) (9). The significance of the finding that in various carcinogen-organ systems the chemical carcinogens react mainly with possibly one target protein or a family of target proteins is yet to be determined.

**ACKNOWLEDGMENTS**

We thank Dovile M. Cooper for assistance in the determination of the content of blood complexes in the organ cytosols.
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