Cytoplasmic Proteins*
A Partial Physical and Chemical Characterization of the Cytoplasmic Proteins

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Sufficient attention has yet to be given to the cytoplasmic proteins in the problem of carcinogenesis. In fact, the mechanism or site of action of the various carcinogens within a normal cell in the process of malignant transformation has, for the most part, remained unknown; and the possibility still exists that carcinogenesis may be an altered protein metabolism. In a recent review of cancer proteins, Toennies (28) has stressed the need for additional information concerning the proteins of normal and malignant tissues. The further examination of these proteins might yield information on the relationship between tissue proteins and those of blood plasma, as well as on the protein differences existing between normal and malignant tissues.

The purpose of the investigations reported here was to establish a procedure for the examination of the soluble tissue proteins which would be rapid, allow a minimum of handling, give uniform results, and permit the isolation of sufficient material for further study. By following the differential centrifugation method of Claude (4), the soluble cytoplasmic proteins can be obtained in the supernate fraction of a tissue homogenate. An adequate characterization of the complex protein mixture found in the supernate requires the parallel application of both physical and chemical methods of analysis. The work has now progressed to a point allowing a preliminary report to be made of the electrophoretic and chemical analyses of two rat fibrosarcoma extracts and of fractions of these extracts prepared by ammonium sulfate precipitation.

Of the two rat fibrosarcomas employed in this study, one was originally induced with benzyopyrene in this laboratory, while the other was induced with methylcholanthrene by W. H. Lewis. These tumors were chosen because of their rapid growth and the high percentage of positive takes on transfer into rats of the Wistar strain. The growths do not become excessively necrotic, and they have a fairly uniform cell population.

During recent years, electrophoretic analyses of tissue extracts have been made by numerous investigators—for example, by Abrams and Cohen (1); Holmes and Morrison (11); Jacob (12); Luck, Nimino, and Tostado (15); Miller, Green, Kolb, and Miller (18); Roberts and White (21); Sorof and Cohen (23); Stern, Reiner, and Silber (24); Taylor, Green, and Cori (25); White and Doughtery (29); and others.

METHODS
The supply of tumors was maintained by inoculating batches of twenty-five rats with tumor material at 3-week intervals. Nitrogen analyses were determined by the micro-Kjeldahl procedure, and nonprotein nitrogen was calculated on the material not precipitated by 10 per cent trichloroacetic acid. Protein concentrations were assumed to be the difference between total nitrogen and the nonprotein nitrogen \(\times 6.25\). Because of the incomplete separation of the phospholipid moiety attached to the precipitated protein, the values for protein nitrogen concentration are somewhat high. The method of Berenblum and Chain (3) was used in determining phosphorus, while pentose- and desoxypentose-nucleic acids were estimated by the orcinol and diphenylamine reactions as modified by Schneider (22). Dry weight was determined by drying to constant weight at 25° C., and pH was measured with the glass electrode. Centrifugal force values were calculated at the centers of the centrifuge tubes.

All electrophoretic experiments were carried out in veronal buffer of pH 8.6 and 0.1 ionic strength at 0.4° C. in the Tiselius apparatus, with the schlieren scanning method of Longsworth (14). Be-
fore electrophoresis, the protein samples were di-
dalyzed by means of a cellophane sack versus two
separate 200-ml. portions of buffer, and finally
the sack was suspended for 24 hours in the bulk
buffer fluid. The dialyzed protein solutions were
clarified, if necessary, by centrifugation. Mobilities
and relative compositions were determined by the
method of Tiselius and Kabat (27) on patterns of
the descending limb.

EXPERIMENTAL

About 18–23 days following inoculation the
tumors were removed and stored on ice after all
the adhering blood vessels, fat, connective tissue
and necrotic parts were eliminated. Two to three
hundred gm. of tissue were collected in 3–5.5

| TABLE 1 |
|-----------------|-----------------|-----------------|-----------------|
| PER CENT OF THE TOTAL NITROGEN AND PHOSPHORUS EXTRACTED FROM THE TISSUES* |

<table>
<thead>
<tr>
<th>EXTRACT MEDIUM</th>
<th>1st extract</th>
<th>2nd extract</th>
<th>Total</th>
<th>Prot. Nitrogen</th>
<th>Total</th>
<th>NPN</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isotonic 0.14 M potassium chloride</td>
<td>36.0 ± 1.2</td>
<td>36.2 ± 1.2</td>
<td>72.2 ± 2.4</td>
<td>29.0 ± 1.4</td>
<td>7.2 ± 0.4</td>
<td>29.0 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>Isotonic 0.25 M sucrose</td>
<td>36.8 ± 1.1</td>
<td>9.6 ± 0.2</td>
<td>46.4 ± 1.3</td>
<td>30.8 ± 0.1</td>
<td>6.9 ± 0.1</td>
<td>37.7 ± 0.1</td>
<td>6.0 ± 0.1</td>
</tr>
<tr>
<td>Isotonic 0.25 M sucrose + 0.03 M sodium bicarbonate, pH 8.0</td>
<td>38.8 ± 1.3</td>
<td>14.4 ± 0.2</td>
<td>53.2 ± 1.1</td>
<td>31.1 ± 0.1</td>
<td>7.8 ± 0.1</td>
<td>38.9 ± 0.1</td>
<td>7.2 ± 0.1</td>
</tr>
<tr>
<td>Bulk 0.14 M potassium chloride</td>
<td>36.8 ± 1.1</td>
<td>9.6 ± 0.2</td>
<td>46.4 ± 1.3</td>
<td>30.8 ± 0.1</td>
<td>6.9 ± 0.1</td>
<td>37.7 ± 0.1</td>
<td>6.0 ± 0.1</td>
</tr>
<tr>
<td>*Results are expressed as the mean ± the standard deviation based on 3 or more values.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| St. dev. = \sqrt{\sum_{n=1}^{N} (x - \bar{x})^2} |

The elimination of most of the unbroken cells, cell
nuclei, cellular debris, and some of the mito-
chondria. After the supernatant fluids were de-
canted, each residue was re-extracted with a fresh
50- to 80-ml. of medium, centrifuged as before,
and the supernates decanted. To remove the
remaining large granules and mitochondria, the first
and second supernates were centrifuged at 3,000 g
for 30 minutes, either separately or as a pool, in
50-ml. centrifuge bottles. These resulting super-
nates were further centrifuged for 2 hours in 50-ml.
lusteroid tubes in a refrigerated angle centrifuge
at 35,000 g. The solution temperature usually rose
from 0° to 9° C. during the last centrifugation. On
the assumption that the mean diameter of the
microsomes is 5 x 10^{-6} cm., it was calculated
that

\[ V = \frac{2/9 \cdot \delta - r}{\eta} \cdot \pi^2 (2\pi S)^2 \times R \text{ cm/sec.} \]

Where R = radius of arm in cm.  
S = No. of rev/sec.  
\( \delta \) = particle density.  
r = medium density.  
\( \eta \) = viscosity in poises.  
\( a \) = radius of particle.
media. The second extracts gave yields, consistent for all media, of 68–74 per cent of the volume used in the re-extraction. On the pooled extracts 50 per cent yields were obtained. These recoveries are low, since only part of the supernates was collected. The total extract volume obtained, in all cases, was between 215 and 375 ml.

All precipitates and residual fluids remaining after each centrifuge operation were added to the original residue fraction. The total time required for preparation of the final extract varied between 13 and 15 hours.

RESULTS

General properties of the tumor homogenate and extracts.—No difference in the dry weight and in the total nitrogen or phosphorus contents of the methylcholanthrene- or benzpyrene-induced tumors could be detected. Tests carried out on the homogenates showed that the tumors contained 15.75 ± 0.20 per cent solids; the solid material contained 14.19 ± 0.57 per cent nitrogen and 2.27 ± 0.01 per cent phosphorus. The amounts of nitrogen and phosphorus extracted by the various media are shown in Table 1. When controlled by the alkaline extraction media, the pH of the homogenates was between 7.7 and 7.8, and when not controlled it was between 6.9 and 7.0.

Electrophoretic properties.—Typical patterns of the extracts obtained from the benzpyrene- and methylcholanthrene-induced tumors are shown in Figure 1. For both tumors the patterns are reproducible from preparation to preparation and are not altered by storage of the extracts in sealed ampoules at —25° C. for 2 months. The mobilities and relative compositions of the designated component groups are shown in Table 3. Patterns of the extracts prepared from the benzpyrene-induced tumors showed the presence of four clearly defined components—i.e., A, A', B, and C: while the patterns of the extracts from the methylcholanthrene-induced tumors showed three —i.e., A, B, and C. Also, different relative compositions for components B and C appeared in the patterns of the two tumor extracts. No differences in the electrophoretic patterns of the first and second extracts of the methylcholanthrene-induced tumors could be detected.

In the region of low mobility where the resolution is poor, the patterns were arbitrarily divided into two component groups, D and E. Consequently, the division of the area in this portion of the patterns was somewhat subjective, and the recorded values for the mobilities and relative compositions of these components have only qualitative significance.

The dissimilarity of the protein distribution in rat plasma to that in the tumor extracts is apparent when their respective electrophoretic patterns are compared (cf. Fig. 1). Several components with similar mobilities are present in both

<table>
<thead>
<tr>
<th>pH*</th>
<th>NPN mg/cc</th>
<th>ΔN NPN mg/cc</th>
<th>Prot. N</th>
<th>Per cent loss</th>
<th>prot. N</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.7† (initial)</td>
<td>0.92</td>
<td>0.458</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.0</td>
<td>1.41</td>
<td>0.49</td>
<td>0.406</td>
<td>10.6</td>
<td></td>
</tr>
<tr>
<td>6.5</td>
<td>1.06</td>
<td>0.14</td>
<td>0.446</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>0.98</td>
<td>0.06</td>
<td>0.435</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>8.0</td>
<td>0.93</td>
<td>0.01</td>
<td>0.437</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>9.0</td>
<td>1.12</td>
<td>0.20</td>
<td>0.438</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>9.5</td>
<td>0.98</td>
<td>0.06</td>
<td>0.439</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>10.0</td>
<td>0.94</td>
<td>0.04</td>
<td>0.436</td>
<td>0.4</td>
<td></td>
</tr>
</tbody>
</table>

* Two of extract ml. 4.5 ml. of Sörensen or carbonate buffer of 0.1 ionic strength, NPN determined, after the addition of 30 per cent trichloroacetic acid, on the centrifuged supernate.
† Values adjusted for 1/7 dilution of the fresh extract.

Table 2

Nonprotein Nitrogen Increase in the Extract Stored at Different pH for 10 Days at 0° C.

At pH 5.75 ± 0.05 all extracts showed an incipient cloudiness and at lower pH yielded a copious precipitate. Pentosenucleoprotein occurred in all the extracts and accounted for 10–15 per cent of the original amount present in the homogenate.

A study was made of the nonprotein nitrogen increase in the extract when stored at various pH for 10 days at 0° C. Extracts kept at a pH < 6.0 showed a gradual precipitation of protein. From the results recorded in Table 2 minima occur for the NPN content at two pH values. However, for storage of the extract, the lower pH 7.8–8.0 is to be preferred, since the more alkaline pH is likely to result in some protein denaturation.

Electrophoretic properties.—Typical patterns of the extracts obtained from the benzpyrene- and methylcholanthrene-induced tumors are shown in Figure 1. For both tumors the patterns are reproducible from preparation to preparation and are not altered by storage of the extracts in sealed ampoules at —25° C. for 2 months. The mobilities and relative compositions of the designated component groups are shown in Table 3.

Electrophoretic patterns of the first extracts were taken at a protein concentration of 1.9–2.6 per cent, while in the second extracts the concentration varied between 1.0 and 1.3 per cent. The protein concentration of the combined extracts was 2.0–2.1 per cent.

Patterns of the extracts prepared from the benzpyrene-induced tumors showed the presence of four clearly defined components—i.e., A', A, B, and C: while the patterns of the extracts from the methylcholanthrene-induced tumors showed three —i.e., A, B, and C. Also, different relative compositions for components B and C appeared in the patterns of the two tumor extracts. No differences in the electrophoretic patterns of the first and second extracts of the methylcholanthrene-induced tumors could be detected.

In the region of low mobility where the resolution is poor, the patterns were arbitrarily divided into two component groups, D and E. Consequently, the division of the area in this portion of the patterns was somewhat subjective, and the recorded values for the mobilities and relative compositions of these components have only qualitative significance.

The dissimilarity of the protein distribution in rat plasma to that in the tumor extracts is apparent when their respective electrophoretic patterns are compared (cf. Fig. 1). Several components with similar mobilities are present in both
patterns, but on the basis of this evidence alone they cannot be considered identical. Plasma from tumorous rats has approximately 8 per cent less \(\gamma\)-globulin than normal rat plasma and shows the presence of the "f-component" reported by Deutsch and Goodloe (5) in plasma of the Sprague-Dawley rats.

The rat plasma electrophoretic patterns were obtained on plasma (dil. 1:2) of both normal and tumor-bearing animals, collected as two separate pools made up of 2 ml. from each of twenty rats. The relative composition values recorded here for electrophoretic analysis. The results of a typical fractionation of a 125-ml. sample of a benzpyrene tumor extract containing 1.9 gm. of protein, using 0.5, 0.75, and saturated 3.87 M ammonium sulfate successively, are shown in Table 4. The electrophoretic pattern obtained for each fraction is shown in Figure 2, and the per cent recovery of each component within the fractions, as compared to the initial amount present, in Table 5.

The altered mobilities of the components within the fractions, as compared to those values obtained in the original extract, may be due to the

### TABLE 3

<table>
<thead>
<tr>
<th>Extract Medium</th>
<th>Extract</th>
<th>Mobilities</th>
<th>Relative Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylocholanthrene-induced fibrosarcoma*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st</td>
<td>2nd</td>
<td>3rd</td>
<td>4th</td>
</tr>
<tr>
<td>Isotonic sucrose (4)</td>
<td>1st</td>
<td>2nd</td>
<td>3rd</td>
</tr>
<tr>
<td>Isotonic potassium chloride (4)</td>
<td>1st</td>
<td>2nd</td>
<td>3rd</td>
</tr>
<tr>
<td>0.5 M potassium chloride + 0.05 M sodium bicarbonate (4)</td>
<td>1st</td>
<td>2nd</td>
<td>3rd</td>
</tr>
<tr>
<td>Isotonic sucrose (2)</td>
<td>1st</td>
<td>2nd</td>
<td>3rd</td>
</tr>
<tr>
<td>Isotonic sucrose (3)</td>
<td>1st</td>
<td>2nd</td>
<td>3rd</td>
</tr>
</tbody>
</table>

### DISCUSSION

It has here been assumed that the extracted proteins are mainly cytoplasmic in origin and fractionation procedure but could also result from the influence of the changed protein composition on the apparent mobilities.

Although relatively little material is precipitated at one-third saturation, the concentration of C in this fraction is high, i.e., 70–75 per cent. As the salt concentration was further increased, fractions containing correspondingly larger amounts of B were obtained. No apparent concentration or preferential separation of the other components was apparent in any of the fractions.

It has here been assumed that the extracted proteins are mainly cytoplasmic in origin and
that the amount of protein material leached from the nuclei, mitochondria, granules and microsomes or contributed by blood is small. Some lymph contamination of the extracts is possible. However, extracts of the tissue made with alkaline media brought considerable amounts of deoxyribonucleoprotein into solution. Microscopic examination of smears prepared from these homogenates and residues indicated that some nuclear dissolution was taking place. Per gram of material extracted, these extracts also gave increased phosphorus and deoxyribonucleic acid values and ultraviolet absorption values at 257 mкр. These effects were especially noticeable in the second extracts. The varying amounts of pentose-nucleoprotein in all the extracts may indicate either an incomplete separation or a partial solubilization of the microsomes.

Because electrolytes tend to agglutinate the cell particulates (20), thereby making sharp centrifuge separation of the fractions difficult, sucrose was substituted in the extraction media (10). However, this substitution did not affect the gross protein yields obtained in the extracts. In order to obtain an extract with a protein concentration suitable for both fractionation and electrophoretic determinations, it was necessary to work with a concentrated homogenate. As efficient centrifugation of the particulates is dependent upon the viscosity of the solution, a 0.35 M sucrose concentration was used for extraction rather than the more desirable 0.88 M (10).

The most outstanding property of the tumor tissue extracts is the remarkable reproducibility of their electrophoretic patterns, both in the number

---

**TABLE 4**

<table>
<thead>
<tr>
<th>CONC. OF AMMONIUM SULFATE</th>
<th>MOBILITIES IN CMS/SEC X 10^{-2}</th>
<th>RELATIVE COMPOSITION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A1</td>
<td>A</td>
</tr>
<tr>
<td>Initial extract</td>
<td>0</td>
<td>11.4</td>
</tr>
<tr>
<td>F-I</td>
<td>0.50 sat.</td>
<td>11.9</td>
</tr>
<tr>
<td>F-II</td>
<td>0.75 sat.</td>
<td>10.8</td>
</tr>
<tr>
<td>F-III</td>
<td>sat.</td>
<td>11.0</td>
</tr>
</tbody>
</table>

**TABLE 5**

<table>
<thead>
<tr>
<th>PER CENT RECOVERY OF ORIGINAL EXTRACT ELECTROPHORETIC COMPONENTS WITHIN THE AMMONIUM SULFATE FRACTIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRACTION</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Original extract</td>
</tr>
<tr>
<td>F-I</td>
</tr>
<tr>
<td>F-II</td>
</tr>
<tr>
<td>F-III</td>
</tr>
<tr>
<td>Total in fractions</td>
</tr>
<tr>
<td>Per cent loss or gain</td>
</tr>
</tbody>
</table>
of components and in their relative compositions. However, the apparent complexity of the extracts and the relatively poor electrophoretic resolution obtained for some of the components leave much to be desired in the analysis of crude tissue extracts. The fast component of mobility $-1.1 \times 10^{-4}$ sq cm/v/sec obtained in the electrophoretic patterns of the benzpyrene-induced tumor extracts may correspond to hyaluronic acid (9, 13, 17). This material has been isolated from mucilaginous tumors and tumor fluids by Kabat (13) and by Meyer and Chaffee (17). These authors (17) claim that hyaluronic acid is elaborated by the tumor cells themselves. The complete solubility of the extract proteins in distilled water also suggests they are pseudo-globulins (8).

The electrophoretic studies made by Abrams and Cohen (1) of human lymphoid tissue and calf thymus, and by Roberts and White (21) of rat lymphoid and lymphosarcoma extracts, showed components with mobilities and relative compositions similar to those reported here. A more recent study (2) of a human lymphosarcoma tissue extract indicated the presence, in appreciable concentration, of a component having a mobility between that of D and E.

The presence of A'B in the benzpyrene-induced tumor, and its absence in the case of the methylcholanthrene, is, therefore, of some interest. At the present time, no conclusions can be drawn from the differences reported here in the relative electrophoretic distributions of the components in the extracts obtained from the two induced rat fibrosarcomas. Whether the carcinogenic agents employed acted on the normal cells by two different pathways or whether two different cell types were involved in the malignant transformation cannot, at present, be determined. Histologically and morphologically, the cells of these two tumors appear identical. Although two similar neoplastic tissues have here been compared, investigations of this type are usually faced with the difficulty of obtaining suitable controls. This situation remains as the stumbling block in making any comparative studies with normal tissues. Certainly, further information concerning the tissue proteins of normal tissues is urgently needed before any generalizations are possible.

It might be of interest to compare the protein distributions of extracts prepared from histologically similar and equally malignant tumors which were induced by a wide variety of carcinogens and also to find out whether the same carcinogen can repeat the same type of transformation. The analysis of the protein distributions in histologically similar tumors of varying degrees of malignancy is also indicated. The number of pathways leading to a malignant transformation may be limited.

**SUMMARY**

Extracts prepared from two rat fibrosarcomas were examined by both physical and chemical procedures. The amount of nitrogen and phosphorus extracted and the nature of the electrophoretic patterns of the extracts were independent of the extraction media employed. Three clearly defined electrophoretic components were observed in the methylcholanthrene- and four in the benzpyrene-induced tumor extracts. The number of electrophoretic components, their mobilities, and their relative compositions were readily reproducible from preparation to preparation, and no
changes in the electrophoretic patterns were detected when the extracts were stored at -20°C for 2 months. If kept at 0°C, the extracts were most stable at pH 7.8-8.0.

Electrophoretic patterns taken on plasma of normal and tumor-bearing rats were similar, except that for the latter animals a slight γ-globulin decrease was indicated. The relative compositions of the electrophoretic components found in the tumor extracts and in normal rat plasma were dissimilar, but several of the components had similar electrophoretic mobilities in both patterns.

The apparent complexity of the extracts and the incomplete electrophoretic resolution obtained suggest that other components are associated with each peak. Fractions were prepared by ammonium sulfate precipitation, and these were electrophoretically examined.

ACKNOWLEDGMENTS

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REFERENCES

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