Ehrlich-reacting Protein in Kidneys of Tumor-bearing Rats*

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SUMMARY

A basic protein has been isolated from the kidneys of Jensen sarcoma-bearing Sprague-Dawley rats which is not present in the kidneys of normal animals of the same strain. The purification and isolation of this protein can be analytically followed because it gives a strong colored reaction with Ehrlich reagent.

The protein, obtained in crystalline form, has been shown to behave as a single component electrophoretically and in the ultracentrifuge. A significant positive correlation has been shown to exist between the amount of this protein present in the kidney extracts and the weight of the tumor.

During the course of an investigation to study the distribution of “free” tryptophan in organs of normal and tumor-bearing animals, it was observed that abnormal levels of tryptophan were consistently obtained from kidney extracts of tumor-bearing animals.

Several methods of protein precipitation were then compared in order to obtain maximum recovery of tryptophan from the organ extracts.

When 1 per cent acetic acid in 95 per cent ethanol was used as a protein precipitant, at room temperature, maximal and consistent values for tryptophan were obtained.

Further study showed that the color reaction given by the kidney extracts with the Ehrlich reagent was only partially due to the presence of free tryptophan; thus only 15 per cent of the total tryptophan calculated from the colorimetric data could be accounted for by paper chromatography.

The remainder of the “chromogenic” material was found to have a different Rf value in our chromatographic system.

This material isolated from the extracts appears to be of protein nature; it gives a strong reaction with the Ehrlich reagent and accounts for 85 per cent of the color obtained in the deproteinized extracts of kidneys from tumor-bearing animals.

By the same extraction procedure from the kidney of normal animals small amounts of proteins are obtained; however, their electrophoretic behavior, as well as their tryptophan content, is different from that shown by the protein isolated from kidneys of tumor-bearing animals. The present paper deals with the isolation and the determination of some physico-chemical properties of the acid-soluble protein isolated from the kidney of tumor-bearing animals.

Our attempt to evaluate a correlation between the presence of this protein in the kidneys and tumor growth will be presented.

MATERIALS AND METHODS

Sprague-Dawley rats maintained on a Purina Chow diet were used for these experiments. Jensen sarcoma implants were introduced by trocar into the axillary region of the animals; the rats were sacrificed at different time intervals by decapitation under chloroform narcosis.

Blood was collected and allowed to clot; the serum was diluted with 2 volumes of M/15 phosphate buffer (pH 7.8). To this mixture an equal volume of 1 per cent solution of acetic acid in 95 per cent ethanol was added to precipitate the bulk of the proteins. Of the organs: spleen, liver, kidney, and tumor were rapidly excised, weighed, and homogenized for 5 minutes in a glass Potter homogenizer with the addition of 2.33 ml. of M/15 phosphate buffer, pH 7.3, for each gram of wet tissue.

The proteins were precipitated by addition to the homogenate of equal volumes of 1 per cent

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acetic acid in 95 per cent ethanol, and the entire suspension was rehomogenized. The final mixture was allowed to stand for 24 hours at 4° C, centrifuged, and the supernatant collected (AES fraction), one ml. of the AES fraction corresponding to 150 mg. of the original tissue. (In Table 1 the isolation procedure is summarized.)

Tryptophan determinations were performed according to Udenfriend (6); nitrogen was determined by the Kjeldahl semi-micro method (3). Ascending paper chromatograms on Whatman No. 1 paper were run with n-butanol saturated with 1 N HCl used as solvent. A Tiselius apparatus was employed for moving boundary electrophoresis, and a Spinco electrophoretic apparatus for paper electrophoresis. The buffers used for paper electrophoresis were all at \( \Gamma/2 = 0.1 \) and were at the following pH values: 4.0, acetate buffer; 5.5, acetate buffer; 7.3, phosphate buffer; and 8.6, veronal buffer.

Paper electrophoresis was carried out at a current intensity of 40 milliamperes and 800 volts for 3 hours.

Moving boundary electrophoresis was carried out with \( \text{pH} \ 5.5 \) acetate buffer \( \Gamma/2 = 0.1 \) for 120 minutes. Ultracentrifugal analysis was performed with a Spinco analytical centrifuge at \( \text{pH} \ 5.5 \) in acetate buffer.

### RESULTS

The average “tryptophan” content found in the AES fractions of various organ extracts from normal and tumor-bearing animals are shown in Table 2. No statistically significant differences can be demonstrated between the two groups of values for most of the organs.

The values found in the AES fractions from tumor tissue were low and were not different from those of some of the normal tissues.

The serum AES fractions were higher in “tryptophan” content in the tumor-bearing animals group, but the variations from the control values were not significant (\( P > 5 \) per cent).

### TABLE 1

**ISOLATION PROCEDURE FOR THE JENSEN SARCOMA KIDNEY PROTEIN**

<table>
<thead>
<tr>
<th>Weighted Tissue</th>
<th>NDF (tumor-bearing rat kidneys)</th>
<th>NDF (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Add 2.33× weight of 1/15M phosphate buffer</td>
<td>Add 6 volumes of absolute ethanol</td>
<td>Dialyze against 55% saturated (NH₄)₂SO₄</td>
</tr>
<tr>
<td>Homogenize for 5 minutes</td>
<td>Let stand 24 hours at 4° C.</td>
<td>Precipitate (JSKP)</td>
</tr>
<tr>
<td>Add equal volume 1% acetic acid in 95% ethanol</td>
<td>Centrifuge</td>
<td>Centrifuge</td>
</tr>
<tr>
<td>Homogenize for 5 minutes</td>
<td>(Supernatant discard)</td>
<td>Precipitate JSKP</td>
</tr>
<tr>
<td>Let stand 24 hours at 4° C.</td>
<td>Dissolve in ( \text{H}_2\text{O} )</td>
<td>Precipitate JSKP</td>
</tr>
<tr>
<td>Centrifuge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supernatant AES</td>
<td></td>
<td>Dialyze against 15% ethanol at 4° C.</td>
</tr>
<tr>
<td>Dialyze against ( \text{H}_2\text{O} )</td>
<td></td>
<td>Crystalline JSKP</td>
</tr>
</tbody>
</table>

* The procedure up to this step has been used also for extracts of organs from normal animals.

NDF = nondialyzable fraction.

JSKP = Jensen sarcoma kidney protein.

However, a statistically significant difference (\( P < 1 \) per cent) is found between “tryptophan” content of the kidney AES fraction extracts from tumor-bearing animals, as compared with the content of the same fraction from normal animals.

An attempt was made to isolate the free tryptophan from the AES kidney fraction of tumor-bearing animals by paper chromatography; only 15 per cent of the color obtained by the Ehrlich reaction could be accounted for as free tryptophan. The

1 Because we do not have definitive proof that the Ehrlich reaction of the isolated protein is given by tryptophan only, the expression “tryptophan” will be used for simplicity and will correspond to the amount of color obtained by the Ehrlich reagent against a tryptophan standard.
rest of the color was given by a "chromogenic" material which in several solvent systems remained at the origin.

Dialysis of the AES fraction from kidney extract for 72 hours at 4° C. against saline removed approximately 15 per cent of the Ehrlich reacting material, a quantity which would correspond to the amount of free tryptophan found by paper chromatography (v.s.). The nondialyzable fraction was precipitated by dialysis against ammonium sulfate at 55 per cent saturation (Jensen sarcoma kidney protein). This precipitate was collected by centrifugation, then was diluted in water, and finally was dialyzed against distilled water.

Jensen sarcoma kidney protein gave a positive Ehrlich and ninhydrin reaction and a weak positive Molish test. Upon alkaline and acid hydrolysis of the Jensen sarcoma kidney protein, a number of amino acids were obtained. A material with the same physico-chemical properties was precipitated directly from the original kidney extracts from tumor-bearing animals by a further addition of 6 volumes of absolute ethanol (Jensen sarcoma kidney protein). When this precipitate was dissolved in water and then dialyzed against slowly increasing concentrations of ethanol (from 10 to 15 per cent by volume) at 0° C., a crystalline material was obtained with identical physico-chemical properties as the nondialyzable fraction (crystalline Jensen sarcoma kidney protein).

This material (crystalline Jensen sarcoma kidney protein) appears to be a basic protein, soluble in water, in acids, and in 40 per cent ethanol in the presence of acids (acetic, hydrochloric, trichloroacetic).

The amorphous precipitate obtained by addition of 6 volumes of ethanol from the kidney extracts, as well as the crystalline material, has been analyzed by paper electrophoresis. From pH 4.0 to pH 8.6 both materials are positively charged and behave as a single component, moving to the cathode. Moving boundary electrophoresis at pH 5.5 has confirmed that the amorphous and crystalline materials are identical.

Ultracentrifuge analysis on both materials showed identical S0 values of 1.85.

When the extraction procedure described above was applied to kidneys from normal animals, the extracts contained a small amount of material which could be precipitated by addition of 6 volumes of ethanol. The yield of this material was about one-third of that obtained from the tumor-bearing animals' kidney extracts, and on dialysis 40–50 per cent of the Ehrlich-reacting material passed through the cellophane bag. A similar percentage of Ehrlich-reacting material was lost by alcohol precipitation of the original extracts.

The nondialyzable fraction from normal kidney extracts contained about 50 per cent less "tryptophan" per mg. of N than the material obtained from tumor-bearing animals. Furthermore, on dialysis against ammonium sulfate this material did not precipitate at a definite salt concentration but behaved as though it were a mixture of proteins. This has been confirmed by electrophoresis, as can be seen in Chart 2, where the boundaries of the two materials are compared.

The concentrations of "tryptophan" found in the kidney extracts (AES fractions) from each of the tumor-bearing animals can be plotted against the corresponding weights of the tumors, and a positive statistical correlation can be observed; a correlation coefficient (r) of 0.710 was obtained (Chart 1).

A similar correlation coefficient was obtained by plotting the nitrogen and "tryptophan" content of the Jensen sarcoma kidney protein precipitates against tumor weight. The correlation curves of "tryptophan" and nitrogen against tumor weight are not parallel but converge. This is shown in Chart 3. The probability that these curves are not parallel by chance is less than 1 per cent.

**DISCUSSION**

It is a commonly accepted notion that the growth of a tumor produces systemic changes in the hosts' metabolism. However, the nature of these changes is relatively unknown, and characteristic differences in composition between tumor-host tissue and normal tissue have seldom been described.

The presence of increased amounts of nitrogenous material (large polypeptides or small proteins) in filtrates of blood deproteinized with tri-
chloroacetic, acetic, perchloric acid, and heat has been described by several authors (1) in cancer patients and in patients with other systemic diseases (4). From the sera of these groups of patients Winzler (5) has isolated a mucoprotein.

From our experience with Jensen sarcoma-bearing Sprague-Dawley rats we have found a general increase of nitrogenous material in all the organ extracts in the AES fraction and the nondialyzable fraction. However, only from the kidney extracts of tumor-bearing animals have we been able to isolate a protein with the characteristics described above. We have not been able to find such a protein in any other organ extract from tumor-bearing animals or from normal organs.

Different rat strains bearing other types of transplantable tumors were analyzed, but the results were negative.

A direct correlation, statistically significant, has been demonstrated between tumor size and the amount of this protein in the kidney of tumor-bearing animals. The fact that this protein is found only in the kidney and not in other organs (at least with the present extraction technic) points to a local effect due to the presence of the tumor in the host. Either the protein is produced by the kidney or it is accumulated there by concentration from the blood. Extracts of the tumor tissue do not appear to contain demonstrable amounts of this protein.

It is too early to speculate as to the biochemical significance of this protein. However, some points can be discussed: The proteins which are present in the kidney of normal animals and remain in the AES-nondialyzable fraction seem to disappear with the growth of the tumor. We do not know how soon they disappear during the growth of the neoplastic mass, and we were not able to demonstrate the simultaneous presence of these proteins and of Jensen sarcoma kidney protein in kidney extracts from tumor-bearing animals in any sample analyzed. Because of the small yield of any other organ extract from tumor-bearing animals or from normal organs.

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Jensen sarcoma kidney protein from the kidneys of one animal, we had to utilize as an analytical tool the "tryptophan" and nitrogen content of the AES-nondialyzable fraction from each animal. Using the data thus obtained (Table 3), we found fairly consistent values for the AES-nondialyzable fractions of kidney from normal animals and an average nitrogen/"tryptophan" ratio of 8.63. The same ratio calculated for AES-nondialyzable fraction from tumor-bearing kidneys gave an average value of 4.86.

This point is of a certain interest if we consider the correlation curves of nitrogen and of "tryptophan" versus tumor weight for all the experimental animals considered as a group in which the normals have tumor weight equal to zero (Chart 2).

The two correlation curves are not parallel, and the slope of the "tryptophan" curve is steeper than that of the nitrogen. In other words, the "tryptophan" content in the AES-nondialyzable fraction of kidney increases faster with tumor growth than does the nitrogen content.

These considerations are in accord with the experimental data of a lower nitrogen/"tryptophan" ratio in the tumor-bearing animals' AES-nondialyzable fractions. However, it is difficult for the moment to give a satisfactory explanation of these data.

One possibility is that, with the growth of the tumor, a protein could be formed containing gradually increasing amounts of "tryptophan." Another possibility could be that a protein which binds tryptophan in increasing amounts forms in the kidney. In this regard McMenamy and Oneley (2) have demonstrated the binding of tryptophan by a fraction of blood serum. Further research is in progress to clarify some of these points.

We feel, however, that the finding of a protein in the kidney of tumor-bearing animals, correlated with the growth of the neoplastic tissue, is of interest. The study of the mechanism of formation of this characteristic protein and its eventual biochemical significance in the host tissue will help to clarify some of the problems of the tumor-host relationship.

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