The Effect of Protein Depletion on the Host Response to Transplantable Rat Tumor Walker 256

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INTRODUCTION

The nutritional state of the host has been established as an important factor in the growth of transplantable rat and mouse tumors (7, 11) and in the induction and spontaneous occurrence of several types of mouse tumors (7, 8, 12, 13, 16). It has been shown that transplantable tumors in the mouse grow at the expense of body nitrogen if insufficient dietary nutrition is available (14, 15). In addition to the influence of the host on the tumor, several changes in the host have been described that apparently result from the presence of a transplantable or induced tumor. The livers of tumor-bearing rats have been shown to increase in weight (6, 18) and water content (6, 9, 10). Increased blood water content has also been described (9). Transplantable granulosa-cell tumors in the mouse may cause an increase in blood volume (4). The present experiments were undertaken to study the liver water and blood volume changes simultaneously in the tumor-bearing rat, and to determine if the host response to the tumor could be modified by protein depletion.

MATERIALS AND METHODS

Young male rats of the Sprague-Dawley strain ranging in weight from 200 to 225 gm. were used in these experiments. Protein depletion was accomplished by the method of Cannon and his group (17). This consisted of feeding a diet adequate in all nutritional factors but protein for 8–12 weeks, until the animals had lost 25–35 per cent of their pre-depletion body weight. The depletion diet contained about 2 per cent protein as carrots, brewer’s yeast, and liver powder concentrate, and it was adequate in vitamins, minerals and calories. Nondepleted control rats of the same age were maintained on a high protein casein diet or Purina Laboratory Chow until the experiments were begun. During the experimental period, synthetic diets were used. The depleted animals were fed a low protein diet that contained less than 1 per cent of protein. The nondepleted animals were fed a diet containing 92% casein.

The tumor used in these experiments was the transplantable Walker tumor 256, which was inoculated in 0.2-cc. amounts of a sterile saline suspension into the skin of the back. The details of the technic are given elsewhere (5). Beginning on the fourth day after transplantation and extending until the fourteenth day, the tumors were measured in three dimensions by calipers to obtain an estimate of the volume. The estimates of tumor volume were plotted on log-log graph paper to obtain straight line growth curves. In this way the slopes of the curves of different experiments could easily be compared. The experimental period

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lasted for 14 days after the inoculation of the tumors.

On the day of inoculation of the tumors, depleted and nondepleted animals of the same age and nutritional state as the experimental animals were sacrificed as base-line controls after serum protein concentration and hemoglobin concentration had been obtained. On the fourteenth day of tumor growth, the serum protein concentration, blood volumes, and hemoglobin concentration were determined in both tumor-bearing and non-tumor-bearing animals which then were sacrificed by exsanguination after light ether anesthesia. The tumors and liver were excised and weighed, the gastro-intestinal tract was excised and cleaned of retained food and fecal material. The carcass weight was obtained by including the cleaned gastro-intestinal tract with the carcass and remaining viscera. Duplicate samples of liver and tumor were analyzed for water, fat, water-soluble fraction content, and protein residue by a rapid gravimetric method. The water content was determined by drying to a constant weight at 70° C., the fat content by hot extraction by commercial absolute alcohol followed by cold ether extraction. The water-soluble fraction was obtained by water extraction at 70° C. after the removal of alcohol and ether-soluble material. The residual dried sample consisted of "protein residue." The gravimetric method protein, as determined by the Kjehldahl method, using the factor N X 6.25, ranged between 90 and 95 per cent of the "protein residue." In the data presented, the results of the liver analysis are expressed in per cent, corrected for the fat-free weight of the liver. Previous experiments have shown that fat is the most variable component, and comparability of values of non-lipid constituents is attained when expressed on a fat-free basis.

Carcass analysis was performed by a similar procedure. The protein residue values include the ash content of the carcasses and are expressed as fat-free dry weight.

Serum protein concentration was determined by the falling drop method (1) and hemoglobin concentration with the Dick-Stevens hemoglobinometer. The total blood volumes and plasma volumes were determined by the Benditt et al. modification of the Evans blue dye technic for rats (2).

The data from two separate experiments were pooled and combined in the final evaluation of results, since both procedures and results were comparable in each separate experiment.

EXPERIMENTS AND RESULTS

The results are summarized in Table 1.

Effect of protein depletion on the growth of Walker 256 tumor.—Protein depletion of the host had a profound effect on the growth of the tumor. The maximum tumor weight obtained in the depleted animals was 7.04 gm., compared with a maximum of 31.72 gm. in the nondepleted group. The state of the host apparently did not affect the rate of growth of the tumors but only prolonged the latent period. Figure 1 is a graph of the separate growth curves defined by plotting the estimated volumes of the tumors against time. It can be seen that, although the tumors from depleted animals were always smaller, the slope of the points was approximately the same.

Comparison of the chemical analyses of the tumors from depleted and nondepleted animals revealed a significant lowering of protein content of the depleted tumors compared to those of the non-depleted animals. Tumors from depleted animals showed consistently less necrosis in their center and hence had less extra-vascular leakage of blood and plasma.

Effect of protein depletion on changes in liver composition associated with Walker 256 tumor.—The presence of the tumor in nondepleted rats was associated with a gross increase in liver wet weight compared with nontumor-bearing animals. The mean weight of the livers from tumor-bearing rats was 8.27 gm., compared with the nontumor-bearing control liver weight of 6.33 gm. This increase in liver wet weight was not observed in the depleted tumor-bearing animals. These did not differ significantly from the depleted controls, as is shown in Table 1.

Associated with the increased wet weight of the livers of the nondepleted tumor-bearing animals, there was an increased water content; this was 76.03 per cent compared to 74.28 per cent for the controls. The livers of all depleted animals showed a higher water content than those of the non-depleted group; however, those from tumor-bearing animals also had a greater water content than those from the controls—78.91 per cent, compared to 77.3 per cent.

The liver protein residue was reduced in the presence of the tumor in both depleted and non-depleted animals. The values were 20.38 per cent for the nondepleted tumor-bearing animals, compared to 22.35 per cent for the controls. Depleted tumor-bearing and control protein residues were 17.23 per cent and 18.94 per cent, respectively.

The water-soluble fraction of the liver was 77.3 per cent compared to 76.03 per cent for the controls. The livers of all depleted animals showed a higher water content than those of the non-depleted group; however, those from tumor-bearing animals also had a greater water content than those from the controls—78.91 per cent, compared to 77.3 per cent.

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### TABLE 1

**SUMMARY OF DATA ON THE EFFECT OF PROTEIN DEPLETION ON HOST PHYSIOLOGY IN THE TUMOR-BEARING RAT**

|                | Number of Animals | Wt. of Baseline
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<td></td>
<td></td>
<td>Wt.†</td>
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<td><strong>Blood</strong></td>
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<td>Serum prot.</td>
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<td>concentration</td>
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<td>Plasma vol.</td>
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<td>(gm.)</td>
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<td>Red cell vol.</td>
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<td>(cc.)</td>
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<td>TCSP †</td>
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<td>(mg.)</td>
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<td>% per cent</td>
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<td><strong>Hemoglobin</strong></td>
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<td>(gm. per cent)</td>
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<td><strong>Carcass</strong></td>
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<td>Wet wt.</td>
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<tr>
<td>Water</td>
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<td>Fat</td>
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<td>Fat-free dry wt.</td>
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#### Depleted:
- **Base line**: 9, 146.41
  - Mean Wet wt.: 132.61, 85.60, 12.70, 34.31
  - S.E.: 4.32, 2.30, 0.72, 0.94
  - Mean No tumor: 8, 151.13, 138.0, -13.13
    - S.E.: 4.21, 1.70, 1.61, 0.79
  - Mean With tumor: 20, 147.20, 136.75, -14.92
    - S.E.: 2.11, 1.30, 0.92, 0.44
    - P value: <0.001

#### Nondepleted:
- **Base line**: 10, 228.68
  - Mean Wet wt.: 306.12, 139.33, 19.67, 47.18
  - S.E.: 2.83, 2.46, 1.27, 0.47
  - Mean No tumor: 10, 232.30, 238.0, + 5.70
    - S.E.: 4.18, 2.28, 1.01, 0.70
  - Mean With tumor: 20, 231.85, 255.35, + 4.04
    - S.E.: 2.62, 1.74, 1.18, 1.07

#### Liver Analysis
- **Net Wt. Change**: 146.41
- **Mean Wet Wt.**: 4.37, 77.42, 4.21, 18.92
- **Per cent Water-sol. solids**: 0.88, 0.35, 0.14, 0.31
- **Per cent protein residue**: 0.20, 0.24, 0.16, 0.32
- **Per cent fat**: 0.00, 0.00, 0.00, 0.00

#### Tumor Analysis
- **Net Wt. Change**: 146.41
- **Mean Wet Wt.**: 7.60, 71.05, 2.90, 23.05
- **Excess wt.**: 0.61, 0.15, 0.14, 0.15
- **Per cent protein residue**: 6.53, 74.28, 3.17, 22.53
- **Per cent water-sol. solids**: 0.083, 0.082, 0.08, 0.15
- **Per cent fat**: 0.17, 0.51, 0.08, 0.17
- **P value**: <0.001

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* All weights expressed in grams.
† Net weight of tumor-bearing animals equals total weight minus tumor weight.
‡ All carcass data expressed in grams.

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* Total circulating serum protein.
† Significance of difference of the means of tumor-bearing and nontumor-bearing rats.
‡ Not statistically significant.
slightly increased in the nondepleted tumor-bearing animals only. No difference was noted in the depleted animals. Analysis for fat revealed a slight increase in the tumor-bearing nondepleted animals. Fat levels were similar in the livers of depleted animals with and without tumors.

**Effects of the Walker tumor on blood volume and plasma protein.**—In the nondepleted tumor-bearing rats, there was a consistent increase in plasma volume. The mean plasma volume was 10.86 cc., as compared to 9.11 cc. for the controls. The red cell volume and hemoglobin concentrations were diminished. Associated with the increased plasma volume, there was a decrease in serum protein concentration, although the total circulating serum protein was not affected. In the protein-depleted rat the presence of a tumor had no effect on the plasma volume response, and there was no change in the red cell volume or hemoglobin concentration. However, the serum protein concentration and total circulating serum protein were diminished in the tumor-bearing depleted animals.

**Effect of protein depletion on the carcass of tumor-bearing rats.**—Presence of the tumor did not consistently alter the chemical content of the carcass of either the depleted or nondepleted animals, except for a diminution of carcass protein residue and wet weight in the depleted group.

**DISCUSSION**

The protein-depleted state has a profound effect on many physiological activities. Since a tumor-bearing animal on a low protein diet has a strictly limited exogenous source of protein and is in negative nitrogen balance, it must supply the requirements of the tumor by depleting its own tissues. It is not surprising that the maximum size of the tumors is much smaller than those in nondepleted hosts. With the smaller tumors in the depleted animals one might expect a less profound effect of the tumor upon the host. This is seen in these experiments by the failure of the liver to increase in weight in the depleted tumor-bearing animals and by the failure of the tumors to elicit an increased plasma volume and hemodilution. It is possible that our methods failed to detect minor changes in plasma volume.

The effect produced on the host may be a simple function of the mass of the tumor. Yeakel (18) noted that the weight of the livers of tumor-bearing rats was a function of tumor weight and body length and that the effect was greater in females than in males. McEwen and Haven (6) observed a significant increase in the percentage of water in the livers of rats bearing the Walker 256 tumor. A critical tumor weight of 10 gm. or more was thought to be necessary before liver water content was increased. Schlottman and Rubenow (9) found a higher percentage of water in the livers of rats bearing the Jensen sarcoma and the Flexner carcinoma. In their experiments the increased liver water was associated with an increased water content of the blood, while the wet weight of the livers was not increased. The present study has confirmed the increase in liver water of tumor-bearing rats, both depleted and nondepleted. Our experiments have shown that the water content of the rat liver was increased in nondepleted rats with large tumors and in depleted animals whose mean tumor weight was less than 5 gm.

Furth and Sobel (4) reported that greatly increased blood volume occurred in mice bearing some types of granulosa-cell tumors. They found an absolute increase in both plasma and red cell volume and suggested that the hypervolemia might be related to a substance elaborated...
by the granulosa cells of the tumors. Mice bearing mammary tumors of the same size or leukemia did not show the increase in blood volume. In the present study increased plasma volume with decreased red cell volume was observed only in the nondepleted tumor-bearing rats. The determining factor here may be tumor size, since the tumors in the depleted animals were much smaller. The increased plasma volume does not appear to be a compensatory mechanism for the lowering of serum proteins, but rather a phenomenon of hemodilution, since the total circulating serum protein was not significantly changed.

The lowering of the liver protein residue in both depleted and nondepleted tumor-bearing animals is to be expected, since the liver is a ready source of immediately available protein. In the depleted animals, the carcass appears to supply the majority of the protein for tumor protein synthesis. Obviously, the high protein intake provided a primary source in the nondepleted animals.

There is a significant difference in protein residue of the tumors of the depleted and nondepleted hosts. The tumors from depleted animals appear to contain less protein (Table 1). The cause of this difference is not apparent, but it seems likely that more necrosis and hence more leakage of blood into large tumors would give them an apparently higher total protein content.

Statistical correlation of the important variables in these experiments clarifies these differences (3). The pooled correlation for all depleted and nondepleted animals of the liver weight with the plasma volume, tumor weight, or carcass weight was approximately the same: 0.562, 0.565, and 0.548, respectively. The plasma volume was highly correlated with the tumor weight (0.669) and showed essentially no correlation with the carcass weight (0.297), nor was the tumor correlated with the carcass weight (−0.0349). By partial correlation it was found that the correlation of plasma volume and weight of the tumor increased to 0.710 at constant carcass weight. This analysis indicates that the plasma volume and the liver water changes are closely related to the presence of the tumor. The increased plasma volume in the animals bearing large tumors may simply be an attempt to keep full the rapidly expanding vascular bed of the tumor, and the increment may approximate the volume of the vascular bed of the tumor. Other factors which might cause a rise in plasma volume by hormonal mechanisms cannot be excluded. About 75 per cent of the difference in liver weight between nondepleted tumor-bearing and control rats is due to the increased water content of the livers of the tumor-bearing rats.

**SUMMARY**

The effects of protein depletion on tumor growth and the response of the host to the presence of transplantable Walker 256 tumor were studied in rats. Protein depletion had a profound effect on the growth of the tumor in a 14-day period. Tumors in rats depleted of protein were less than one-fourth as large as those in nondepleted rats. The primary effect seemed to be on the latent period of the tumors of the depleted animals, since the relative rates of growth of tumors in depleted and nondepleted rats were comparable. In the nondepleted tumor-bearing animals, there was an increased plasma volume, liver wet weight, and increased water content of the liver. The presence of the tumor in the depleted host did not cause an increase in plasma volume or liver wet weight, but it was associated with an increased water content of the liver. Total circulating serum protein was not altered in conjunction with the increased plasma volume of the nondepleted rats. The concentration of liver protein was decreased in both the nondepleted and depleted tumor-bearing animals.

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**REFERENCES**

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