The Weight and Lipid Content of the Intestines in Rats with Walker Carcinoma 256*

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Patients with severe cancer lose weight and often die of what is essentially starvation. Similarly, in our work on rats with Walker carcinoma 256 we have observed that the rats ate well until the tumor reached a certain size (generally 40–50 per cent of the body weight); then they ceased to eat and died in a day or two. Blood taken during the active growth of the tumor was generally lipemic owing to mobilization of fat from the stores. The lipemia subsided terminally, and it was found that the fat stores were then empty.

In these animals, as in human cancer patients, the needs of the animal are no longer supplied by the food. Since food is available at all times, the digestive tract is a logical place to look for nutritional failure. A study was accordingly undertaken of the intestinal tract and constituent lipids in rats near death from large tumors, as compared with the intestines in normal rats of approximately the same age and weight. In a study of the effects of this tumor on the lipid composition of body tissues, Boyd et al. (3) found in agreement with Greenstein (6) that the composition of the body tissues approached the composition of the tumor. Boyd et al. (8) used only the duodenum as representative of the intestine. In the present work the whole intestine from stomach to anus was used.

METHODS

Male albino rats of the Wistar strain and Walker carcinoma 256 grown subcutaneously were used throughout the experiments. The rats, which weighed from 70 to 100 gm. at the time of transplantation of the tumor, had access to a diet of Purina Fox Chow meal. The tumor was allowed to grow for 4–6 weeks (average = 37 days) after transplantation.

The rats were killed by decapitation; the whole intestine from stomach to anus was removed, slit throughout its length, washed out with warm water, dried between filter paper, and weighed. The intestine was then lyophilized and the dry tissue again weighed, after which it was ground in a mortar and extracted twice with several volumes of alcohol-ether (3:1) and once with chloroform-methanol (1:1). The remainder was dried and weighed as dry extracted tissue. The combined extracts were evaporated to dryness on the steam bath; the lipid was then taken up with petroleum ether-chloroform (4:1) and the liquid (generally cloudy) washed with an equal volume of 50 per cent ethyl alcohol to remove nonlipid material. The petroleum ether-chloroform solution was separated, the solvent evaporated, and the residue weighed as total lipid.

Analyses.—The total lipid was dissolved in petroleum ether-chloroform solution and made to a volume of 25 ml. Of this volume an aliquot of 3 ml. was taken for saponification and one of 2 ml. for ashing for lipid phosphorus.

The aliquot for saponification was measured into a small Erlenmeyer flask, the solvent evaporated, and about 25 ml. of 95 per cent alcohol and 1 gm. of solid NaOH were added. The mixture was heated until the alcohol had evaporated (sticky dryness) and to it were added in succession 20 ml. of 95 per cent alcohol and 20 ml. of petroleum ether and, after the mixture was well shaken and stirred, 20 ml. of water. Centrifugation hastened the separation into a top layer of petroleum ether containing the cholesterol and other unsaponifiable material, and a lower alcoholic layer containing the fatty acids as soaps. After the lower layer was drawn off, the petroleum ether solution of unsaponifiable was washed with 50 per cent alcohol and then made to a volume of 25 ml. Aliquots of 5 ml. each were taken for determination of the amount of total unsaponifiable material by the oxidative procedure (2) and of cholesterol by the Liebermann-Burchard color production (2).

The aliquot for lipid phosphorus was wet ashed with sulfuric acid and hydrogen peroxide (superoxol), and the phosphorus was measured by the method of Kuttner and Lichtenstein (9). The value

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173
for phosphorus multiplied by 25 was called the phospholipid (PL). The value for neutral fat was obtained by subtracting from the total lipid the sum of the unsaponifiable and two-thirds of the weight of the phospholipid (the fatty acid content).

RESULTS AND DISCUSSION

The results of the examination of the lipids of the intestine of tumor-bearing as compared with normal rats are given in Table 1. The following outstanding similarities and differences may be noted.

**TABLE 1**

<table>
<thead>
<tr>
<th>WEIGHT OF INTESTINE</th>
<th>PER CENT OF DRY AND FAT-FREE INTESTINE</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. RATS</td>
<td>AV. WT. (gm.)</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>28</td>
<td>209</td>
</tr>
<tr>
<td>26</td>
<td>206</td>
</tr>
<tr>
<td>P value†</td>
<td>.01</td>
</tr>
<tr>
<td>Significance</td>
<td>H.S.‡</td>
</tr>
</tbody>
</table>

* Body weight.
† Significance of the difference between the averages for normal and for tumor-bearing rats.
‡ Highly significant.
§ Not significant.
$ Significant.

a) In terms of final body weight (including the tumor), the percentage of intestine is much lower in tumor-bearing animals (2 per cent) as compared with the normal controls (3 per cent). The same is shown for the value of dry and fat-free intestine as percentage of body weight (0.49 for controls as compared with 0.36 for tumor-bearing animals).

b) The percentage of cholesterol is the same in the intestines of both tumor-bearing and normal rats. The percentage of phospholipid is significantly higher in the intestine of the tumor-bearing animals.

c) The neutral fat content of the intestines of the animals bearing tumors is much below that of the controls (1.1 per cent of the dry and fat-free intestine weight as compared with 19 per cent in the controls).

d) The value for unsaponifiable matter other than cholesterol is much lower in the tumor animals, namely, 1.1 per cent of the dry and fat-free intestine, as compared with 1.9 per cent in the controls.

The significance of the differences in (a) is brought out when a comparison is made between the dry extracted intestines, expressed as percent-

**TABLE 2**

| THE DRY EXTRACTED WEIGHT OF INTESTINE IN NORMAL AND TUMOR-BEARING RATS |
|-----------------------------|-----------------------------|
| NORMAL | TUMOR-BEARING |
| Gm. | B.W. | Gm. | B.W. | C.W. |
| 1.09 | 0.62 | 0.96 | 0.64 | 0.25 |
| 0.99 | 0.59 | 0.68 | 0.89 | 0.58 |
| 0.94 | 0.50 | 0.75 | 0.50 | 0.46 |
| 0.90 | 0.47 | 0.74 | 0.31 | 0.42 |
| 0.88 | 0.45 | 0.87 | 0.33 | 0.47 |
| 0.85 | 0.52 | 0.83 | 0.51 | 0.47 |
| 0.84 | 0.47 | 0.68 | 0.91 | 0.50 |
| 0.83 | 0.47 | 0.55 | 0.89 | 0.50 |
| 0.82 | 0.44 | 0.75 | 0.55 | 0.53 |
| 0.74 | 0.54 | 0.74 | 0.54 | 0.54 |
| 0.96 | 0.50 | 0.96 | 0.50 | 0.76 |
| 0.79 | 0.50 | 0.79 | 0.50 | 0.82 |

* Body weight.
† Body weight of rat plus tumor.
‡ Carcass weight equals weight of rat minus tumor.
animals may mean, first, an increased activity of the intestine similar to that found in earlier work with muscle (1); second, a greater production of phospholipid for use in the tumor where lipid phosphorus has been shown to be concentrated (7); or, third, a shift in the metabolism of the tissues toward that of the tumor as indicated by Boyd et al. (3) and earlier workers (6).

The lower values for neutral fat in (c) mean merely that the fat of the intestine, like that of the rest of the body, has been mobilized for energy in the starving animal. The very low values for the fraction in (d) consisting of total unsaponifiable fat less cholesterol, also found to be low in earlier work with adrenals and blood (8), indicate that this fraction is important in connection with the growing tumor. It may be simply stored energy like the neutral fat but is more probably concerned with growth, either of the tumor or of the host body.

In these young animals the intestine seems to grow along with the rest of the body until the tumor becomes established. Then the tumor presumably takes the material needed for growth of the intestine, which then stops growing. Some interesting light on this problem is obtained by examination of Donaldson's (4) charts on the rat. Thus, our tumor-bearing animals at 250—300 gm. body weight have intestinal weights of only 4—5 gm., values which correspond to a body weight in the normal animal of about 100 gm. On the other hand, the control rats of the same weight have an intestinal weight of 6—7 gm., which corresponds to their body weight in the Donaldson chart. The inference is that the intestines of the tumor-bearing rats do not grow after the tumor has become established or after the weight of the carcass less the tumor has reached 100—125 gm. The following calculation may then be made: in a tumor-bearing animal of 250 gm. in which 40 per cent of the weight is tumor, the weight of the carcass less the tumor would be 150 gm. The tumor of 100 gm. is growing fast and therefore requires more food—perhaps twice as much as would be required for maintenance. At death, then, the tumor animal consists of a slowly growing or stationary host body of about 150 gm. and a fast-growing 100-gm. tumor. The total energy requirement would then be that of an animal of more than 350 gm. (body requirement of 150 gm. and a tumor requirement of 200 gm.). Another factor is the possibly increased basal metabolic rate in tumor animals as discussed by Fenninger and Mider (5). To meet these requirements there is intestine enough to supply only a 150-gm. rat, i.e., the carcass alone. The result is starvation. The stored fat is mobilized (lipemia) and burned; probably body tissue is also burned down to a starvation minimum, after which the animal dies. From the results of other experiments we know that the food intake continues up until 2 or 3 days before death at a level equal to that of the normal control.

The growth impulse in the tumors is obviously much stronger than that in the normal body tissues, since it stops growth in the animal's body and consumes the stored energy. What is the basis of the growth impulse? What is the growth factor? The pituitary has been implicated, either directly or indirectly through other organs, for example, the adrenal. As an indication of adrenal involvement, the “other sterol” fraction has been found in earlier work (8) to be notably less in the adrenals of tumor animals than in the controls. The same was found for blood plasma (8). In the present work the unsaponifiable fraction of the intestine is much lower in the tumor-bearing animals than in the controls. This fraction can be assumed to be related to the growth of the tumor, perhaps as a growth-promoting substance or as raw material for growth. The fraction contains desoxycorticosterone1 to the extent of about 50 µg/intestine in the normal animal, and also the fat-soluble vitamins which would appear in the extract from the alkaline saponification mixture. Further study of this fraction is indicated and will be undertaken.

From the considerations noted above, the inference may be made that the starvation and final death of the rats with tumors are due to insufficient intestine, perhaps with exhaustion of the “other unsaponifiable” fraction. The intestine probably stops growing soon after transplantation (10 days), while the tumor grows rapidly, with the result that not enough intestine is present to supply the needs of the animal. The stored fat is used and finally the animal dies, although abundant food is available. The part played by the “other unsaponifiable” fraction in these events remains to be determined.

SUMMARY

In this study of the intestine of rats bearing Walker carcinoma 256 as compared with that of normal rats, the following differences were noted: (a) the small amount of intestine relative to body weight; (b) low values for neutral fat; (c) significantly higher phospholipid content and normal cholesterol; and (d) the low values of the “other unsaponifiable” fraction (unsaponifiable other than cholesterol).

These results indicate that the animal dies be-

1 Values obtained through the kindness of Dr. Ethel Ashworth of the Department of Medicine.
cause the amount of intestinal tissue is insufficient to support life and growth in the face of the competition of the tumor. The deficiency in the "unsaponifiable" fraction is probably a factor.

REFERENCES
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