Studies on the Cachexia of Tumor-bearing Animals

I. Body Weight Changes, Carcass Composition, and Metabolic Studies*

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SUMMARY

During the period of most rapid tumor growth, rats bearing Lymphosarcoma R2788 gained more weight than did control animals, although their food consumption was almost identical. Carcass and tumor analyses showed that there was an increased percentage of water in the tumor and the carcass when compared with normal controls, sufficient to explain the increased weight gain. An equally high degree of hydration was observed in rats bearing Hepatoma 3688, although in this instance the loss of fat and body nitrogen exceeded the gain in water resulting in a loss of weight.

In confirmation of these findings, balance studies showed that in the period of most rapid tumor growth rats bearing Lymphosarcoma R2788 retained nearly the same amount of nitrogen as did control animals but an increased amount of water and sodium. Terminally, rats bearing tumors went into negative nitrogen and potassium balance. Although water retention was reduced during this period below the normal level, sodium retention persisted.

Neither the terminal loss of appetite, increased excretion of nitrogen, nor the shortened life span of the tumor-bearing rats was corrected by increasing the salt intake above that given in the standard diet.

Previous studies (19, 21, 22, 35) from this laboratory dealt with the etiology and nature of anemia of tumor-bearing animals. These studies demonstrated that the anemia of rats with various tumors could be quantitatively correlated with the loss of cells by hemorrhage into the area of the tumor. In the course of this work it was observed that rats with marked anemia lost weight more rapidly, became cachectic, and died, when the tumors were in the range of 20-30 per cent of the body weight; in animals without anemia the onset of weight loss and cachexia was much slower, and the tumors frequently reached 50-65 per cent of the body weight. However, in further study on the relationship of the anemia to the cachectic process it was observed that, during the period of most rapid tumor growth and despite the onset of anemia, rats bearing the Lymphosarcoma R2788 gained appreciably more weight than did the normal controls, although they both had almost identical food consumption.

Other investigators have made similar observations of the greater body weight gains in animals bearing Walker 256 carcinosarcoma (5, 7, 9, 11, 30, 43, 45) and Baboon (2) has reported comparable findings for animals bearing Sarcoma R-1 and Flexner-Jobling rat tumors.

In an attempt to determine whether the increased weight gain of tumor-bearing animals, when compared with that of the normal controls, resulted from an increased nitrogen retention or from accumulation of fluid, the present studies were undertaken. The nature of the body weight gain of the tumor-bearing animals was examined...
by the use of carcass and tumor analyses, and the efficiency with which the animals utilized nutrients was investigated by the use of metabolic balance studies.

MATERIALS AND METHODS

Animals.—Male F1 hybrid rats of the M520 × (AXC/9935) strain were used in all experiments of the present study. Animals were housed in separate cages with raised screen bottoms and were allowed to eat the pelleted NCI stock diet and drink water ad libitum. Changes in body weights and food consumption were followed daily.

Tumors.—Dunning Lymphosarcoma R2788 and Morris Hepatoma 3883 in F1 hybrids of M520 × (AXC/9935) female rats were used as tumor transplants. The tumors were implanted subcutaneously, bilaterally, in the pectoral region of the experimental animals. Tumors were removed aseptically, and three pieces of about 3 mm. in diameter were placed in a trocar. The hair on a recipient rat was washed down with water to prevent contamination from the hair. By the use of these technics and inbred rats the rate of tumor growth was sufficiently uniform to give satisfactory results without resorting to selection or the use of large numbers of animals.

Carcass analyses.—A modification of the method outlined by Mickelsen and Anderson (27) was used for preparation of rat carcasses for chemical analyses. The animals were killed by a blow on the head, after which they were shaven, their gastrointestinal tracts were removed and discarded, and their remaining carcasses were weighed and placed in a wide-mouth Mason jar. Tumor-bearing rats were treated similarly after their tumors were removed and weighed. The jars were then covered and autoclaved for 2 hours under 15 pounds' pressure. After being autoclaved, the jars were tightly sealed and stored in a cold room until the contents were homogenized. Homogenization was done in a Waring Blender of 1-gallon mixing capacity, with approximately 1 liter of distilled water, for 30 seconds each at low and medium speeds and 2 minutes at high speed. Each homogenate was transferred into an Erlenmeyer flask and was allowed to settle. The liquid suspension was poured into a graduated cylinder, while the remaining sediment on the bottom of the flask was rehomogenized in the Waring Blender with additional water. This second homogenate was then quantitatively transferred to the same cylinder. The blender was washed several times with small amounts of water, and the washings were added to the homogenate, the final volume of which was adjusted to 2 l. with distilled water. The homogenates were poured into 1-quart plastic bottles and stored in frozen state until analyzed.

At the time of analyses the homogenates were allowed to defrost overnight in the refrigerator, and then they were rehomogenized in a Waring Blender.

For moisture determination 10-ml aliquots of homogenates were pipetted into aluminum weighing dishes and dried in a vacuum oven to constant weight. The loss of weight on drying was assumed to be equivalent to the moisture content of the sample.

Nitrogen was determined on 2-ml aliquots of homogenates by the Kjeldahl procedure (12, 34).

The method for fat analysis was essentially that of Bligh and Dyer (6); 85 ml of the carcass homogenate was mixed in a Waring Blender with 100 ml. chloroform and 200 ml. methanol, followed by homogenization with 100 ml. of chloroform and then with 100 ml. of distilled water. The mixture was filtered with suction through Whatman No. 4 filter paper, and the sediment was washed with small amounts of chloroform. The filtrate was transferred to a graduated cylinder; after distinct separation of layers, the volume of the chloroform (the bottom layer) was recorded, and the alcholic layer was removed by aspiration. A 10-ml aliquot of the chloroform layer, containing the lipides, was evaporated and dried in the vacuum oven to constant weight.

Tumor analyses.—After being weighed, the tumors were homogenized in a Waring Blender with 9 volumes of distilled water. Moisture and nitrogen determinations were carried out on 10-ml and 2-ml samples, respectively, by the methods described above. Fat content was determined on a 1-ml aliquot of the tumor homogenate by the procedure of Sperry and Brand (42).

Blood analyses.—The blood was labeled with Fe59-tagged erythrocytes, and the blood samples were taken from the tail as described previously (19). The hemoglobin was measured as cyanmethemoglobin (16), and the concentration of Fe59 in the blood was measured with a scintillating
counter. Hematocrits were measured by a microtechnic with the Drummond microhematocrit.

Liver catalase.—The catalase activity was determined on 1:50 liver homogenates using the spectrophotometric method of Beers and Sizer (9) as modified by Greenfield and Price (20).

Plasma aldolase.—The method employed for the assay of aldolase is the one described by Sibley and Lehninger (41) with some modifications as suggested by Cook and Dounce (14).

Metabolic balances.—In the balance studies the animals were placed in the glass metabolism cages which allowed a separation of the urine and the feces. Urine was collected daily under toluene, diluted to 50 ml. with distilled water, and then stored at —4°C. until analyzed. Urine samples were analyzed for nitrogen by the standard micro-Kjeldahl procedure, and for sodium and potassium by the Beckman DU spectrophotometer with the flame photometric attachment (13).

RESULTS

Systemic Effects of Tumors on the Host

Studies with Lymphosarcoma R2788.—This experiment was designed to compare the tumor-bearing rat with a normal animal in regard to the growth rate, food consumption, the degree of anemia and other systemic effects exerted by the tumor on the host. For this purpose twelve rats with an average weight of 196 gm. were selected, half of which were given inoculations of the Lymphosarcoma R2788, while the other half served as controls.

Chart 1 shows the correlation between the changes occurring in the blood and the growth of experimental animals. During the first week after the tumor implantation no apparent differences were observed between the rats bearing the lymphosarcoma and the control animals. Following this period, when the tumor became palpable, the tumor-bearing rats began to gain weight at an increasing rate when compared with the controls and continued to do so for at least 18 days. It should be noted that the greater growth rate of tumor-bearing rats occurred even in the presence of a progressive decrease in the hematocrit, hemoglobin, and Fe59-labeled erythrocytes. It is of interest that the rapid gain of the tumor-bearing rats continued until the anemia became very severe, i.e., when hemoglobin dropped below 7 gm. per cent. This experiment was ended before the animals reached the terminal stage. When the animals were allowed to live until the terminal stage, however, they were observed to suffer from anorexia, loss in total body weight, and they finally died.

On the 21st day of the experiment the animals were sacrificed and subjected to autopsy. The average weight of the tumor was found to be 52 gm., which is equivalent to the body weight gain of the tumor-bearing rats. Since the carcass weight of the host remained unchanged during the experiment while the normal rat gained an average of 31 gm. in weight, it is apparent that the weight of the tumor exceeded the difference in weight of the tumor-bearing and normal rats.

On the center portion of Chart 1 are shown the daily changes in the food consumption of the respective groups of rats. It is apparent from the graphic representation that the animals of both groups consumed approximately the same amount of food throughout the greater part of the experiment. Thus on the 20th day the total food consumption of either lymphosarcoma-bearing or normal control rats totaled 253 gm. Since the tumor-bearing animals gained almost twice as much in weight as did the controls during this same period, the finding that the food consumption was similar in the two groups is noteworthy.

Other systemic changes of the rats bearing the lymphosarcoma are compared with the values found in normal rats in Table 1. Besides anemia, the tumor-bearing rat had an enlarged adrenal gland, involuted thymus, elevated plasma aldolase, and depressed liver catalase activity. Liver and spleen weights varied markedly with tumor size. When the tumor weight exceeded 25 per cent of the final body weight, there was a decrease in the weights of these organs, whereas with smaller tumors the liver and spleen were somewhat enlarged.

Studies with Hepatoma 3683.—A similar experiment conducted with rats bearing the Hepatoma 3683 showed comparable changes with respect to the hemoglobin, hematocrit, and Fe59 concentration. These changes occurred, however, about 1 week later than in the case of the lymphosarcoma. The food intake of these animals was less than that of the controls, which was reflected in smaller body weight gains of hepatoma-bearing rats. Systemic changes in these animals were marked and closely resembled the changes observed in the rats bearing the lymphosarcomas. For this reason the data were included with previous findings in Table 1.

The effect of hepatoma on the body weight of animals was reexamined in a separate experiment with ten tumor-bearing and ten control rats, which was continued until the death of the animals. It
TABLE 1

SYSTEMIC CHANGES IN TUMOR-BEARING RAT

<table>
<thead>
<tr>
<th>Group</th>
<th>Tumor (percent)</th>
<th>Hemoglobin (gm/100 ml)</th>
<th>Hematocrit (percent)</th>
<th>Plasma aldolase (U/ml)</th>
<th>Liver catalase (U/liver)</th>
<th>Liver nitrogen (mg/liver)</th>
<th>Liver weight (gm.)</th>
<th>Kidney weight (gm.)</th>
<th>Spleen weight (gm.)</th>
<th>Adrenal weight (gm.)</th>
<th>Thymus weight (gm.)</th>
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</thead>
<tbody>
<tr>
<td>Lymphosarcoma (6)†</td>
<td>21.0</td>
<td>5.80</td>
<td>30</td>
<td>185.4</td>
<td>605</td>
<td>963</td>
<td>8.57</td>
<td>1.80</td>
<td>1.28</td>
<td>0.058</td>
<td>0.118</td>
</tr>
<tr>
<td>Control (6)</td>
<td>13.51</td>
<td>49</td>
<td>23.9</td>
<td>168.4</td>
<td>261</td>
<td>168</td>
<td>7.96</td>
<td>1.56</td>
<td>0.71</td>
<td>0.040</td>
<td>0.380</td>
</tr>
<tr>
<td>Hepatoma (3)</td>
<td>28.8</td>
<td>5.15</td>
<td>52</td>
<td>208.5</td>
<td>911</td>
<td>917</td>
<td>9.70</td>
<td>1.62</td>
<td>2.55</td>
<td>0.051</td>
<td>0.151</td>
</tr>
<tr>
<td>Control (3)</td>
<td>14.84</td>
<td>52</td>
<td>26.2</td>
<td>1600</td>
<td>8.03</td>
<td>1.50</td>
<td>0.65</td>
<td>0.035</td>
<td>0.296</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Rats in this group were killed 21 days following the tumor implantation.
† Figures in parentheses designate the number of animals.
‡ Rats in this group were killed 28 days following the tumor implantation.
may be seen from the data shown in Chart 2 that the hepatoma-bearing rats grew at a slower rate than normal animals during the first part of the experiment, followed by a slight increase in the latter part and a sudden loss in the body weight just before death. There was a great variation in the time of death of the tumor-bearing animals which was not related to tumor size. Whereas the first death occurred 18 days after the tumor implantation, there was still one animal alive after 37 days. Tumor weight varied from 20 to 40 per cent of the final body weight while the carcass lost 8–25 per cent of the initial body weight. Both food and water consumption of the hepatoma-bearing animals were lower than in the control animals, and this was especially marked in the later stage of the tumor growth.

**Changes of Body Weights and Body Composition in Tumor-Bearing Animals**

**Growth rate, food consumption, and water consumption.**—The above experiments showed that rats given implants of lymphosarcoma gained more weight (carcass and tumor) than did comparable control animals despite almost identical food consumption in both groups and that rats implanted with hepatoma had nearly the same body weight as the control rats from the 19th day of tumor growth till death, in spite of a decrease in the intake of food. There are two possible mechanisms which could account for the greater weight gain of these animals when compared with their food intake: (a) a more efficient utilization of food with increased retention of nitrogen or (b) an accumulation of water.

In an attempt to determine to what degree each mechanism was involved, a study was undertaken in which the carcass was analyzed for nitrogen, water, and fat at the end of 15 days of tumor growth. For this purpose, 30 rats were divided into three comparable groups. One group of rats was implanted with the Lymphosarcoma R2788, another group with the Hepatoma 3683, while the remaining ten rats served as controls. The food and water intake and the growth rate of these animals were carefully measured. These data are presented in Table 2.

The tumor weight in both groups of the tumor-bearing animals exceeded the gain in total body weight. The carcasses of the rats bearing lympho-
sarcoma and hepatoma weighed only 96 ± 2 per cent and 91 ± 1 per cent of the initial body weight, respectively, whereas the control rats gained 12 ± 1 per cent of the initial body weight. The growth curves on these animals, until they were killed on the 15th day, were so similar to those presented in Charts 1 and 2 that they are not presented here for the sake of brevity.

The food consumption of the lymphosarcoma rats in this study was slightly lowered, especially toward the end of the experiment. In the hepatoma group, on the other hand, the food consumption began to drop 4 days after the tumor implantation, was lowest on the 8th day, and then slightly but consistently increased.

The water consumption of animals varied from day to day and from animal to animal in all groups. In general, however, the intake of the lymphosarcoma-bearing rats was similar to that of control animals, while the intake of rats in the hepatoma group was somewhat lower.

Carcass and tumor composition.—The results of the chemical analyses are given in Table 3. It is evident from the data that the greater body weight gains of the lymphosarcoma-bearing rats when compared with the controls were not due to greater retention of nitrogen but, rather, to increased water retention. Despite almost equal water intakes, rats bearing the lymphosarcomas contained an average of 32.5 gm. more water than did the control animals. The difference in the content of water between rats bearing the lymphosarcoma and control rats amounted to 32.5 gm. (25 per cent), whereas the difference between the average body weights of these groups was only 19.6 gm. (10 per cent).

The increase in the average water content of the

**TABLE 2**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>AV. BODY WEIGHT</th>
<th>AV. BODY WEIGHT CHANGE (15 DAYS)</th>
<th>AV. FOOD INTAKE (gm.)</th>
<th>AV. WATER INTAKE (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial (gm.)</td>
<td>Final (gm.)</td>
<td>Total (gm.)</td>
<td>Host (gm.)</td>
</tr>
<tr>
<td>Normal</td>
<td>193.5 ± 3.8†</td>
<td>225.8 ± 6.6</td>
<td>27.3 ± 3.4</td>
<td>27.3 ± 3.4</td>
</tr>
<tr>
<td>Lymphosarcoma</td>
<td>194.0 ± 4.3</td>
<td>244.2 ± 5.6</td>
<td>50.2 ± 1.2</td>
<td>12.6 ± 3.2</td>
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<tr>
<td>Change from normal</td>
<td></td>
<td></td>
<td>22.9</td>
<td>34.9</td>
</tr>
<tr>
<td>Hepatoma</td>
<td>192.5 ± 3.7</td>
<td>208.0 ± 3.0</td>
<td>15.6 ± 3.4</td>
<td>18.0 ± 3.7</td>
</tr>
<tr>
<td>Change from normal</td>
<td></td>
<td></td>
<td>-11.8</td>
<td>-45.8</td>
</tr>
</tbody>
</table>

* Each group consisted of ten animals.
† Standard error of the mean.

**TABLE 3**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>AV. FINAL WEIGHT</th>
<th>NITROGEN</th>
<th>WATER</th>
<th>FAT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Carcass</td>
<td>Tumor</td>
<td>Total</td>
</tr>
<tr>
<td>Normal (gm.)</td>
<td>195.5 ± 5.4</td>
<td>195.5</td>
<td>6.41</td>
<td>6.41</td>
</tr>
<tr>
<td>S.E. ‡</td>
<td>± 5.4 ± 5.4</td>
<td>± 0.30</td>
<td>± 0.30</td>
<td>± 5.3</td>
</tr>
<tr>
<td>Per cent</td>
<td>± 2.7 ± 2.7</td>
<td>± 0.27</td>
<td>± 0.27</td>
<td>± 2.9</td>
</tr>
<tr>
<td>Lymphosarcoma</td>
<td>115.1 ± 4.3</td>
<td>157.4</td>
<td>57.8</td>
<td>6.19</td>
</tr>
<tr>
<td>(gm.)</td>
<td>± 4.3 ± 3.5</td>
<td>± 1.4</td>
<td>± 0.15</td>
<td>± 0.15</td>
</tr>
<tr>
<td>S.E. ‡</td>
<td>± 0.5 ± 0.4</td>
<td>± 0.28</td>
<td>± 0.28</td>
<td>± 2.84</td>
</tr>
<tr>
<td>Per cent</td>
<td>± 2.4 ± 1.4</td>
<td>± 2.52</td>
<td>± 2.52</td>
<td>5.71</td>
</tr>
<tr>
<td>Hepatoma (gm.)</td>
<td>185.6 ± 2.5</td>
<td>185.1</td>
<td>23.5</td>
<td>5.17</td>
</tr>
<tr>
<td>S.E. ‡</td>
<td>± 1.7 ± 1.7</td>
<td>± 0.17</td>
<td>± 0.17</td>
<td>± 3.08</td>
</tr>
<tr>
<td>Per cent</td>
<td>± 1.2 ± 1.2</td>
<td>± 0.28</td>
<td>± 0.28</td>
<td>3.08</td>
</tr>
</tbody>
</table>

* Each group consisted of ten animals.
† Carcasses exclude the hair and the gastrointestinal tract.
‡ Standard error of the mean.
lymphosarcoma-bearing rats was actually greater than their average gain in body weight. The amount of water in the lymphosarcoma-bearing rats was especially high in the tumor compartment. Thus, the tumor with an average weight of 57.8 ± 1.4 gm. contained 47.6 ± 1.2 gm. or 83 per cent of water. The carcass of the tumor-bearing rats also contained an increased percentage of water, for a higher proportion of the fat and protein were lost than that of water. The carcass of the tumor-bearing rat was found to contain 74 per cent water, whereas the water content of the body of a normal rat was only 67 per cent. Although part of this increased percentage of water arises from the loss of adipose tissue which contains little water, the carcass of rats bearing lymphosarcoma contained 79.4 ± 0.9 per cent of water on a fat-free wet weight basis, whereas the control rats contained 75.1 ± 0.8 per cent water on a comparable basis. Since previous studies from this laboratory (19) have never found the increase of blood volume arising in these tumor-bearing animals to reach 2 per cent of the fat-free carcass and since it can be assumed that part of the increase of blood volume arises from vessels within the tumor, the increase in tissue water found in the present experiments, although admittedly small, exceeds that which can be attributed to a change in blood volume alone.

Table 3 also shows the results of nitrogen and fat analyses. In no case did the tumor-bearing animals (carcass and tumor) contain more nitrogen than did the control rats. In fact the tumor-bearing animals, whether of the lymphosarcoma or the hepatoma type, actually stored less nitrogen when compared with normal rats. This is consistent with the lower nitrogen consumption of the tumor-bearing animals in this experiment (Table 2). Contrary to the observations on water, the difference in the nitrogen content of carcasses between the tumor-bearing and the control rats paralleled the changes in the carcass weights. The carcasses of the lymphosarcoma-bearing animals contained an average of 1.6 gm. (24 per cent) less nitrogen than the control animals. This difference could partly be accounted for by the nitrogen content of the tumor which amounted to 1.3 gm. and partly by the lower nitrogen consumption of the animals, the first factor being the more important. In rats bearing the hepatomas, on the other hand, anorexia accounted for nearly as great a loss in carcass nitrogen as did translocation of nitrogen to the tumor.

With respect to the fat content, both groups of tumor-bearing animals stored about 35 per cent less total fat than did the control rats. The carcass fat in the tumor-bearing host amounted to less than 60 per cent of that found in the normal animal, and the amount of fat deposited in the tumor was negligible. Similar observations were made previously by Mider (29, 30) and others.

It can be seen from the above discussion that the shift of water tends to obscure the losses of nitrogen and fat, making gross weight changes unreliable when used to evaluate any single compartment. Craig and Waterhouse (15) have emphasized the danger of using gross weight changes in human patients because of the gain in total body-water.

**METABOLIC BALANCE STUDIES**

The above experiments demonstrated that tumor-bearing animals were retaining greater amounts of water than were the control rats. To gain a better understanding of the phenomenon, several metabolic balance studies were conducted with normal rats and rats bearing Lymphosarcoma R2788. The animals were kept in glass metabolism cages throughout the period of tumor growth until they died. The daily intake of food and water by the animals and their daily urinary output was carefully measured. Every 3-day composite sample of urine was analyzed for nitrogen, sodium, and potassium.

Body weight changes of the tumor-bearing rats showed the increase in the weight gain above the normal control animals characteristic of Lymphosarcoma R2788. The food consumption dropped about 12 days after the tumor implantation, and the animals died at the end of 21 days.

Nitrogen intake and urinary nitrogen excretion of the normal and the lymphosarcoma-bearing
rats is shown in Chart 3. It can be seen that, whereas no apparent differences were observed in the normal animals throughout the experimental period, there was a gradual decrease in the nitrogen intake and an increase in the nitrogen excretion of the tumor-bearing animals. Normal animals consumed an average of 330 mg. of nitrogen and excreted 155 mg. nitrogen in the urine per day. Tumor-bearing animals, on the other hand, consumed only 297 mg. of nitrogen and excreted 144 mg. of nitrogen during the 15 days of the experiment. When expressed as per cent of the dietary nitrogen, the normal animal excreted 40 per cent nitrogen in the urine while the tumor-bearing rat excreted an average of 49 per cent.

Daily fluid balance of the animals is plotted in Chart 4. The normal animals consumed an average of 21.5 ml. of water and excreted 8.3 ml. of urine per day. The tumor-bearing rats in this experiment were consuming somewhat greater amounts of water than were the control animals. it is striking. Note especially the decrease in the water retention concomitant with an increase in the percentage of the dietary nitrogen recovered in the urine.

Sodium intake and urinary sodium excretion of the animals are plotted in Chart 6. In general, it was observed that tumor-bearing animals retained more sodium than did the control animals. With the onset of anorexia in the tumor-bearing animals, the sodium intake gradually fell, which was followed by a decrease in sodium excretion. Terminally, tumor-bearing rats were still retaining a greater proportion of ingested sodium than were the control animals, although it can be seen in Chart 4 that they were excreting more fluid than the latter.

Potassium balance data are shown in Chart 7. In general, it may be concluded that tumor-bearing and normal rats were excreting approximately the same amount of potassium in the urine. If the potassium intake is taken into account it will be-
come apparent, however, that, while the normal animals were consuming almost constant amounts of this ion throughout the experiment, the tumor-bearing rats were progressively consuming less and less potassium. This trend was responsible for the negative potassium balance observed in the final stage of tumor growth.

**EFFECT OF EXCESS SALT ON BODY WEIGHT CHANGES AND APPETITE OF TUMOR-BEARING ANIMALS**

*Comparative response of rats to saline and water.*

White and his associates (31, 33, 46) have shown that an inclusion of lyophilized tissue of the Walker 256 carcinoma or other tumor in the diet as a source of nitrogen stimulated appetite and growth of rats bearing this tumor subcutaneously.

The effects of feeding tumor tissue could be grossly simulated by the addition of extra NaCl to the basal diet (32), which supplied 0.3 per cent NaCl (33).

Although our animals were given a diet containing adequate amounts of NaCl (1.14 per cent), experiments were set up to determine whether the loss of appetite could be overcome by further addition of salt. For this purpose 24 rats were divided into two comparable groups according to weight, twelve of which were inoculated with the Lymphosarcoma R2788 while the other twelve served as controls. Half of the animals in each group were fed water, and the other half received the fluid in the form of physiological saline (0.9 per cent NaCl). Both saline and water, together with the usual pelleted food, were offered ad libitum. The experiment was terminated at the time of death of the tumor-bearing animals.

The results of the experiment are shown in Table 4. Lymphosarcoma-bearing rats ingesting saline were found to gain more weight than those fed water, the difference being primarily due to greater carcass weight of the former. The body weight gain of control animals, on the other hand, was not affected by the ingestion of saline. Rats bearing the lymphosarcoma on either regimen gained more weight than did the control animals. Prior to death of the tumor-bearing animals, regardless of whether they were ingesting water or saline, there was a sudden loss in the total body weight.

With respect to the intake of water, both normal and tumor-bearing animals were observed to consume almost identical amounts. Rats ingesting
saline, whether normal or tumor-bearing, consumed more fluid than those fed water. This was especially marked, however, in the case of the tumor-bearing animals. Tumor-bearing animals, whether they were fed saline or water, progressively lost appetite and became markedly anoretic in the terminal stages of tumor growth. While these animals were consuming less than 4 gm. food per day in the final period, the normal controls were eating more than 10 gm. It should also be noted that rats ingesting saline had somewhat shorter survival time than animals ingesting water.

Since there was marked anorexia in the terminal stages of tumor growth, it was probable that the increased weight gain of the lymphosarcoma-bearing animals after saline ingestion was primarily due to fluid retention. The validity of this idea is supported by the results of the metabolic studies which were conducted with these animals. It is apparent from Charts 8 and 9 that neither normal nor lymphosarcoma-bearing rats which were ingesting saline showed any increase in the nitrogen retention above that observed in the animals consuming water. In fact, when the urinary nitrogen was expressed as per cent of nitrogen intake there was a tendency for a greater nitrogen loss among the animals ingesting saline, both normal and tumor-bearing. On the other hand, there was a gradual increase in the fluid retention of the tumor-bearing rats ingesting saline when compared with that of those consuming water. Thus, on the 15th day, the saline-fed rats were retaining as much as 25 ml. fluid, whereas those consuming water retained only 11 ml/day.

### TABLE 4

**AVERAGE BODY WEIGHT CHANGES AND FOOD CONSUMPTION OF NORMAL AND TUMOR-BEARING RATS INGESTING EITHER SALINE OR WATER**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>FLUID INTAKE</th>
<th>FOOD INTAKE (G/M. DAY)</th>
<th>INITIAL WEIGHT (G/M.)</th>
<th>MAXIMUM WEIGHT (G/M.)</th>
<th>FINAL WEIGHT (G/M.)</th>
<th>TUMOR WEIGHT (PER CENT)</th>
<th>SURVIVAL TIME (DAYS)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Kind</td>
<td>Ml/day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphosarcoma</td>
<td>Water</td>
<td>31.2 ± 1.3</td>
<td>8.5 ± 0.1</td>
<td>175 ± 2</td>
<td>233 ± 6</td>
<td>223 ± 9</td>
<td>25 ± 2</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>36.0 ± 1.6</td>
<td>10.6 ± 0.3</td>
<td>178 ± 1</td>
<td>265 ± 7</td>
<td>243 ± 11</td>
<td>25 ± 1</td>
</tr>
<tr>
<td>Normal</td>
<td>Water</td>
<td>21.0 ± 0.7</td>
<td>10.4 ± 0.2</td>
<td>178 ± 5</td>
<td>209 ± 5</td>
<td>204 ± 6</td>
<td>20 ± 4</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>30.6 ± 1.8</td>
<td>10.1 ± 0.3</td>
<td>178 ± 6</td>
<td>204 ± 6</td>
<td>204 ± 6</td>
<td>20 ± 2</td>
</tr>
<tr>
<td>Hepatoma</td>
<td>Water</td>
<td>21.7 ± 1.4</td>
<td>10.8 ± 0.4</td>
<td>167 ± 7</td>
<td>230 ± 10</td>
<td>209 ± 15</td>
<td>31 ± 2</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>30.5 ± 3.3</td>
<td>11.1 ± 0.3</td>
<td>164 ± 16</td>
<td>226 ± 10</td>
<td>210 ± 14</td>
<td>29 ± 2</td>
</tr>
<tr>
<td>Normal</td>
<td>Water</td>
<td>34.8 ± 1.4</td>
<td>13.6 ± 0.3</td>
<td>165 ± 7</td>
<td>233 ± 11</td>
<td>223 ± 11</td>
<td>31 ± 2</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>37.2 ± 0.9</td>
<td>14.8 ± 0.2</td>
<td>172 ± 6</td>
<td>246 ± 10</td>
<td>246 ± 10</td>
<td>29 ± 2</td>
</tr>
</tbody>
</table>

* Figures in parentheses designate the number of animals.
† Standard error of the mean.
‡ The experiment was terminated at the time of death of the last tumor-bearing animal.
In view of the marked anorexia observed previously in rats bearing Hepatoma 3683, it was of interest to determine whether the increased salt intake by these animals would prevent their loss of appetite. The experiment was similar to the one above, and the results are shown in the lower portion of Table 4. It is apparent from the results of the experiment that the ingestion of saline did not correct anorexia of the animals. Tumor-bearing rats were again observed to die earlier when ingesting saline than those fed water. Contrary to observations in the lymphosarcoma-bearing animals, however, ingestion of saline by the hepatoma-bearing rats had no apparent effect on the body weight gain. In this respect the hepatoma-bearing rats resembled the normal control animals.

Hepatoma 3683 were retaining greater amounts of water than were the normal animals. This effect was readily observed in rats bearing the lymphosarcoma, in which case the increased water retention actually resulted in greater body weight gains than those of the controls. In the hepatoma-bearing rats the hydration effect was masked by anorexia of the animals, especially during the initial phases of tumor growth.

The greater body weight gain of the tumor-bearing animals when compared with the normal controls has been observed previously by several investigators, both under ad libitum (7, 9, 11), paired (2, 30), or force-feeding (5, 43, 45) conditions, as long as the animals maintained their food consumption at normal levels.

The water content of tumors (1, 2, 10, 18, 23, 40, 44), with the possible exception of the interstitial tumors of the dog testis (24), has been found to be greatly increased over that found in normal tissues. Many investigators who have noted an increase in the total body weight of the tumor-bearing animal concomitant with a loss of lipid and total nitrogen have attributed the weight gain wholly or in part to the water content of the tumor.

Other investigators (5, 9, 11, 15, 45) have suggested that the water content of the other tissues may also play a role in the weight gain of the tumor-bearing host.

Mider (30) in his excellent studies on the nitrogen balance of rats bearing Walker 256 tumor reported that the loss of carcass nitrogen equaled the nitrogen lost into the tumor plus the nitrogen balance. He found that the nitrogen concentration on a fat-free wet weight basis of the tumor-bearing host was nearly the same as that of the control rats but 40 per cent greater than that of the tumor.

**DISCUSSION**

The above experiments demonstrated that the rats bearing the Lymphosarcoma R2788 and Hepatoma 3683 were retaining greater amounts of water than were the normal animals. This effect was readily observed in rats bearing the lymphosarcoma, in which case the increased water retention actually resulted in greater body weight gains than those of the controls. In the hepatoma-bearing rats the hydration effect was masked by anorexia of the animals, especially during the initial phases of tumor growth.

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**TABLE 5**

**FLUID CONSUMPTION, FOOD INTAKE, AND BODY WEIGHT CHANGES OF NORMAL AND TUMOR-BEARING RATS GIVEN FREE CHOICE OF WATER AND SALINE**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Fluid Intake</th>
<th>Food Intake</th>
<th>Initial Weight (g)</th>
<th>Maximum Weight (g)</th>
<th>Final Weight (g)</th>
<th>Tumor Weight (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water (ml/day)</td>
<td>Saline (ml/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphosarcoma</td>
<td>16.2 ± 1.2†</td>
<td>12.2 ± 2.6</td>
<td>18.0 ± 0.5</td>
<td>265 ± 15</td>
<td>251 ± 25</td>
<td>24 ± 3</td>
</tr>
<tr>
<td>Normal</td>
<td>17.5 ± 1.6</td>
<td>16.2 ± 1.9</td>
<td>13.6 ± 0.4</td>
<td>197 ± 6</td>
<td>234 ± 5</td>
<td>234 ± 6</td>
</tr>
<tr>
<td>Hepatoma</td>
<td>14.3 ± 1.5</td>
<td>16.3 ± 2.9</td>
<td>11.5 ± 0.2</td>
<td>167 ± 5</td>
<td>246 ± 10</td>
<td>294 ± 10</td>
</tr>
<tr>
<td>Normal</td>
<td>17.4 ± 1.1</td>
<td>15.7 ± 0.4</td>
<td>14.8 ± 0.3</td>
<td>165 ± 7</td>
<td>243 ± 10</td>
<td>243 ± 10</td>
</tr>
</tbody>
</table>

* Five animals were used in each group.
† Standard error of the mean.
‡ The experiment was terminated at the time of death of the last tumor-bearing animal.
itself. These data would suggest that the greater weight gain was primarily due to the lower nitrogen content or higher water content of the tumor. His observation that normal rats failed to increase their weight on a dietary intake of 50 mg N/gm rat/day, whereas some of the tumor-bearing rats gained well though they ingested as little as 35-40 mg N/gm would support this concept. Babson (2) gained well though they ingested as little as 35-40 mg N/gm would support this concept. Babson (2) came to similar conclusions.

The findings in the present study would be consistent with the view that the gain in body weight arises predominantly but not entirely from the water content of the tumor for a small but significant increase was found in the concentration of water in other body tissues on a fat-free wet weight basis. This conclusion agrees well with the data of others on the water content of the tumor-bearing carcass. If the data of Boyd et al. (8) on the water content of the carcass are calculated on a fat-free basis, the carcass of the tumor-bearing animal has 4.3 per cent more water than that of the normal control rat. This value is the same as that calculated from the present data. Sugimura et al. (45) found that the carcass of force-fed animals bearing the Walker carcinosarcoma did not lose weight and actually might have gained slightly in weight while losing 462 mg N to the tumor. They suggested that the failure to show a weight loss may have arisen because of a high degree of hydration of the non-neoplastic tissues. These findings on the carcass composition are not surprising, since there have been many reports of increased water content of the organs of tumor-bearing animals (1, 23, 25, 26, 38, 39). A greater concentration of water in the livers of tumor-bearing animals has been especially noted (1, 5, 18, 25, 26, 38-40, 44).

In this regard, the edematous area immediately around the tumor deserves further study, since in many tumors there is a rather extensive area of gelatinous material of high water content.

The results obtained on the water balance studies suggest that the water content of the tumor-bearing animal may be expected to vary with the stage of tumor growth. Early in tumor growth the urinary excretion of water is decreased while the water consumption may be actually elevated. In the terminal stages of tumor growth the rate of urine excretion increases more rapidly than the water consumption. Although the present data give no evidence as to the source of the water terminally excreted, the tissues of the host would seem to be the most likely location in view of the continued growth of the tumor. The positive sodium balance, together with the decrease in the potassium retention, indicates the possibility that the terminal dehydration of the tumor-bearing animals may at least partially arise from the loss of intracellular fluid.

Previous investigators (5, 28) have reported a higher retention of salt in the tumor-bearing rat. Craig and Waterhouse (15) have reported a similar retention of sodium in human cancer patients but point out that the gain in total body-water found in every patient studied could not be completely accounted for by the water associated with chloride retention. It was suggested that both intracellular and extracellular water must be increased. It would appear that additional studies must be carried out in which both sodium and potassium are measured before this question can be discussed further.

The finding by Millar et al. (31-33) and White et al. (46) that tumor tissues will increase the appetite and the body weight of rats bearing Walker carcinosarcoma 256 and their recent reports that the effects of feeding tumor tissue can be simulated by the addition of extra NaCl to the basal diet are of special interest. Millar et al. (31) pointed out that the large weight gain of these animals could not be accounted for by food intake alone, but was accompanied by increases in water consumption. The question arises if the NaCl in the diet does not determine to some extent the degree of hydration of the tissues of the tumor-bearing animal. The present data are not sufficient for any generalizations to be made on this point. Providing additional salt as 0.9 per cent NaCl in the drinking water increased the body weight of the animals bearing the Lymphosarcoma R2788 but had little effect on the rats bearing Hepatoma 3683. Further experiments in which salt-deficient diets are fed would be of interest.

The relation of salt appetite to the physiologic need for salt has been demonstrated (37). After adrenalectomy rats ameliorate their condition by seeking and ingesting large amounts of NaCl when offered a choice between salt solution and water. It has also been shown that high salt intake increases the longevity of adrenalectomized animals. In our studies, the rats bearing Lymphosarcoma R2788 and Hepatoma 3683 showed no preference for saline over water, and moreover it has been observed that tumor-bearing animals ingesting only saline died earlier than those fed water. These observations, together with the facts that the lymphosarcoma-bearing rats were not in negative sodium balance and actually seemed to retain more sodium than the normal controls, give support to the idea (4) that the enlargement of the adrenal gland of the tumor-bearing animals may represent persistent hyperfunction rather than hypofunction of the gland at least in regard to its
secretion of aldosterone. In view of the increased aldosterone secretion reported following acute hemorrhage (17), it would be of interest to study the aldosterone secretion in tumor-bearing animals with and without anemia.

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Studies on the Cachexia of Tumor-bearing Animals: I. Body Weight Changes, Carcass Composition, and Metabolic Studies

Miloslav Rechcigl, Jr., Flora Grantham and Robert E. Greenfield


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