Growth Hormone and Tumor Phospholipide Effects on Tumor and Body Growth*

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Growth hormone.—The changes occurring in the normal rat following the administration of growth hormone and those evoked by the growth of Walker carcinoma 256 in the rat are similar in many respects. In both instances there is an increase in energy expenditure and a decrease in respiratory quotient (5, 15, 17). Accompanying both is an increase in water content (2, 10) and a decrease in fat stores (2, 5, 7, 10), with suggestion of increased fat mobilization (6, 7, 9). These factors, besides the obvious fact that growth is occurring in each instance, suggest a relationship between tumor growth and the growth stimulated by growth hormone.

The effect of the administration of growth hormone on the relation of growth of Walker carcinoma 256 to the growth of the rat bearing the tumor, with proper consideration for food intake, has not been adequately investigated. The present experiment was designed, therefore, to determine the partition of total body weight between carcass and tumor in the rat receiving growth hormone injections in relation to food consumption.

Phospholipide.—Haven and Bloor (8) supplemented the diet of Wistar rats bearing Walker carcinoma 256 with mixed phospholipide from the tumor, after preterminal anorexia and weight loss had occurred, and demonstrated a restoration of appetite and a gain in body weight. This material was labeled by them “a lipid appetite factor.” Their experiments indicated that, in the preterminal phase at least, this “factor” was capable of stimulating appetite and body growth. These studies have been expanded in the following experiment to detect any effects that this “factor” might have on the distribution of weight between carcass and tumor at an earlier stage of tumor development.

Growth hormone and phospholipide.—Phospholipides, as integral components of β-lipoproteins, probably play a role in fatty acid transport and seemingly facilitate the oxidation of fats (1). As mentioned above, an increase in mobilization and utilization of fatty acids occurs in both the tumor-bearing animal (9, 13) and the one receiving growth hormone (6). In addition, Greenbaum and McLean (6) showed that growth hormone stimulates the synthesis of phospholipides by the liver. Therefore, some common ground may exist between growth hormone, tumor growth, and phospholipide. Accordingly, in the following experiment, treatment with the combination of growth hormone injections and phospholipide diet was carried out in the tumor-bearing and nontumor-bearing rat.

MATERIALS AND METHODS

Treatment of rats.—Female rats of the Sprague-Dawley strain† that had reached a plateau in body weight were housed individually in wire-mesh, basket-type cages. When the average weight was 235 gm. (range: 212-261), 83 of the rats received transplants, by trocar, of fragments of Walker carcinoma 256 in the subcutaneous tissues of the mid-back. Two weeks later 72 of these animals had palpable tumors² of an estimated average weight of 1-2 gm. After being weighed, the rats were divided into four groups on the basis of their tumor size and body weight so that the average of each group approximated the others as closely as possible. The four groups of tumor animals were matched by four groups without tumor. The groups, their subsequent treatments, and average weights on the day of matching, are listed in Table 1. Food and water were available ad libitum; the food consumption of all rats was measured, and body weights were recorded 3 times a week. The tumors of all tumor-bearing rats were measured in 3 dimensions (length, width, and depth) periodically throughout the growth of the tumors. The control groups were started on the experiment 15 days after transplantation with the tumor; the PH (tumor-phospholipide diet) groups, 16 days; and the GH (growth hormone) groups, 17 days.

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† Obtained from the Hormone Assay Laboratories, Inc., Chicago 29, Illinois.

² Tumors arose subsequently in six of the remaining animals. Thus, 94 per cent of the rats that received transplants grew tumors.
mone-injected) and GHPL (growth hormone-injected and phospholipide diet) groups, 17 days after transplantation.

At the completion of 14 days of experimental time for each group, the animals were weighed and sacrificed by decapitation. In the animals bearing tumors, the tumors were measured, each animal was sacrificed, and the tumor was removed and weighed.

Treatment of data.—The data analyzed and presented here were collected in the 10-day period between 17 and 27 days after transplantation of the tumor-bearing rats. Thus, the animals were living throughout the entire experimental period.

To calculate the distribution of the total body weight between tumor and carcass (total body weight minus tumor weight) during the experimental period, a formula was derived for converting tumor mass to weight. The equation was evolved as the product of the three dimensions of each tumor obtained just before sacrifice against the corresponding tumor weight determined immediately after sacrifice. The plot yielded a straight line, the equation of which was used to derive an estimate of their actual weights in the living animals.

Between tumor and carcass (total body weight minus tumor weight) during the experimental period, a formula was derived for converting tumor mass to weight. The equation was evolved which fitted the line of regression derived by plotting the product of the three dimensions of each tumor obtained just before sacrifice against the corresponding tumor weight determined immediately after sacrifice. The plot yielded a straight line, with only a moderate amount of scatter. The standard error of estimate of this line of regression (8) was only 2.01 for tumors under 40 gm. Since most of the tumors during the experimental period were below this weight, their calculated weights are believed to represent an accurate estimate of their actual weights in the living animals.

Table 1: Group, Number, and Treatment of Rats in the Experiment

<table>
<thead>
<tr>
<th>Group</th>
<th>No. Rats</th>
<th>Normal Tumor (gm)</th>
<th>Diet</th>
<th>Treatment</th>
<th>Injections*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>26</td>
<td>27†</td>
<td>Mixture of 985 gm. Purina Fox Chow meal and 15 gm. Wesson oil</td>
<td>0.2 cc. isotonic saline</td>
<td></td>
</tr>
<tr>
<td>PL†</td>
<td>18</td>
<td>21†</td>
<td>Mixture of 985 gm. Purina Fox Chow meal, 13.2 gm. Wesson oil, and 2.3 gm. tumor phospholipide</td>
<td>0.2 cc. isotonic saline</td>
<td></td>
</tr>
<tr>
<td>GH</td>
<td>12</td>
<td>12</td>
<td>Mixture of 985 gm. Purina Fox Chow meal and 15 gm. Wesson oil</td>
<td>0.2 cc. growth hormone§</td>
<td></td>
</tr>
<tr>
<td>GHPL</td>
<td>12</td>
<td>12</td>
<td>Mixture of 985 gm. Purina Fox Chow meal, 13.2 gm. Wesson oil, and 2.3 gm. tumor phospholipide</td>
<td>0.2 cc. growth hormone§</td>
<td></td>
</tr>
</tbody>
</table>

*Volume given was injected intraperitoneally, daily, 6 days per week.
† Only animals with progressively growing tumors were included in the final statistical analyses with the result that three control and two PL rats were excluded from the number of rats given above. Hence, the final control group contained 24 rats and the PL group nineteen rats.
PL = tumor-phospholipide diet.
GH = growth hormone-injected.
GHPL = growth hormone-injected and phospholipide diet.
‡ Lyophile-dried, whole Walker carcinoma 256 tissue was extracted exhaustively with boiling 95 per cent ethanol followed by boiling chloroform-methanol (1:1). The solvent was removed from the combined extract below 50° C. under partial vacuum; the residue was extracted with petroleum ether-chloroform (6:1). The washed extract was concentrated to one-third volume and the phospholipide precipitated with 20 vols. acetone. The calorific value of the crude tumor phospholipide and of the Wesson oil was determined by bomb calorimetry. The amount of phospholipide used was substituted for a calorically equivalent amount of Wesson oil. An emulsion of the phospholipide in the remainder of the Wesson oil was mixed with the Fox Chow meal.
§ Armour somatotropin courteously supplied by Dr. Sanford L. Steelman of the Armour Laboratories, Chicago 9, Ill. This Lot (no. M-208) was listed as having an activity of 75 per cent of the Armour Standard, and its main contaminant as TSH 0.15 USP units/mg. Each 0.2-cc. dose injected contained 0.4 mg. of this Armour somatotropin.

RESULTS AND DISCUSSION

Control and PL groups.—During the 10-day period, the tumor-free rats fed the PL diet gained a significant amount of weight over the control animals without tumor (Chart 1 and Table 2). Thus, the presence of tumor phospholipide in the PL diet has led to more efficient weight gain. This gain is significant (P < 0.01; > 0.001) whether expressed simply on the basis of an actual weight gain or as the weight gain per gram of food (Chart 2). The gain of the control tumor-free animals was similar, quantitatively, to the gain of the carcass (total body minus tumor) of the control tumor animal. Thus, during the period studied, the control rats were able to maintain body weight as well as average 17.4 gm. tumor growth on
the same amount of food as the control rats without tumor.

The tumor-bearing animals fed the PL diet gained approximately the same average amount of total weight as the control tumor-bearers (Chart 1 and Table 2). However, the distribution of this weight gain between tumor and carcass was quite different. Not only was the weight gain in the PL group represented in its entirety by the tumor, but 1.4 gm. of the tumor size represented actual carcass loss. Their tumors were larger than those of the control rats, but, because of the variation in tumor size, the difference is not significant (P < 0.2; > 0.1) (Table 2). The carcass change, however, is significantly less than that of the control tumor-bearer, and even further below the body change of the tumor-free PL rats. Indeed, of our eight groups the PL tumor-bearer was the only one to lose carcass weight. This loss is even more remarkable when one recalls (see above) that the PL diet in the group without tumor resulted in an increased weight gain/gm food over the control group without tumor.

There are two possible explanations for this seeming paradox: Since carcass loss has been shown to occur (14) when the tumor reaches a critical size, have the slightly larger tumors in the phospholipide-fed animals caused the carcass loss observed? With this in mind, data were selected from the control rats with the nine largest tumors, which averaged 26.5 gm., slightly greater than the average of 22.4 gm. for the tumor rats of the PL group (Chart 1). However, these nine animals showed a carcass gain of 5.3 gm. (approximately that of the entire control group) as opposed to the loss of 1.4 gm. in the phospholipide-fed animals with even smaller tumors. This suggests that tumor size alone was not responsible for the carcass loss in the rats of the PL group.

Secondly, the tumor-bearers of the PL group ate significantly less food than any other group (all P values < 0.01). This offered another possible explanation for their paradoxical carcass loss. The food intake of the six smallest eaters in the PL tumor-free group was found to average 14.9 gm/day or 149 gm. over the “10-day experiment”; this amount is identical with the average of the tumor-bearing group fed phospholipide. However, these six smallest eaters had a weight gain of 8.8 gm. during the experiment, a figure more closely allied with the 9.8 gm. gain of their entire group than with the carcass loss of the PL animals with tumor. Thus, it appears that the decreased food intake alone was not responsible for the carcass loss of the PL group with tumor. There was then something in the combination of the tumor phospholipide in the diet and the growing tumor that resulted in the carcass loss, since this combination was the only specific treatment that this group received which differed from the treatment of the other three groups currently under discussion. In the search for an explanation for the mechanism of this combined influence, an interesting possibility arises.

The combined influence of the fed phospho-
lipide and the growing tumor produced gross changes in the rat, namely, carcass loss and decreased food intake, which have been observed in rats with tumors somewhat larger than those with which we have been dealing. Did the fed tumor phospholipide have sufficient “tumor-like” effect in itself so that, in combination with the tumor growing in the rat, it was able to bring about changes in the host (carcass loss and decreased food intake) which are seen in rats that bear larger tumors? In any event, the possibility that the tumor phospholipide contained material which, when incorporated into the diet, had an effect on the host animal similar to the growing tumor itself, merits further confirmation and elaboration. Such studies are being carried out.

As mentioned in the introduction, Haven and Bloor (8) found that supplementing the diet of preterminal tumor-bearing rats with a tumor phospholipide material, prepared in a manner similar to that used in this experiment, resulted in restoration of appetite and gain in body weight. The findings of this experiment (loss of appetite and body weight) appear to be the antithesis of the original observation. The experiment presented in this paper represents work done on rats during the first and second phases of growth of the Walker carcinoma 256 as defined by Sherman et al. (16) and Mider (11, 12). In the experiment cited by Haven and Bloor (8), the rats had tumors in the third phase of growth. The nature of the tumor-host relationship may be quite different during these phases of tumor growth. The relation of the dietary phospholipide to these differences is not clear at present, but will be investigated in the future.

**Growth hormone effects.**—The treatment with growth hormone was definitive, since it produced in the tumor-free animals receiving it (GH group) a highly significant weight gain over the control tumor-free animals (Chart 1, Table 2). This was true whether the gain was expressed simply as absolute weight gain or in terms of gain/gm food consumed (Chart 2, P < 0.001). Their food intake was not significantly increased (P > 0.1) over the control animals. In the tumor-free animals of the GHPL group, the combination of GH injections and the PL diet produced a gain in weight in the range of that for the animals receiving GH alone. The addition of GH seemed to mask the gain in weight induced by the PL diet that was seen in those animals that received PL diet alone. In any event, the two effects were certainly not additive. It is interesting that, in comparing the GHPL animals with the PL animals, the greater increase in weight of the former is only

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**TABLE 2**

<table>
<thead>
<tr>
<th>Group*</th>
<th>Control (gm)</th>
<th>GH (2.5), (gm)</th>
<th>GHPL, (2.5)</th>
<th>GHPL, (2.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Body weight (gm)</td>
<td>80.4</td>
<td>81.0</td>
<td>84.3</td>
<td>88.3</td>
</tr>
<tr>
<td>Tumor weight (gm)</td>
<td>17.4</td>
<td>9.4</td>
<td>10.8</td>
<td>15.1</td>
</tr>
<tr>
<td>Carcass weight (gm)</td>
<td>63.0</td>
<td>71.6</td>
<td>73.5</td>
<td>73.2</td>
</tr>
</tbody>
</table>

*See Table 1.

†Probability that the difference between the means as compared is due to chance.

---

**TABLE 1**

<table>
<thead>
<tr>
<th>Group*</th>
<th>Control (gm)</th>
<th>GH (2.5), (gm)</th>
<th>GHPL, (2.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Body weight (gm)</td>
<td>80.4</td>
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</tr>
</tbody>
</table>

*See Table 2.

†Probability that the difference between the means as compared is due to chance.
of questionable significance ($P < 0.1; > 0.05$) when absolute weight gain is considered (Table 2), and not significant ($P < 0.2; > 0.1$) when the gain is expressed per gram of food (Chart 2). No significant difference exists between the GH and GHPL groups themselves.

In the tumor animals that received growth hormone (GH and GHPL groups) the gain in total body weight was significantly greater than that of the control and PL tumor animals (Chart 1; Table 2). There is contradiction in the literature concerning the distribution of the weight gain in the tumor-host organism under the influence of growth hormone. Within the time limits of our experiment there seemed to be little preference for the tumor or the carcass when compared with the control animals (Table 3): the expression of $\Delta$ tumor/$\Delta$ total body weight $\times 100$ gives figures of $75.8$, $64.3$, and $56.3$ per cent for the control, GH, and GHPL animals, respectively. The GH figure is not significantly lower, and the GHPL figure is of questionable significance ($P < 0.1; > 0.05$) in relation to the control. Thus, if there were any preference in the partition under the stimulus of growth hormone alone, it favored the carcass rather than the tumor under the conditions of our experiment. Likewise, if the PL diet, when combined with the growth hormone injections, exerted any influence, it was also in favor of the host, although the difference between the control and GHPL groups in regard to tumor/total-body ratios is not of sufficient significance to do more than indicate that this may have been true. This suggested preference for the host carcass in the matter of weight gain is interesting in the light of the extreme “tumor-favoring” of the PL diet in the tumor animals without growth hormone, where the ratio of $\Delta$ tumor/$\Delta$ body weight was $101.9$ per cent (Table 3); this was the only group in our experiment which showed actual carcass loss. However, when the PL diet was added to the growth hormone injections (GHPL group), an actual increase in the carcass weight over the tumor animal receiving growth hormone alone (GH group) is suggested, rather than a decrease as one might expect. Thus, the growth hormone injections not only overcame the effect of the dietary phospholipide on the carcass, as seen in the tumor animal (PL group), but the hormone may somehow have utilized such phospholipide for greater carcass growth than occurred in the tumor animals receiving growth hormone alone.

In summary, then, the growth hormone in the tumor animal increased the gain in total body weight but caused little or no change in the relative partition of that gain between carcass and tumor. The data suggest that the combination of growth hormone and PL diet may have favored, somewhat, the carcass over the tumor, an effect that was exactly opposite to that exerted by the PL diet alone.

### Table 3

The means of the ratios* of change in tumor weight to change in total-body weight

<table>
<thead>
<tr>
<th>Group</th>
<th>Tumor weight×100</th>
<th>Standard deviation</th>
<th>Compared with</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>75.8</td>
<td>21.8</td>
<td>PL</td>
<td>$&lt;0.05; &gt;0.02$</td>
</tr>
<tr>
<td>PL</td>
<td>101.9</td>
<td>39.1</td>
<td>GHPL</td>
<td>$&lt;0.01; &gt;0.001$</td>
</tr>
<tr>
<td>GH</td>
<td>64.3</td>
<td>26.4</td>
<td>Control</td>
<td>$=0.3$</td>
</tr>
<tr>
<td>GHPL</td>
<td>56.3</td>
<td>23.0</td>
<td>Control</td>
<td>$&lt;0.1; &gt;0.05$</td>
</tr>
</tbody>
</table>

* Computed for each animal.
† See Table 1.
‡ Probability that the difference between the means as compared is due to chance.

### SUMMARY

The effect of dietary tumor phospholipide and of administered growth hormone on the partition of total body weight between carcass and tumor has been studied over a 10-day period of growth of Walker carcinoma 256 in adult female rats that had reached a plateau in body weight.

The tumor phospholipide decreased appetite and carcass weight and favored the growth of tumor, as compared with groups not fed phospholipide. Tumor-free rats, maintained on the same phospholipide-containing diet, not only did not lose appetite but gained significantly more weight than rats on diet without phospholipide. Thus, in the tumor-bearers, dietary tumor phospholipide seemed to enhance the effect of the growing tumor upon the host rat. The possible significance of these findings is discussed.

Growth hormone increased the gain in total body weight of the animals with and without tumor, as compared with rats not given hormone. In the tumor-bearing rats the partition of weight gain between tumor and carcass was little affected by growth hormone. When dietary tumor phospholipide and growth hormone were combined,
the gain in weight of the carcass tended to exceed that of the tumor.

REFERENCES
Growth Hormone and Tumor Phospholipide Effects on Tumor and Body Growth


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