Subsequent to the observation of Schott, Samuels, and Ball (21) that the slow-growing Walker 256 tumors of hypophysectomized rats contained considerably greater concentrations of glycogen than were found in the more actively growing tumors of intact animals, it became of interest to determine whether a high glycogen percentage was associated generally with slow tumor growth or was a special effect of hypophysectomy.

The significance of glycogen in normal cells, whether static or proliferating, has never been satisfactorily defined. Its significance in neoplastic cells is, therefore, bound to be in doubt. Two conflicting views are held; one, that glycogen is most abundant in rapidly proliferating neoplasms, and the contrary, that it is more abundant in the relatively static growths.

Brault (5), using a histological technique, studied malignant tissues and found glycogen most abundant in the areas of greatest proliferation. He concluded that the degree of malignancy was proportional to the amount of glycogen present and reiterated his belief in 1938 (6). Gierke (10) made observations on a variety of tumors and concluded that glycogen was present on the same basis as its occurrence in normal cells. Lubarsch (14) observed more glycogen in sarcomas than in carcinomas, while Best (3) found glycogen to be more abundant in slow-growing tumors. Although further early observations are on record, little knowledge was added until after a chemical technique for determination was available (18) and interest in the subject was renewed.

Cori and Cori (7), using a modification of Pfüger's chemical method, reported glycogen values for 6 spontaneous carcinomas in mice within the range of 0.171 to 0.303 per cent, and for 1 Jensen rat sarcoma of 0.122 per cent. They stated that ingestion of glucose seemed to increase the glycogen content of tumors. Tesuuro (22) reported values for 5 cases of cancer of the cervix and 1 case of carcinoma of the fundus. These values averaged 0.759 per cent of the dry weight of the sample, corresponding to about 0.114 per cent of the fresh weight. Fahrig (8) also worked with human tumors. Bernhard (2) reported low values (0.014 to 0.066 per cent) for mammary carcinoma and myoma of the uterus, and high values (0.436 to 0.683 per cent) for carcinoma of skin, bladder, and stomach. The value for one spindle cell sarcoma is given as 0.172 per cent. Twelve tumors were analyzed and the conclusion was drawn that malignant tumors contain more glycogen than benign ones. Borghi (4) found an average value of 0.01 per cent for 5 adenocarcinomas of mice. Roussy and Cracien (19, 20), using a histological procedure, attempted to describe the distribution of this material in the Jensen sarcoma and the Rous sarcoma. They distinguished between a "reversible" and "irreversible" form, and claimed to discern five separate zones in the Jensen tumor. Goldfeder (12) made numerous analyses of implanted tumors of mice and chickens, and ascertained that the glycogen content varied inversely with the tumor size. In the Rous sarcoma she found that the periphery of the tumor contained about three times the amount detected in the necrotic center. Feeding glucose or giving it subcutaneously caused higher glycogen values in the tumors but was accompanied by more active tumor growth and an earlier decline of the host. Haendel and Maler (13) found less histological evidence of glycogen in the treated skin and livers of tarred mice than in controls. Babes and Pantzu-Lazarescu (1) found no granules of glycogen in undifferentiated cervical carcinomas but considerable quantities in the partially differentiated forms. Since normal mucous membrane contains this material, it was considered evidence of the degree of differentiation. Faroy (9) supports Brault's contention that glycogen concentration increases with the malignancy of the tumor, and thinks that the determination of glycogen in a given tumor might be of prognostic value. Petrowa (17) examined 22 myomas of the uterus by the histologic method and found considerably more glycogen in the myomas than in the uterine muscle. Lustig and Wachtel (15) found that of many substances mixed with minced tumor inoculum, glycogen was the only substance which inhibited the usual development of tumors.

Several references dealing with the use of glycogen-forming sugars in tumor-bearing animals are of interest. Cori and Cori (7), Goldfeder (12), and Schott, Samuels, and Ball (21), cited above, observed more glycogen in the tumors of animals fed glucose. Parfentjev et al. (16) found no effect on the growth rate of sarcoma 180 from glucose or arabinose injected subcutaneously at a distance from the tumor site. Vannaitl (23) reported a significantly longer life and fewer metastases in a large series of tarred mice to which a 50 per cent glucose solution was available for drinking. It seems, therefore, that information on the significance of glycogen and the influence of carbohydrates on tumor growth is still problematical.

**MATERIALS AND METHODS**

Male rats were inoculated with tumor fragments by trocar and one to two weeks later a portion of each group was hypophysectomized. In experiments I to III, inclusive, all were fed twice daily by stomach tube a mixture of 200 gm. of powdered milk, 200 gm. of glucose, 220 ml. water, and 5 Abbott ABD capsules,
in amounts sufficient to maintain body weight at a constant level. All received subcutaneously 0.1 ml of adrenal cortex extract daily. In experiment IV, a stock diet was available to the animals ad libitum. Tumors were measured at daily to twice weekly intervals and the growth curve plotted as the best fit straight line. The slope was then estimated.

Tumors were removed under amytal anesthesia at a stipulated time after inoculation or after having reached a certain size, roughly bisected, and a thin slice from one cut surface reserved for histological study by Best's carmine stain after fixation in absolute ethyl alcohol. The remaining tissue was divided into two samples for chemical assay: a small peripheral slice of viable tissue by a micro method, and the remainder including the necrotic center by the standard technic of Good, Kramer, and Somogyi (11) using the micro sugar reagents of Shaffer and Somogyi. The hydrolyzates were subjected to yeast fermentation in every case to exclude errors due to nonfermentable reducing substances.

Completeness of pituitary ablation was checked on the formalin-fixed heads of the animals by removing the superior part of the skull, retracting the brain upwards and back, and examining the sella by direct vision or with the aid of a lens.

The following special conditions apply: Experiments I and II—tumors were removed for analysis on a given day after inoculation. Experiment III—the tumors were taken for analysis when they had attained a given size. Experiment IV—tumors from intact animals on a stock diet were selected from fast and slow-growing transplants or picked at random at regular intervals.

RESULTS

The data in Table I reveal an inverse correlation between the glycogen content of the tumors and their size and rate of growth. The chance of occurrence from random sampling is indicated and in general

### Table I: Correlation Coefficients (r) Between Glycogen Content and Size and Rate of Growth of Tumors

<table>
<thead>
<tr>
<th></th>
<th>Experiment I</th>
<th>Experiment II</th>
<th>Experiment III</th>
<th>Experiment IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>( r_g ) (Glycogen and tumor size)</td>
<td>-0.43 ± 0.12</td>
<td>-0.59 ± 0.14</td>
<td>-0.33 ± 0.09</td>
<td>-0.35 ± 0.07</td>
</tr>
<tr>
<td>( r_g ) (Glycogen and growth rate)</td>
<td>-0.68 ± 0.11</td>
<td>-0.34 ± 0.09</td>
<td>-0.30 ± 0.07</td>
<td>-0.40 ± 0.11</td>
</tr>
<tr>
<td>( r_{g+d} ) (Glycogen and growth rate, excluding the influence of hypophysectomy)</td>
<td>-0.61 ± 0.14</td>
<td>-0.32 ± 0.08</td>
<td>-0.34 ± 0.11</td>
<td>-0.32 ± 0.09</td>
</tr>
<tr>
<td>( r_{s} ) (Size and growth rate)</td>
<td>+0.92 ± 0.03</td>
<td>+0.60 ± 0.09</td>
<td>+0.60 ± 0.10</td>
<td>+0.60 ± 0.10</td>
</tr>
<tr>
<td>Number of animals</td>
<td>20</td>
<td>15</td>
<td>46</td>
<td>15</td>
</tr>
</tbody>
</table>

Coefficients for occurrence from random sampling:
- 5 per cent chance: 0.44
- 1 per cent chance: 0.56

\( x_0 \) = glycogen percentage in total tumor.
\( x_1 \) = size (weight).
\( x_2 \) = growth rate (mm. mean diameter per day).
\( x_3 \) = hypophysectomy (expressed as unity for hypophysectomized rats and zero for intact animals).

### Table II: Data from Experiment III

<table>
<thead>
<tr>
<th></th>
<th>Controls (32)</th>
<th>Hypophysectomized (14)</th>
<th>Difference of means</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor weight in gm</td>
<td>3.8 ± 16.2</td>
<td>6.57 ± 0.33</td>
<td>2.79 ± 3.7</td>
</tr>
<tr>
<td>Growth rate, mm. per day</td>
<td>0.84 ± 0.24</td>
<td>0.59 ± 0.07</td>
<td>0.25 ± 0.09</td>
</tr>
<tr>
<td>Glycogen in total tumor, per cent</td>
<td>0.001 ± 0.01</td>
<td>0.62 ± 0.013</td>
<td>0.62 ± 0.013</td>
</tr>
<tr>
<td>Glycogen in peripheral slice, per cent</td>
<td>0.005 ± 0.012</td>
<td>0.63 ± 0.013</td>
<td>0.62 ± 0.013</td>
</tr>
</tbody>
</table>

An attempt at correlation of histological and chemical determinations of glycogen is given in Table III for 32 tumors. The results are disappointing for the microscopist. Apparently a large proportion of the glycogen must be distributed in solution throughout the tissue. Only in those tumors which showed the largest amounts of glycogen granules is there any significant correlation with the chemical analyses.
**DISCUSSION**

The increased glycogen concentration in the slow-growing tumors may be either a result or a cause of slow growth. The first assumption seems more probable. Since slow-growing tumors, whether produced by hypophysectomy or other causes, require less energy, the increase in glycogen values is probably a matter of accumulation incident to decreased utilization. Since glycogen accumulates when tumor utilization is probably decreased, it follows that glycogenesis is a more constant process than utilization, and is not influenced to an equal extent by the same factors. Glycogenesis appears to be primarily dependent on the supply of materials from which glycogen can be formed since the concentrations within both fast- and slow-growing tumors are increased by feeding large amounts of carbohydrates (21).

Even though relatively large areas of necrosis were present centrally and absent peripherally, glycogen was rather uniformly distributed throughout the tumor.

It would seem, then, that the enzyme system involved is not dependent on the immediate presence of viable cells, or that greater diffusion of glycogen has occurred than would ordinarily be supposed. Our findings in this respect differ sharply from those of Goldfeder (12). It seems possible that the difficulty to be encountered in separating the peripheral part of the Rous sarcoma from the adjacent muscles might reasonably account for this difference.

**SUMMARY AND CONCLUSIONS**

1. The glycogen content of Walker 256 tumor is inversely correlated with its size and rate of growth.

2. The increased glycogen concentrations which have been observed in the tumors of hypophysectomized rats are due to slower growth, since elimination of the influence of hypophysectomy did not significantly alter the correlation between glycogen content and rate of tumor growth.

3. Necrotic areas of tumor contain glycogen in amounts comparable with those found in proliferating areas.

4. Little correlation was found between the amount of material histologically distinguished by its carmine staining reaction, and polysaccharide which can be separated from tumor tissue by the usual biochemical technic. Only when the concentration of glycogen is relatively high is there a significant increase in glycogen granules in tissue preparations.

**REFERENCES**


15. Lustig, B., and H. Wachtel. Versuch einer Methodik zur 
Prüfung von Substanzen auf ihre Wirksamkeit bei Carci-
The Influence of Various Preparations of Lactic Acid and 
Sugars on the Growth of Transplanted Tumors: Mouse 
17. Piatrowa, E. Zur Frage über den Glycogengehalt in den 
18. Pfeiffer, E. Methode der quantitativen Analyse des 
Glykogens und die Arteigentlichkeit der Substanzen 
1909.
19. Roussy, G., and E.-C. Cracium. La glycogénie du sarcome 
1928.
20. Roussy, G., and E.-C. Cracium. Glycogen of the sarcoma 
de Pousson-Rous. Compt. rend. Soc. de biol., 99:1720-
1722. 1928.
21. Schott, H. F., L. T. Samuels, and H. A. Ball. Effect of 
Hyphophysectomy on Glycogen Distribution in Tumor-Bearing 
412. 1937.
22. Tsujiro, G. Il glicogeno nei carcinomi dell'utero. Arch. di 
23. Vannfält, K. A. Über die Wirkung von Glykose auf Teer-
1917-18.

Corrections


The following corrections of errors in the manuscript are published at the request of the authors:
1. P. 799, column 2, line 14: For "(No. 3, Table I)" substitute "(No. 2, Table I)."
2. P. 799, footnote 2: For "No. 3" substitute "No. 2"; for "0.017" substitute "0.17."
3. P. 800, column 1, paragraph 2, line 9: Insert "in the filtrate" after "it was present."
4. P. 804, column 2, last line: Delete "nor" and insert "and is."
5. P. 806, column 1, line 1: Insert "not" after "The active material is."
6. P. 800, Table I, substitute the following:

Table I: Summary of Tests of Fractions of Yeast Extract

<table>
<thead>
<tr>
<th>Extract No.</th>
<th>Preparation</th>
<th>Dose mgm.</th>
<th>Percent of solids compared with yeast extract</th>
<th>Total number of animals</th>
<th>Complete regressions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Ethanol precipitate after dialysis</td>
<td>...</td>
<td>3</td>
<td>28</td>
<td>37</td>
<td>9</td>
</tr>
<tr>
<td>2. Lead filtrate from No. 1</td>
<td>...</td>
<td>3</td>
<td>32</td>
<td>27</td>
<td>9</td>
</tr>
<tr>
<td>3. Silver filtrate from No. 2</td>
<td>...</td>
<td>3</td>
<td>11</td>
<td>23</td>
<td>3</td>
</tr>
<tr>
<td>4. Lead filtrate from undialized yeast extract</td>
<td>...</td>
<td>4</td>
<td>25</td>
<td>19</td>
<td>5</td>
</tr>
<tr>
<td>5. Lead precipitate from undialized yeast extract</td>
<td>...</td>
<td>4</td>
<td>37</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>6. Silver filtrate from No. 4</td>
<td>...</td>
<td>4-5</td>
<td>16</td>
<td>16†</td>
<td>5</td>
</tr>
<tr>
<td>7. Silver precipitate from No. 4</td>
<td>...</td>
<td>1</td>
<td>11</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>8. Ethanol-insoluble barium salts from No. 6</td>
<td>...</td>
<td>0.9</td>
<td>5.2</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>9. Phosphotungstate from No. 6</td>
<td>...</td>
<td>0.7</td>
<td>2.5</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>10. No. 1 after nitric acid treatment</td>
<td>...</td>
<td>7.5</td>
<td>...</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>11. No. 1 after treatment with N2HCl</td>
<td>...</td>
<td>4.5</td>
<td>...</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

* Temporary regressions with subsequent recurrences are not included in these statistics.
† 8 mice received 4.5 mgm., and 8 received 1.5 mgm. in single doses.
Glycogen in Walker Tumor 256

Howard A. Ball, Hermann F. Schott and Leo T. Samuels

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