Blood Cell Factors and Tumor Growth in the Cheek Pouch of the Golden Hamster (*Mesocricetus auratus)*

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Previous studies on the hematological changes accompanying tumor growth have not been correlated with the various phases of growth as determined from an accurate growth curve. With the introduction of the membranous cheek pouch of the hamster as a transplantation site (16), it became possible to relate blood cell changes to the morphological changes undergone by a transplant as it grows, viz., the macroscopic tissue reaction observed in the early stages of tumor growth and the logarithmic phase, ulceration, and external necrosis, which are characteristic of the later stages.

The purpose of this study is to establish relationships between all phases of an accurate growth curve of a 100 per cent transplantable methylcholanthrene-induced sarcoma of the hamster (16) and the changes in the formed elements of the blood.

MATERIALS AND METHODS

The transplantation technic, the tumor, the apparatus, the method of animal care, and the method of tumor observation used in the present investigation were the same as those described by Luts et al. (16) and by Handler (10). The original microscopic description of the tumor is still tenable, but mitotic figures are twice as numerous.

The average growth curves of the tumors (10th—50th transplantation generation) used in the present investigation show a latent period of 2 days, and vascularization appears to become established during the first 3 days. The methylcholanthrene-induced sarcomas gradually increase in size until the 9th day and show accelerated growth until the 10th day, and then growth proceeds exponentially, with ulceration occurring between the 13th and 30th days. Using standard hematological techniques, the following blood cell factors were studied in relation to the average growth curve and to phenomena associated therewith; differential white blood cells, total white blood cells, and sedimentation rates (determinations were made on separate groups of twenty hamsters of both sexes, 8—10 weeks old); total red blood cell counts, red blood cell volumes, hemoglobin and platelet determinations (performed on each of twenty hamsters of both sexes, 8—10 weeks old).

Two blood determinations taken 10 days apart were averaged to establish the normal in any one animal. Blood was obtained by cardiac puncture at 5-day intervals for 50 days for all of the hematological tests except for differential white counts, in which case blood was obtained by tail puncture on the 1st and 5th days and at the 5-day intervals as above. Immediately following the second "normal" determination, a 1.0-c.mm. fragment of the tumor selected uniformly 10 days after external ulceration was transplanted to the hamster cheek pouch.

The following control experiments were performed:
(a) A group of 40 hamsters of both sexes, 8—10 weeks old, was used to preclude the possibility that the effects observed and results obtained might be related to the chronic blood loss rather than to tumor growth. (b) Benzidine tests were performed every 5 days for 30 days on fifteen hamsters with tumors to detect occult fecal blood. (c) Blood was drawn from ten hamsters 30 days after tumor transplantation and was plated on blood agar. (d) Food consumption (gm/24 hours) was measured at 2-day intervals in fifteen experimental and ten control hamsters over a period of 30 days.

Spleens and livers were removed from 30 hamsters that possessed tumor transplants in their cheek pouches but had had no heart punctures, from 30 hamsters with sarcoma transplants and which had one to twelve cardiac punctures made at 5-day intervals, and from 27 nontumor-bearing hamsters which received one to twelve cardiac punctures at similar intervals. The spleens and livers were removed from the three groups of animals at comparable days.

All material prepared for histological study was stained with Harris’ hematoxylin and eosin Y and phosphine b. Marrow smears were made from femoral bone marrow and stained with a combination of Wright’s stain and Giemsa’s stain. The Lepehne-Pickworth technic (13) was used to demonstrate the hemolysis associated with the macroscopic tissue reaction.

RESULTS

Table 1 summarizes the normal blood values of male and female hamsters of 80—100 grams body weight. Chart 1 shows that, as the methylcholanthrene-induced tumor grew, the average neutrophil percentages increased while the average lymphocyte percentages decreased during the 50 days of tumor growth. Ten days after transplantation a statistically significant increase (P < 0.01) in the average neutrophil percentage and a statistically significant decrease (P < 0.01) in the average lymphocyte percentage, as compared with the normal averages, were demonstrated by the Fisher-Student “t” test (8). Reversal of the average of these percentages occurred by the 17th day.

* This work has been supported in part by the National Cancer Institute, U.S.P.H.S. Grant No. C-1644 M & G.
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Received for publication July 9, 1954.
In all animals the rate of increase in the percentage of neutrophils and the rate of decrease in the percentage of lymphocytes appeared to be more pronounced after ulceration of the cheek pouch.

In about 40 per cent of the experimental animals, areas of hemolysis were macroscopically observable over and around the implant during the first 10 days following transplantation. This reaction is referred to as the macroscopic tissue reaction, and it was observed only in hamsters with statistically significant increase (P < 0.01). Neutrophils with "toxic" granules first appeared in 50 per cent of the hamsters after 30 days of tumor growth, and cytoplasmic vacuoles were observed after 35 days of tumor growth. The number of neutrophils with "toxic" granules increased from 0.60 to 6.60 per cent during the last 20 days of tumor growth.

### TABLE 1

**Hematological Values for Normal Hamsters (Mesocricetus auratus)**

<table>
<thead>
<tr>
<th></th>
<th>Average</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total white blood cells (thousands/c mm)</td>
<td>3.78</td>
<td>1.29</td>
</tr>
<tr>
<td>Differential white blood cells (per cent):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total neutrophils</td>
<td>29.9</td>
<td>5.5</td>
</tr>
<tr>
<td>Filamentous neutrophils</td>
<td>22.6</td>
<td>2.5</td>
</tr>
<tr>
<td>Nonfilamentous neutrophils</td>
<td>7.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>66.4</td>
<td>5.9</td>
</tr>
<tr>
<td>Monocytes</td>
<td>2.6</td>
<td>0.8</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>1.1</td>
<td>0.02</td>
</tr>
<tr>
<td>Total red blood cells (millions/c mm)</td>
<td>5.96</td>
<td>0.70</td>
</tr>
<tr>
<td>Hemoglobin* (grams/100 ml)</td>
<td>16.2</td>
<td>1.8</td>
</tr>
<tr>
<td>Red blood cell packed volume* (hematocrit) (per cent)</td>
<td>50.7</td>
<td>3.0</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin* (jug)</td>
<td>23.3</td>
<td>2.0</td>
</tr>
<tr>
<td>Mean corpuscular volume* (cp)</td>
<td>72.4</td>
<td>4.2</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin concentration* (per cent)</td>
<td>32.1</td>
<td>1.8</td>
</tr>
<tr>
<td>Platelets* (thousands/c mm)</td>
<td>2.97</td>
<td>0.73</td>
</tr>
<tr>
<td>Sedimentation rate (mm/hour)</td>
<td>1.6</td>
<td>0.81</td>
</tr>
</tbody>
</table>

* Determinations made on same animals used for total red blood cell counts.

† Temperature varied between 69° and 75° F.

Pretransplantation lymphocyte counts higher than one standard deviation above the normal average. Metamyelocytes, myelocytes, and progranulocytes were observed in the peripheral blood of 15 per cent of the hamsters with 25-day tumors. During the last 5 days of tumor growth the average percentage of monocytes and eosinophils was significantly less than the normal average at the 5 per cent level of significance (23).

Chart 2 shows the bone marrow response to tumor transplants. Both the filamentous and non-filamentous neutrophils are included in these percentages of neutrophils with "toxic" granules. From the 25th day after tumor transplantation to the conclusion of the experiment, the average percentage of nonfilamentous neutrophils showed a 1 filamentous and nonfilamentous forms of neutrophils compare with the segmented and band forms of the human, respectively. The latter nomenclature is recommended by the Committee on Clarification of the Nomenclature of Cells and Diseases of the Blood and Blood-forming Organs (Blood, 4:90, 1940).
Chart 3 shows the progressive increase in the average total white blood cell counts as the tumor grew. From the 15th to the 50th day after tumor transplantation, the average values reported for total white blood cell counts were significantly greater than the normal value \( (P = 0.05) \). Occasionally, a variable amount of edema was observed around the transplants in the experimental animals and was related to a low pretransplantation total white blood cell value and to a low hemoglobin value (both at least one standard deviation below the normal average). These low blood values were found even at 20 and 30 days after tumor transplantation in all hamsters which bore nonulcerated, edematous tumors, and such transplants possessed a small volume.

Chart 4 shows the average hematocrit values for normal and tumor-bearing hamsters. From the 20th day to the conclusion of the experiment, the average hematocrit percentages were significantly different from the normal \( (P = 0.05) \). The average normal hemoglobin value as well as changes in this value associated with 50 days of tumor growth are shown in Chart 5. The averages established between the 40th and 50th day are significantly lower than the established normal value \( (P < 0.01) \), and all other apparent deviations from the normal are not statistically significant. Chart 6 represents the average total red blood cell counts for both normal hamsters and those bearing tumors. Changes in the average total red blood cell counts for the first 35 days after tumor transplantation lack statistical significance, but after this time they are significantly decreased \( (P = 0.05) \).

The three red blood cell indices showed fluctuations during the period of tumor growth. The percentage of anemic hamsters increased with time, and the later stages of tumor growth were associated with a hypochromic type of anemia. Sixty per cent of the hamsters were anemic after 50 days of tumor growth.

Control hamsters which were repeatedly bled showed minor fluctuations from the established
normal averages for the total red blood cell, hemoglobin, and hematocrit values. The red blood cell indices in this control group revealed that a microcytic, normochromic anemia was the only morphological form of anemia present; 30 per cent of these hamsters possessed this type of anemia after four cardiac punctures, and after eight cardiac punctures 56 per cent were anemic. The percentage of hamsters with this type of anemia tended to decrease after eight punctures and, after twelve punctures, none of those surviving was anemic.

The benzidine tests performed on the feces and the bacteriological tests performed on the blood of control groups of tumor-bearing hamsters were all negative. The percentages and forms of anemia in these groups were approximately the same as those in the experimental group. Normal hamsters of both sexes, 8–10 weeks old, consumed an average of 6.94 ± 1.52 gm. of Purina Laboratory Chow per 24 hours. After 50 days of tumor growth, the average amount of food consumed in 24 hours was found to be 5.00 ± 2.77 gm., and the difference is not statistically significant.

The tumor-bearing hamsters which were chronically bled generally showed two types of bone marrow response after 50 days of tumor growth and twelve cardiac punctures: (a) a normal cellular content with a hyperplasia of either the erythrocytic or granulocytic series and increased megakaryocytes and (b) a hypercellular content with a hyperplasia of the erythrocytic series and of the megakaryocytes.

After five or more cardiac punctures the non-tumor-bearing hamsters showed a hyperplasia of the erythrocytic series and megakaryocytes in their bone marrow. The cellularity of the bone marrow remained normal regardless of the number of cardiac punctures.

Chart 7 depicts the average normal sedimentation rate of both normal and tumor-bearing hamsters and shows a statistically significant increase after 30 days of tumor growth (P = 0.05). Thirty per cent of the hamsters were found to have normal sedimentation rates even after 50 days of tumor growth.

The data for the average platelet counts in normal and tumor-bearing hamsters are presented in Chart 8. No statistically significant difference could be demonstrated between the average platelet counts obtained before tumor transplantation and the average platelet counts obtained for any phase of tumor growth.

Extramedullary hematopoiesis was observed in the spleens of all experimental animals. It was also observed in the livers of all tumor-bearing hamsters but not in the livers of control hamsters subjected to repeated heart punctures. Table 2 is a tabulation of the data on the extramedullary hematopoietic studies in the three experimental groups of hamsters.

Microscopically, the spleens with extramedullary hematopoiesis possessed indistinct red pulp...
areas. The hematopoietic foci appeared as small groups of deeply staining cells scattered throughout the splenic tissue. Megakaryocytes were observed, and mitotic figures were quite numerous. Animals which possessed extramedullary hematopoiesis in their livers had areas of deeply staining nuclei generally situated about their intralobular veins, and sometimes in the parenchyma. Microscopically, a splenic enlargement was noted in some hamsters in Groups 1 and 3 which possessed advanced tumors but in none of the animals in Group 2.

DISCUSSION

In discussing the blood cells of the hamster in relation to tumor growth, the state of the tumor must be kept clearly in mind, since internal necrosis, ulceration of the cheek pouch, and external necrosis take place.

Since internal necrosis was found to be related to tumor size, the larger 10-day tumors may have had small, internal areas of necrosis. This necrotic material might result in a stimulation of the bone marrow with the resulting changes (24, 30). However, it is doubtful whether this is adequate to explain the changes associated with the small 10-day tumors. The methylcholanthrene-induced hamster sarcoma may have a direct effect upon hematopoietic centers to increase the neutrophils even in the absence of internal necrosis. After ulceration of the cheek pouch, the changes observed were most probably related to the absorption of necrotic and bacterial products. Other investigators (1, 3, 4, 6, 7, 9, 13, 17, 21, 28, 30) have noted similar changes in the number of neutrophils and lymphocytes in other animals and with other tumors.

The increases in the average percentages of nonfilamentous neutrophils before the day of ulceration probably indicate a growing activity of the bone marrow and not necessarily an infection, which was not the case after ulceration. The inability of the bone marrow to keep pace with the demand for neutrophils can be seen, for after 35 days of tumor growth the average percentage of nonfilamentous neutrophils and the total white blood cell counts declined, and at this time there was a progressive increase in the percentage of neutrophils with "toxic" granules. The presence of cytoplasmic vacuoles indicated that a severe infectious and degenerative processes were taking place (2, 22, 25).

The correlation between high lymphocyte values before tumor transplantation and a marked macroscopic tissue reaction is interesting. The association of the lymphocytes with transplantation resistance has been reported by numerous investigators (5, 14, 18—20). The macroscopic tissue reaction was probably a function of the inherent genetic dissimilarities between the host and the tumor.

We found no evidence that the tumor per se produces a substance which interrupts the formation of hemoglobin, as has been shown by other investigators (26, 27). On the contrary, in the animals with ulcerated tumors, the drop in the total red blood cell volume, the hemoglobin, and the total red blood cell count became statistically significant at approximately the same time that the animals became severely infected.

The absence of bacteria in the blood stream of the hamsters bearing tumors and the absence of occult fecal blood indicated that the anemia, regardless of type, was not related to the presence of bacteria per se nor to hemorrhage, but was probably directly related to the tumor transplant and/or internal necrosis in the slow-growing nonulcerating tumors.

Tumor-bearing hamsters possessed various types of anemia. The early stages of tumor growth were generally associated with a microcytic, normochromic type, which decreased with tumor growth, and the later stages with hypochromic types that were probably produced by factors other than chronic bleeding. Extramedullary hematopoiesis may have been present in these hamsters, decreasing the anemic tendency, but because of the increasing volume and increasing

![Chart 8. Correlation of changes in the average platelet counts with tumor growth.](chart8.png)
<table>
<thead>
<tr>
<th>Days after transplantation</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tumor-bearing hamsters with cardiac punctures</td>
<td>Non-tumor-bearing hamsters with cardiac punctures</td>
<td>Tumor-bearing hamsters without cardiac punctures</td>
</tr>
<tr>
<td></td>
<td>No. animals with extramedullary hematopoiesis</td>
<td>No. animals with extramedullary hematopoiesis</td>
<td>No. animals without extramedullary hematopoiesis</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>Liver</td>
<td>Spleen</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>15</td>
<td>5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>20</td>
<td>6</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>25</td>
<td>7</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>30</td>
<td>8</td>
<td>2,1?</td>
<td>1</td>
</tr>
<tr>
<td>35</td>
<td>9</td>
<td>3,1?</td>
<td>1</td>
</tr>
<tr>
<td>40</td>
<td>10</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>45</td>
<td>11</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>50</td>
<td>12</td>
<td>10</td>
<td>12</td>
</tr>
</tbody>
</table>

* Numbers with question marks refer to animals with either questionable extramedullary hematopoiesis or the beginning of this condition.
necrotic mass of a growing tumor, this factor may not have been effective in preventing anemia.

The control hamsters which were chronically bled showed an increasing frequency of microcytic, normochromic anemia as the number of cardiac punctures increased up to eight. Thereafter the number of animals with this type of anemia diminished, and at the conclusion of the experiment none of the surviving hamsters was anemic. These hamsters were apparently able to overcome the cumulative effects of chronic blood loss with the aid of extramedullary hematopoiesis.

The sedimentation rate was first observed to increase at about the time that the differential white blood cell picture reflected a marked infection and at approximately the same time that the total red blood cell, hemoglobin, and hematocrit values were declining. The increase in the sedimentation rate more closely paralleled the anemic condition of the red blood cell picture than it did the infectious state.

Lutz, Fulton, and Akers (15) reported white blood cell thrombi and platelet plugs in the blood vessels of hamsters bearing advanced methylcholanthrene-induced sarcoma transplants in their cheek pouches. Though a decrease in the platelet values might be expected, the presence of normal platelet counts in animals with advanced tumors can be explained. First, there was an increased number of megakaryocytes observed in the bone marrows of the tumor-bearing hamsters which were chronically bled. Secondly, masses of platelets that form around a foreign body or a portion of a vessel may subsequently float off into the circulation and break up again into their constituent parts (29). Thirdly, sites of extramedullary hematopoiesis with megakaryocytes may also maintain normal platelet values in these hamsters.

The results of this study show that extramedullary hematopoiesis can be produced in the hamster spleen by chronic bleeding. Since the tumor growth process also resulted in hematopoietic activity in some livers, as well as in all spleens, it can be assumed that tumor growth produced more pronounced changes in the hematopoietic centers than did chronic bleeding alone.

Kelsall (11) stated that the cells produced in the extramedullary foci of her hamsters were functionally identical with those produced in the bone marrow. The extramedullary production of red blood cells could have been functionally capable of maintaining the total red blood cell count and the hemoglobin level after ulceration. However, in the very advanced stages of tumor growth, in spite of extramedullary hematopoiesis in the spleen and possibly the liver, hamsters with tumors were unable to produce a sufficient number of normal red blood cells, and, therefore, they developed a hypochromic anemia.

**SUMMARY**

1. Associated with the growth of the methylcholanthrene-induced hamster sarcoma in the cheek pouch of the hamster, there was an increase in the neutrophils and total white blood cells before ulceration, and the sedimentation rate increased during the later stages of tumor growth.

2. Nonfilamentous neutrophils increased after tumor transplantation, and then they decreased; neutrophils with “toxic” granules increased at this time.

3. The lymphocyte, hemoglobin, total red blood cell, and hematocrit values decreased during tumor growth, while monocyte, eosinophil, and platelet values remained unchanged.

4. The early stages of tumor growth were associated with a microcytic, normochromic type of anemia, whereas the later stages were associated with a hypochromic type.

5. Extramedullary hematopoiesis was observed in the spleens and livers of tumor-bearing hamsters regardless of whether or not they received cardiac punctures. This condition was never observed in the livers of control animals with repeated heart punctures but was observed in the spleens of these animals.

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