Hemoglobin Level and Tumor Growth*

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The association of anemia with cancer has been reported for many different types of animals. It has been repeatedly observed that the later stages of malignant growths are characterized by a severe and progressive depression in the blood hemoglobin level. This has been found to be true of many different types and locations of cancer in man, mice, rats, rabbits, birds, etc. (1, 2, 4, 5, 9, 13, 15).

In reporting their observations of this effect most workers have described it as a "secondary anemia" and in general consider it to be the result of the general toxic action of the cancer on the host. Such conditions as hemorrhage, infection, the presence of necrotic tissue, and general debilitation have been suggested as mediating factors (1, 3, 14).

The possibility that cancerous growths may have a direct and destructive effect on hemoglobin or inhibit its production has not apparently been seriously considered.

It has been recently shown by Craig et al. (8) that the concentration of cytochrome C in cancer tissue is lower than in normal tissue. Greenstein and co-workers (10) also recently reported that the introduction of liver tumor tissue into an animal produced a very rapid and striking decrease in the liver catalase concentration. Both of these observations indicate that cancer tissue has an antagonistic action on enzyme systems of this type and since both cytochrome and catalase enzymes have the same type of hemin prosthetic groups as hemoglobin, it would not be surprising to find that hemoglobin was acted upon in the same manner by tumorous tissue. These considerations could conceivably account for the striking anemia characteristic of the later stages of cancer, which is referred to above. However, in the advanced period it is difficult to dissociate the primary from the secondary effects of tumor growth. For that reason the possible mechanism of the precancerous and early cancerous conditions. Under these circumstances, significant decreases in the blood hemoglobin concentration should constitute fairly valid indications of an antagonism between cancer tissue and hemoglobin.

Strong has painted a clear picture of the blood hemoglobin in relation to inherited cancer susceptibility and resistance in mice. In a series of investigations (16-19) he has shown that the hemoglobin level of mice of high spontaneous tumor incidence falls off markedly long before cancerous growths appear, in contrast to cancer-resistant strains which do not react in this manner. However, he considers this genetically-determined effect to be unrelated to the anemia which appears after cancer develops and which he speaks of as being secondary in nature. So far as can be ascertained, no one has investigated the effect of a chemically-induced precancerous stage on the hemoglobin level.

In studies which utilized several varieties of rabbits, Casey, Pearce, and co-workers reported (6, 7, 11) that the resistance of these animals to implanted malignant tumors was related to the hemoglobin level which prevailed before tumor inoculation. They found that the most resistant animals were those with "normal" blood hemoglobin concentrations and red blood cell counts while rabbits in which these values were relatively high or low proved to be more susceptible to the growth of the tumor implants. They also investigated the reactions of the blood to the growth of transplanted cancer tissue. Their results disclosed no appreciable change in hemoglobin concentration or red blood cell counts for the early stages of tumor development.

Recently Blumenthal (3) reported the results of an investigation of the reaction of blood cellular elements to the growth of neoplastic implants. Several types of tumors and both mice and rats were utilized. He found, among other things, no significant change in the red cell count while the tumors were comparatively small. Anemia developed after the tumors had attained a large size and the animals had become debilitated. A number of other papers might be cited since the reactions of the blood to cancer have long been and still are a fruitful field for research. But as indicated before, the relation of cancerous growth to the hemoglobin level has been generally regarded as secondary. Such data as can be found in the literature have been obtained in studies which were primarily concerned with other aspects of cancer-blood relationships.

Preliminary work in our laboratory indicated the possibility of a direct relation between the hemoglobin level and not only the early stages of cancer, but also the chemically induced precancerous condition.

MATERIALS AND METHODS

All hemoglobin determinations were made by the method described by Evelyn using an Evelyn photoelectric colorimeter. This technic proved to be sensitive and thoroughly reliable.

Tumor implants.—The method of hypodermatic injection of finely divided tissue was used. The tumor tissue was forced through muslin, the resulting material passing easily through a No. 18 needle. Each animal received 0.05 ml. of this undiluted homogenous tissue subdermally in the mid-dorsal area posterior to the cervical vertebrae.

Mammary gland adenocarcinomas which had become stabilized by many generations of transplants furnished the material for the implants made into the Dba group. The C57 mice received portions of a round cell sarcoma which had originally been produced by the action of methylcholanthrene in this

* This investigation was generously supported by the Clayton Foundation, Houston, Texas.
strain. Only animals without ulcers or any sort of external lesions were included in the experiment. The sarcoma implants used for the rats were also obtained from a tumor induced by methylcholanthrene.

Cancer induction by butter yellow.—White rats of the Wistar strain were fed on two basal diets, each of which contained 0.6 per cent of dimethylaminoazobenzene (butter yellow) incorporated as a 3 per cent solution in olive oil. Diet 1 consisted of Purina dog chow, and diet 2 was cooked whole rice plus raw carrots and salt.

Cancer induction by methylcholanthrene.—Dba and C57 mice received single injections of 0.4 mgm. of methylcholanthrene dissolved in 0.2 ml. of olive oil subcutaneously in the right axilla.

Fig. 3 show clearly the striking decrease in hemoglobin produced in rats when butter yellow (p-dimethyl-aminoazobenzene) was added to the diet. When the basal diet was cooked whole rice and raw carrots, the decrease in hemoglobin content was about 24 per cent in 30 days. At this period the liver of these animals was macroscopically unaffected. Nodular cirrhosis affected the livers of most of the animals before the end of the 148-day period. As the figure shows, the hemoglobin level remained almost constant after the initial drop. With the development of hepatoma nodules, the hemoglobin level fell below 11 gm. per cent or 36 per cent below normal.

The use of a nutritionally more complete basal diet (Purina dog chow) led to an initial drop of about

### Table I: The Effect of Growth of Tumor Implants on the Blood Hemoglobin Concentration

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Animals</th>
<th>Tumor</th>
<th>Days after implantation</th>
<th>Tumor size</th>
<th>Hemoglobin (gm. per 100 ml.)</th>
<th>Relative hemoglobin values (controls = 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dba mice 15</td>
<td>Adenocarcinoma</td>
<td>7</td>
<td>0.5 cm.</td>
<td>15.1 ± 0.7</td>
<td>95.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>14</td>
<td>1.5 cm.</td>
<td>14.4 ± 0.9</td>
<td>88.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>21</td>
<td>3.0 cm.</td>
<td>8.4 ± 0.6</td>
<td>52.0</td>
</tr>
<tr>
<td>2</td>
<td>Dba mice 140</td>
<td>Adenocarcinoma</td>
<td>7</td>
<td>0.59 cm.</td>
<td>15.1</td>
<td>88.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>14</td>
<td>3.85 cm.</td>
<td>12.8</td>
<td>75.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>21</td>
<td>13.3 cm.</td>
<td>6.7</td>
<td>39.7</td>
</tr>
<tr>
<td>3</td>
<td>C57 mice 17</td>
<td>Sarcoma</td>
<td>20</td>
<td>2.3 cm.</td>
<td>10.2 ± 1.1</td>
<td>62.5</td>
</tr>
<tr>
<td>4</td>
<td>Wistar rats 6</td>
<td>Sarcoma</td>
<td>20</td>
<td>4.5 cm.</td>
<td>9.5 ± 2.5</td>
<td>55.7</td>
</tr>
</tbody>
</table>

* The values for experiment 2 were obtained by multiplying diameter measurements in three dimensions directly. In the other experiments, the average diameter of measurements in two dimensions only are recorded.

† The average hemoglobin values of the animals before implantation were used as controls.

‡ Each animal received 0.02 cc. of original cancer tissue diluted to 0.2 cc. with sterile saline solution, instead of the usual 0.05 cc. of undiluted tissue.

### RESULTS

Tumor implants.—The decided drop in hemoglobin level effected by the implantation of tumor tissue in Dba mice is clearly shown in Table I and Fig. 1. At the end of the first week, the tumors were very small, and the animals were vigorous and healthy; nevertheless, 7 to 10 per cent hemoglobin decreases had occurred. This trend was continued during the second week, and accelerated with time until death occurred.

The same general degree of effect was observed with the sarcoma implants in C57 mice and Wistar rats.

Methylcholanthrene injections.—The results (Table II, Fig. 2) show that a 4 per cent average drop in hemoglobin had occurred in 22 Dba mice 50 days after injection of the methylchlolanthrene. That this drop was real was borne out by the fact that the hemoglobin level continued to fall to a level approximately 10 per cent below normal by the time 83 days had elapsed. This same effect was observed with the C57 mice which had received methylcholanthrene implants. No visible tumors were evident at this time, but after 103 days, 21 per cent of the animals showed visible tumors, and the hemoglobin level had fallen to 84 per cent.

Effect of butter yellow and diet.—Table III and

### DISCUSSION

As the results show, there was a definite drop in the hemoglobin level in the very early stages of the growth
of tumor implants. The data obtained from 155 mice left little room for doubt on this point. Records on 25 mice for which the changes in hemoglobin level were followed individually (although not reported individually here) manifested in each individual case a decline in the hemoglobin concentration even before the tumors were large enough to be measured. The later stages of tumor development were associated with an ever-decreasing hemoglobin level. Some mice showed a fall in this respect from 17 gm. per cent to less than 3 gm. per cent before death.

While factors other than the growth of the cancer tissue may play a part in the extreme anemia which developed in the later stages, it is unlikely that such an explanation could be reasonably advanced for the initial moderate hemoglobin depression which accom-

panied the early development of the tumor. The animals at this time appeared vigorous and unimpaired.

The hemoglobin reaction to the methylcholanthrene- and dimethylaminoazobenzene-produced precancerous states was also striking in its uniformity. The effect of the methylcholanthrene deposits on the hemoglobin level appeared to be not unlike the effect in this regard of the genetically determined influence which Strong (19) reported as operating in cancer-susceptible mice. It might well be that the two phenomena are closely related, perhaps differing mainly in the intensity of the carcinogenic stimulus which of course is more intense in mice receiving the chemical. It is especially worthy of note that the cancer-resistant and the cancer-susceptible mice reacted in the same manner to the treatment with methylcholanthrene. As the results show, a group of C57 black mice and a group of Dba mice each manifested a hemoglobin depression of 10 per cent by about the 80th day, before visible tumors had appeared, after receiving the methylcholanthrene deposits. A carcinogen of this type is so powerful that genetic influences tend to be nullified.

The effect of butter yellow ingestion on the blood hemoglobin of rats probably involves some factors not present in the action of methylcholanthrene. As the

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Strain</th>
<th>Number</th>
<th>Days after injection</th>
<th>Per cent bearing visible tumors</th>
<th>Hemoglobin (gm. per cent)</th>
<th>Relative hemoglobin values (controls=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dba</td>
<td>22</td>
<td>50</td>
<td>0</td>
<td>15.6 ± 0.6</td>
<td>96.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>83</td>
<td>0</td>
<td>14.7 ± 0.7</td>
<td>90.0</td>
</tr>
<tr>
<td>2</td>
<td>C57</td>
<td>19</td>
<td>84</td>
<td>0</td>
<td>15.3 ± 1.0</td>
<td>90.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>103</td>
<td>21</td>
<td>14.3 ± 1.2</td>
<td>84.3</td>
</tr>
</tbody>
</table>

* All values based on 16.4 gm. per cent of hemoglobin as 100, except for the first value reported, which was based on 15.4 gm. per cent. In rats of that age and size the hemoglobin level is still rising.

The effect of butter yellow ingestion on the blood hemoglobin of rats probably involves some factors not present in the action of methylcholanthrene. As the
data show, the hemoglobin in this instance dropped suddenly to a new level and then remained constant until the appearance of hepatomas at which time a further drop was recorded. Liver damage does not seem to be a satisfactory explanation since the livers at the 30-day period were macroscopically normal, while in the later period severe cirrhosis developed. However, the hemoglobin concentration remained stabilized at the new level and was apparently unaffected by the progressive damage to the liver tissue. As time went on, there was actually less individual variation in the hemoglobin levels of the individual rats in this experiment.

In this connection, it was significant that a diet (rice-carrot) known to favor the development of liver carcinoma in the presence of butter yellow also effected a more severe decline in the hemoglobin level.

Rats, fed butter yellow, which developed hepatomas did not manifest the extreme anemia which was characteristic of the late stages of external tumors. Subdermal implants of adenocarcinoma may attain a comparatively large size before killing the animal; sometimes as much as 20 gm. of tumor tissue develops in a 30 gm. mouse. Under such circumstances the hemoglobin level may be depressed 85 per cent below normal. Obviously, a liver tumor would cause death long before attaining any such size and, accordingly, the late stages of hepatoma tend to depress the hemoglobin level to a lesser extent; i.e., about 36 per cent below normal.

The underlying mechanism responsible for the results reported here must necessarily remain obscure until this aspect of the problem has been thoroughly investigated. However, it does seem permissible, especially in view of Craig's and Greenstein's work (8, 10) referred to before on the effect of cancer on cytochrome C and catalase, to postulate an antagonistic action between hemoglobin and cancerous tissue, and to a lesser extent precancerous tissue. As far as the precancerous condition is concerned, it seems probable that one of the characteristics of cancer tissue which is gradually assumed by the cells undergoing transformation is that of incompatibility with high hemoglobin levels. It is likely that this is true of mice which have a tendency towards spontaneous cancer as well as animals under the influence of applied chemical carcinogens.

SUMMARY AND CONCLUSIONS

1. Evidence based on results obtained from a study of 155 tumor implants in mice indicated that tumor tissue has an antagonistic action toward the blood hemoglobin of the host. The hemoglobin level was depressed by the time the implant had grown to measurable size and thereafter became progressively lower until death occurred.

2. The precancerous condition as induced by methylcholanthrene was associated with a gradual fall of hemoglobin values which began soon after the mice received injections of this chemical and continued on into the cancerous condition. The hemoglobin of cancer-susceptible and cancer-resistant mice behaved in the same manner to this carcinogen. It was concluded from these results that precancerous tissue was more or less antagonistic to hemoglobin in the degree to which it approached the cancerous state.

3. Ingestion of butter yellow by rats induced a sudden drop in the hemoglobin to a new level by the 30th day of the experiment. Thereafter the hemoglobin concentration remained fairly constant until hepatomas developed, after which a further decline was noted. The hemoglobin of butter yellow-fed rats dropped to a much lower level (76 per cent) on a rice-carrot basal diet then on a Purina dog chow basal diet (83 per cent). Increasing liver damage as manifested by the appearance of cirrhotic nodules did not effect any further change in the hemoglobin level over a 162-day period.

We wish to acknowledge the invaluable assistance and encouragement of Professor Roger J. Williams, Director of the Biochemical Institute. Our thanks are also due to Mrs. Dorothy Pennington and Miss Juanita Thacker for technical assistance rendered during the course of this research.—Authors.

REFERENCES

10. Greenstein, J. P., W. V. Jenkette, and J. White. The Liver Catalase Activity of Tumor-Bearing Rats and the


Correction


In the graphic formula for the split product of compound III in Fig. 7, p. 9, the methyl group shown in the ortho-position should be in the meta-position.
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