Serum Proteins in Mice Bearing Induced and Spontaneous Mammary Gland Carcinomas*

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Despite the efforts of a large number of investigators, the possible relation of immune body formation to the cancer problem is still only poorly understood. This is due, possibly, to experimental difficulties as well as to difficulties encountered in interpreting experimental results. For example, at the present time we do not possess a precise knowledge concerning antigenic differences between normal and neoplastic tissue, and for this reason it has been difficult to use classical serological methods to determine whether or not tumors stimulate the formation of antibodies that differ from antibodies to normal tissue. Studies on the serum proteins in cancer patients have, in general, failed to demonstrate that the levels of antibody-containing serum fractions deviate from the normal in a characteristic manner. A possible exception is the case of multiple myelomas (10, 11, 13, 14).

The investigations of Davidsohn and Stern (4) have shown that hereditary differences may be involved in antibody production, and experiments in this laboratory indicate that hereditary factors are concerned with the level of serum proteins in mice. It is considered possible, therefore, that studies of serum protein levels in a heterogeneous human population might not be expected to yield consistent or easily interpretable results. A number of experiments have been conducted, however, which indicate that immune body formation may be involved in host response to cancer and that the nature of this relation is worthy of further investigation. For example, a decrease in circulating antibodies has been found in both tumor-bearing animals and human cancer patients (6, 12, 16). Preliminary experiments conducted in this laboratory involving the anaphylactic reaction in inbred mice sensitized with horse serum indicated that differences might exist, in the intensity of the antigen-antibody reaction, between animals of high and low cancer incidence strains, and between young and old animals of a high cancer incidence strain. Also, it was shown that either transplanted or spontaneous mammary gland carcinomas in mice induced changes in the phosphorus metabolism and microscopic appearance of the lymph nodes that were consonant with antibody production.

These investigations suggested that it might be useful to explore the antibody production-cancer relation further, using a cancer-bearing animal in which a relatively high degree of genetic control could be maintained, e.g., the mouse. This species presents an additional advantage, in that it is highly resistant to infections. The present report deals with serum protein levels in inbred mice bearing either spontaneous or transplanted tumors, and in both tumor-bearing and control mice that had received injections of antigen.

EXPERIMENTAL

C3H/Sp (3) mice were employed in all the studies to be described. They were bled by severing the great vessels of the neck, and the blood from each animal was collected in a 3-ml. centrifuge tube. The blood was allowed to clot for 1-4 hours, and the serum was obtained by centrifugation. Separation of the serum proteins was accomplished by the paper electrophoresis technic, with No. 8 MM Whatman paper; 0.05 ml. of undiluted serum was used for each analysis. The apparatus employed in the tanks at pH 8.8. A voltage of 4-5 volts/cm was applied for 24 hours. The paper strips were dried, after development, in an oven at 98°—100°C. for 20 minutes. The protein spots on the papers were then stained with 0.1 per cent bromphenol blue in 95 per cent ethanol that had been saturated with HgCl₂, followed by repeated washings with

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The P₂₂ used in these experiments was furnished by the Oak Ridge National Laboratory, Oak Ridge, Tenn.

1 Unpublished experiments.

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0.5 per cent acetic acid. The paper strips were finally air-dried, cut crosswise into 1-inch sections, and the dye from the individual sections eluted with 2.0 ml. of 0.01 N NaOH. The density of the color in each tube was determined at a wave length of 570 μ. The optical density values for the 1-inch sections comprising each isolated protein were summated, and these values taken as indications of the relative amounts of the different proteins present in each serum sample.

The graft-recipient mice employed in the study of the effect of the presence of transplanted tumors on blood serum protein levels were 2-4 months old. The isologous tumor used was a mammary gland carcinoma that had arisen spontaneously in the CSH/Sp strain and was in its 92nd generation of passage when used. The homologous tumor arose spontaneously in a Ma/Sp (8) strain mouse and was in its nineteenth generation of passage. Transplantation was carried out as described previously. Control animals were similar mice that were either untreated or were sham-operated by subcutaneous insertion of an empty trocar.

When lymph node P3 uptake activity was studied, the same procedures described earlier (1) were followed in administering radioactivity, removing lymph nodes, determining P3 and P4, and expressing the results.

### RESULTS

The gamma globulin region in most of the paper electrophoretic patterns obtained appeared to consist of two quite distinct spots. No attempt was made in this study to identify them, and for convenience they have been designated gamma-1 and gamma-2 globulin, the latter having the slightly greater mobility. Except in the case where a differentiation of these two areas is of interest, values for total gamma globulin are given.

In the initial experiment, the serum protein levels found in CSH/Sp female mice bearing spontaneous mammary carcinomas were compared with those of normal control mice. The amount of gamma globulin in the sera of the tumor-bearing mice was significantly lower than that of the normal mice (Table 1). No differences were found in any of the other serum proteins. There was no indication from these data whether a decreased serum gamma globulin preceded the appearance of a tumor, or resulted from it. In an attempt to answer this question the serum protein levels were determined in tumor-free CSH/Sp strain mice of various ages, ranging from 1-11½ months.

There were no significant changes with increasing age in the amounts of either serum albumin, alpha-1, alpha-2, or beta globulin (Table 1). The gamma globulin levels decreased, however. In the 6-7½-month-old mice there was a significant reduction of this component over the amounts found in the younger animals (Table 1). The levels found in the 9-11½-month-old group were increased over those of the 6-7-month-old group, but this is believed to be an artifact, arising as a result of excluding from this group all those animals of a similar age which had tumors and therefore possessed low serum gamma globulin levels.

The experiments just described indicated that in the CSH strain mice, at least, a lowered serum gamma globulin level precedes the appearance of spontaneous mammary carcinomas. The experi-

### TABLE 1

<table>
<thead>
<tr>
<th>Tumor-free mice:</th>
<th>No.</th>
<th>Gamma globulin</th>
<th>Beta globulin</th>
<th>Alpha-I globulin</th>
<th>Alpha-2 globulin</th>
<th>Albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2 months old</td>
<td>10</td>
<td>18.91±1.06</td>
<td>15.75±0.95</td>
<td>4.88±0.33</td>
<td>8.48±0.46</td>
<td>27.52±0.95</td>
</tr>
<tr>
<td>3-5 months old</td>
<td>11</td>
<td>19.00±0.76</td>
<td>16.42±0.64</td>
<td>4.97±0.36</td>
<td>9.10±0.33</td>
<td>27.90±1.45</td>
</tr>
<tr>
<td>6-7½ months old</td>
<td>14</td>
<td>9.85±0.46</td>
<td>17.48±1.49</td>
<td>5.15±0.58</td>
<td>9.06±0.38</td>
<td>28.41±0.87</td>
</tr>
<tr>
<td>9-11½ months old</td>
<td>20</td>
<td>11.25±0.75</td>
<td>17.00±0.60</td>
<td>4.55±0.19</td>
<td>9.50±0.55</td>
<td>28.13±1.10</td>
</tr>
<tr>
<td>Tumor-bearing mice:</td>
<td>34</td>
<td>8.49±0.58</td>
<td>15.70±0.70</td>
<td>4.84±0.29</td>
<td>8.54±0.27</td>
<td>30.09±0.70</td>
</tr>
</tbody>
</table>

* Mean values and standard errors of mean values.
† Spontaneous mammary carcinomas.
The isologous tumors on the other hand caused a decrease in the amount of gamma globulin, a small but significant decrease in the amount of beta globulin, and an increase in the amount of albumin. The influence of the isologous tumors on the level of gamma globulin is demonstrated more clearly in Table 3. When the size of the tumor was compared with the amount of gamma globulin in the serum of the host animal, it was found that, in general, the larger the tumor became, the lower was the gamma globulin level.

When P\textsuperscript{32} uptake was measured in the lymph nodes of the CSH/Sp mice described in Table 1, it was found that there was a good degree of correspondence between lymph node P\textsuperscript{32} uptake and gamma globulin levels in the healthy animals, i.e., a decreased lymph node P\textsuperscript{32} uptake rate accompanied the decreased gamma globulin levels as the mice became older (Table 4). It might be expected, therefore, that the tumor-bearing animals, possessing the lowest gamma globulin levels in this group, would also have the lowest uptake of P\textsuperscript{32} in the lymph nodes. This was not the case, however (Table 4). On the contrary, there was an increased P\textsuperscript{32} uptake in nodes taken from the animals possessing tumors. A similar observation has been made in animals bearing transplanted mammary carcinomas.\textsuperscript{2}

It appears from this that there is an increased metabolic activity in the lymph nodes of the animals bearing spontaneous mammary carcinomas but that the increased activity was ineffective in increasing the level of the serum gamma globulin. This might indicate either that the lymph nodes are not associated with gamma globulin production or that there is an increased destruction of gamma globulin in the presence of the tumor, as suggested by Wharton \textit{et al.} (16). The latter could not explain the decreasing gamma globulin levels associated with aging seen in the animals bearing spontaneous mammary carcinomas but that the increased activity was ineffective in increasing the level of the serum gamma globulin. This might indicate either that the lymph nodes are not associated with gamma globulin production or that there is an increased destruction of gamma globulin in the presence of the tumor, as suggested by Wharton \textit{et al.} (16). The latter could not explain the decreasing gamma globulin levels associated with aging seen in the animals bearing spontaneous mammary carcinomas but that the increased activity was ineffective in increasing the level of the serum gamma globulin. This might indicate either that the lymph nodes are not associated with gamma globulin production or that there is an increased destruction of gamma globulin in the presence of the tumor, as suggested by Wharton \textit{et al.} (16). The latter could not explain the decreasing gamma globulin levels associated with aging seen in the animals bearing spontaneous mammary carcinomas but that the increased activity was ineffective in increasing the level of the serum gamma globulin. This might indicate either that the lymph nodes are not associated with gamma globulin production or that there is an increased destruction of gamma globulin in the presence of the tumor, as suggested by Wharton \textit{et al.} (16). The latter could not explain the decreasing gamma globulin levels associated with aging seen in the animals bearing spontaneous mammary carcinomas but that the increased activity was ineffective in increasing the level of the serum gamma globulin. This might indicate either that the lymph nodes are not associated with gamma globulin production or that there is an increased destruction of gamma globulin in the presence of the tumor, as suggested by Wharton \textit{et al.} (16). The latter could not explain the decreasing gamma globulin levels associated with aging seen in the animals bearing spontaneous mammary carcinomas but that the increased activity was ineffective in increasing the level of the serum gamma globulin. This might indicate either that the lymph nodes are not associated with gamma globulin production or that there is an increased destruction of gamma globulin in the presence of the tumor, as suggested by Wharton \textit{et al.} (16). The latter could not explain the decreasing gamma globulin levels associated with aging seen in the
tumor-free C3H/Sp mice, however (Table 1). If
the lymph nodes are concerned in some way with
gamma globulin production, this function seems
to be lost or markedly depressed in the tumor-
bearing animals, despite the fact that there is
present a stimulated metabolic activity in the
nodes, as indicated by the increased P\sup{32} uptake
and reticulum-cell hyperplasia.\textsuperscript{2}

Previous experiments\textsuperscript{4} had shown that the in-
jection of an antigen into normal mice caused an
increase in the P\sup{32} uptake of lymph nodes. Con-
sidering the foregoing results, it seemed germane
to this study to determine whether this increase
was accompanied by an increase in the level of
serum gamma globulin, and particularly whether
or not the presence of spontaneous mammary
carcinomas in mice affects the response to an
antigen, employing as criteria the changes in
lymph node P\sup{32} uptake and changes in the amount
of serum proteins. Healthy, tumor-free female
C3H/Sp mice, 9–11 months old, and similar mice
bearing spontaneous mammary carcinomas were
selected for this study. Control animals were either
tumor-free or tumor-bearing mice that were not
given injections of the antigen (0.5 ml. of undi-
luted horse serum). One-half of the experimental
animals of each group received one injection of
antigen and were killed 7 days later, and serum
protein and P\sup{32} uptake measurements were made.
The remaining experimental animals received an
initial injection of antigen which was followed
by a second injection 21 days later, and they were
killed 3 days following this. Only serum protein
measurements were determined in this latter
group. Previous experiments\textsuperscript{4} had shown that the
maximum P\sup{32} uptake in the lymph nodes occurred
about 7 days following an initial injection of
antigen, but that the increase in antibodies was
greatest approximately 3 days following a second
injection of the antigen.

The results indicated that the size of the tumor
was a determining factor, i.e., animals possessing
small tumors responded differently from those
with large tumors. An examination of the data
suggested that, as in a previous study,\textsuperscript{2} the mice
possessing tumors under 300 sq. mm. (cross-
section area) could be conveniently classified as a
group possessing small tumors, while those with
tumors over this size logically might be grouped in
the “large tumor” class.

A single injection of antigen produced marked
increases of P\sup{32} uptake in lymph nodes of both the
control animals and those with small tumors
(Table 4). In both groups a second injection of the
antigen was accompanied by large increases in the
amounts of gamma globulin occurring primarily in
the “gamma-I” fraction, small decreases in
serum albumin, and no significant changes in any
other serum proteins. On the other hand, the P\sup{32}
uptake in the lymph nodes of animals bearing large
tumors, which was found earlier\textsuperscript{2} (see also Table 5)
to be higher than that of normal animals or
animals with small tumors, was not increased after
a single injection of the antigen (Table 4). The
second injection of antigen produced slight de-
creases in the levels of beta globulin, alpha-1
globulin, and albumin, and, what may be of
greater importance, it failed to increase the level
of serum gamma globulin in these animals. This
may indicate that the lymph nodes of the animals
were stimulated maximally by the presence of the
large tumors, and the antigen injection did not
elicit any further response. Whether or not this
failure was related to the inability of these animals
to respond to the repeated injection of the antigen
by an increased gamma globulin level cannot be
certified from these experiments. Precipitin
measurements were made, but no correlation was
found between the amounts of precipitin and of
gamma globulin, in agreement with the findings of
Wharton et al. (16).

DISCUSSION

The possibility that a depressed antibody for-
mation is associated in some manner with a
lessened capacity of the host to resist carci-
ogenesis or to successfully overcome a growing
neoplasm is suggested by the investigations of a
number of workers (2, 15, 16). The present experi-
ments appear to offer somewhat more direct evi-
dence favoring this view and suggest that the de-
fect might reside with the antibody-forming
mechanism. The data indicate that the amount
of serum gamma globulin in female mice of the
high cancer C3H/Sp strain decreases as the ani-
mals become older. This is accompanied by a
decreasing P\sup{32} uptake activity in the lymph nodes,
a suggested site of antibody formation. These phe-
nomena were not observed in mice of the low
cancer C37/Sp strain.\textsuperscript{1} It is as though there is an
agent in young C3H/Sp mice which stimulates the
lymph nodes, and the stimulation is accom-
panied by a high serum gamma globulin level. As
the mice grow older, either the stimulation
decreases in intensity, or the nodes as well as other
gamma globulin-producing centers decrease in
their ability to respond to the stimulation. A pos-
sible explanation is that the milk agent which is
present in the C3H/Sp mice, and has been shown
to be antigenic, constantly stimulates the anti-
body-producing centers. It is conceivable that
eventually, under constant stimulation, the anti-
TABLE 5

THE EFFECT OF THE PRESENCE OF SPONTANEOUS MAMMARY GLAND CARCINOMAS IN MICE ON THE RESPONSE TO AN ANTIGEN

<table>
<thead>
<tr>
<th>Treatment†</th>
<th>No. of ANIMALS</th>
<th>Gamma globulin</th>
<th>Gamma-1 globulin</th>
<th>Gamma-2 globulin</th>
<th>Beta globulin</th>
<th>Alpha-1 globulin</th>
<th>Alpha-2 globulin</th>
<th>Albumin</th>
<th>Lymp node</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor-free mice:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>20</td>
<td>11.25 ± 0.75</td>
<td>7.28 ± 0.60</td>
<td>3.97 ± 0.36</td>
<td>17.09 ± 0.60</td>
<td>9.50 ± 0.85</td>
<td>4.53 ± 0.19</td>
<td>23.13 ± 1.10</td>
<td>174 ± 7†</td>
</tr>
<tr>
<td>One inject. antigen</td>
<td>14</td>
<td>11.69 ± 1.06</td>
<td>7.73 ± 0.50</td>
<td>4.08 ± 0.60</td>
<td>15.39 ± 1.05</td>
<td>8.40 ± 0.33</td>
<td>3.85 ± 0.26</td>
<td>24.56 ± 0.90</td>
<td>244 ± 11</td>
</tr>
<tr>
<td>Two inject. antigen</td>
<td>7</td>
<td>19.74 ± 1.56</td>
<td>14.8 ± 1.44</td>
<td>4.94 ± 0.98</td>
<td>17.89 ± 1.44</td>
<td>9.82 ± 1.16</td>
<td>4.39 ± 0.51</td>
<td>23.50 ± 1.81</td>
<td></td>
</tr>
<tr>
<td>Mice with small tumors:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>13</td>
<td>10.03 ± 0.62</td>
<td>6.84 ± 0.57</td>
<td>3.19 ± 0.25</td>
<td>15.73 ± 0.67</td>
<td>8.91 ± 0.42</td>
<td>4.81 ± 0.48</td>
<td>30.28 ± 1.02</td>
<td>180 ± 7†</td>
</tr>
<tr>
<td>One inject. antigen</td>
<td>14</td>
<td>9.94 ± 0.84</td>
<td>6.29 ± 0.57</td>
<td>3.65 ± 0.46</td>
<td>14.07 ± 0.63</td>
<td>8.33 ± 0.46</td>
<td>3.70 ± 0.18</td>
<td>27.58 ± 1.44</td>
<td>255 ± 14</td>
</tr>
<tr>
<td>Two injects. antigen</td>
<td>8</td>
<td>16.12 ± 1.61</td>
<td>11.9 ± 1.11</td>
<td>4.17 ± 0.86</td>
<td>18.20 ± 0.89</td>
<td>9.79 ± 0.58</td>
<td>4.01 ± 0.27</td>
<td>25.48 ± 1.95</td>
<td></td>
</tr>
<tr>
<td>Mice with large tumors:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>11</td>
<td>6.52 ± 0.44</td>
<td>4.13 ± 0.35</td>
<td>2.38 ± 0.28</td>
<td>15.67 ± 0.60</td>
<td>8.09 ± 0.25</td>
<td>4.88 ± 0.30</td>
<td>29.87 ± 1.01</td>
<td>206 ± 6</td>
</tr>
<tr>
<td>One inject. antigen</td>
<td>14</td>
<td>5.07 ± 0.48</td>
<td>3.52 ± 0.81</td>
<td>1.54 ± 0.12</td>
<td>12.05 ± 1.00</td>
<td>5.94 ± 0.46</td>
<td>3.75 ± 0.23</td>
<td>32.01 ± 1.04</td>
<td>209 ± 13</td>
</tr>
<tr>
<td>Two injects. antigen</td>
<td>8</td>
<td>8.02 ± 0.96</td>
<td>5.12 ± 0.65</td>
<td>2.91 ± 0.56</td>
<td>12.26 ± 0.56</td>
<td>5.97 ± 0.30</td>
<td>3.86 ± 0.36</td>
<td>23.01 ± 1.95</td>
<td></td>
</tr>
</tbody>
</table>

* Values are given in arbitrary units. Figures are mean values and standard errors of the means.
† See text for details concerning grouping of animals and administration of antigen.
‡ Figures are biological concentration coefficients (1).
§ These figures are presented elsewhere (Albert et al., Cancer Research, in press). They are repeated here for the sake of convenience, since they are used as control values in the present experiments.
body-producing mechanism might become fatigued or otherwise ineffective. This possibility is being investigated in this laboratory.

If changes in gamma globulin levels are an indication of corresponding changes in circulating antibody, the experiments involving transplanted tumors (Tables 2 and 3) as well as the observation that mice with large spontaneous mammary carcinomas did not exhibit an increased serum gamma globulin upon injection of an antigen, whereas control animals and animals with small tumors did, suggests that the presence of the tumor itself further depresses the defenses of the host. It might do this by overstimulating the antibody-producing centers into a state of fatigue with a subsequent decreased production of gamma globulin-type proteins. This idea finds support in the work of others. For example, Gorer (7) cites evidence that animals with either induced or transplanted tumors are more susceptible to bacterial infection than those without, and Blumenthal (2) demonstrated that animals with spontaneous tumors are more susceptible to heterologous transplants than control animals.

The present experiments leave unsatisfied a number of questions which must be answered before one attempts to explain fully the observations described, or to assess their importance in clarifying the relation of immune body formation and host defense to the cancer problem. For example, it will be necessary to know whether or not an increase or a decrease in the amount of serum gamma globulin indicates corresponding changes in circulating antibodies; whether lymph nodes produce a gamma globulin; and whether observations of the type described herein made on lymph nodes indicate the state of antibody-forming activity or potential in the animal. Experiments are in progress in this laboratory which, it is hoped, will provide more information on these questions.

SUMMARY

1. The amounts of serum proteins have been investigated by paper electrophoresis in normal C3H/Sp mice of different ages and in C3H/Sp mice bearing either spontaneous mammary carcinomas or isologous or homologous mammary carcinoma transplants.

2. Mice bearing spontaneous mammary carcinomas possessed lower serum gamma globulin levels than normal control animals. No differences were found in other protein fractions.

3. Serum gamma globulin levels decreased as the mice became older and approached the age at which tumors began to develop. No changes with age were observed in other serum proteins. The decreasing gamma globulin levels were accompanied by decreased ability to concentrate P32 in the lymph nodes as the animals became older.

4. The presence of growing transplanted mammary carcinoma tissue in mice was accompanied by a decreased gamma globulin and an increased albumin level. Animals bearing the larger tumor transplants had the lowest serum gamma globulin levels.

5. Lymph nodes of mice bearing small spontaneous mammary carcinomas had a P32 uptake similar to that of nodes from normal mice. Nodes from mice with large tumors had an increased uptake of P32 activity. Administration of an antigen (horse serum) to these animals stimulated the uptake of P32 in the nodes and increased the serum gamma globulin levels of both normal mice and those bearing the small tumors. However, neither lymph node P32 uptake nor the serum gamma globulin levels were affected in mice bearing the large spontaneous mammary carcinomas.

ACKNOWLEDGMENTS

We wish to thank Dr. William Prychodko of this institute for assistance in care, selection, and maintenance of the animals used in this study.

REFERENCES


Announcements

Errata

The following corrections should be made in the article by Johnson, Albert, and Pinkus, "Serum Proteins in Mice Bearing Induced and Spontaneous Mammary Gland Carcinomas," which was published in the December, 1954 (Volume 14, No. 11) issue, pp. 880–86:

Page 833, paragraph 2, line 25, should read "killed 7 days following this."

Page 833, paragraph 2, line 31, should read "greatest approximately 7 days."
Serum Proteins in Mice Bearing Induced and Spontaneous Mammary Gland Carcinomas


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