The Development of Metastases from the Rous Sarcoma in Relation to Some Characteristics of Its Causative Virus**

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It was shown in previous studies that both increased age of the host (10) and increased age of the tumor (5, 7, 10) significantly lessened the transmissibility of the Rous sarcoma by cells and by filtrates, and also that filtrability of the sarcoma was significantly higher in tumors induced by cells than in tumors induced by filtrates (10). On the other hand, the incidence, among birds, of metastases was not found to be significantly influenced by any of the above factors (10) but was significantly and directly related to the transmissibility of the tumors by filtrates and also by cells—phenomena which are, in turn, conditioned by the age factor.

The possibility that the effect of the age factor on cell transmissibility and formation of metastases is exerted through an effect on the virus has been discussed (10). In brief, it has been suggested that the virus might undergo changes in the aging original host which would finally result in its masking, as shown by nonfiltrability. This hypothesis would be considerably strengthened if it could be shown that a virus from an original tumor subjected to the effect of the age factor—but still retaining its filtrability—when injected into other test hosts induces tumors which behave in some respects like those from which the virus was obtained. The present investigation presents data pertinent to the point in the case of the formation of metastases.

MATERIALS AND METHODS

Plymouth Rock chicks were used, as in a previous investigation (10). At the age of 14 days, these 479 birds were given subcutaneous injections of the Rous sarcoma materials as follows: 231 with 0.1 cc. of tumor cell suspension at a 1:5 dilution in each side of the breast and 248 with dilutions of tumor filtrates in several sites of the skin. We shall refer to these animals as "test chicks." These chicks were given injections of materials from tumors of varying age grown in chickens of two age groups. The cell suspensions were derived from 41 adult chickens (15.9 ± 4.2 months) and 32 young ones (15.7 ± 5.9 days). The filtrates were obtained from seventeen adult chickens (15.9 ± 4.6 months) and 32 young ones (16.2 ± 6.6 days). We shall refer to these birds as "original hosts" and to their tumors as "original tumors."

All the test chicks developed at least one primary tumor. Appropriate analysis of the data showed that the number of primary tumors was not a factor in the development of metastases. The few cases where visceral growth was due to extension of the primary tumors were excluded. Almost all the test chicks died of their tumors. The time of survival after tumor inoculation was 23.7 ± 11.3 days for chicks injected with cell suspensions and 21.7 ± 5.6 days for chicks injected with filtrates. The incidence of grossly detectable metastases—and also of hemorrhagic lesions (9)—was carefully recorded at death. Histological examination was resorted to only in doubtful cases.

The statistical analysis of the results by one of us (P. M. F.) was carried out for the most part using Bliss's methods for the calculation of dose-response curves (1-4). In a previous study (10), these methods were applied to results of the same kind as those here considered.

RESULTS

Of a total of 479 chicks injected with filtrates or cell suspensions, 170 (35.5 per cent) developed metastases. In Table 1 this incidence of metastases is shown in relation to characteristics of the

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original tumor material inoculated. The possible influence of age of the original hosts, age of the original tumors, filtrability of the original tumors, and kind of material—cells or filtrates—inoculated into the test chicks were analyzed statistically.

The analysis of the data showed, as a main finding, that the number of test chicks with evident metastases decreased significantly with an increase in the age of the original tumors used for inoculation, this holding true even when only chicks inoculated with filtrates were considered. Furthermore, when the original tumors had grown in young chickens and the effect of different ages of these tumors was taken into account, test chicks with tumors induced by filtrates developed metastases at a significantly higher rate (45.6 per cent) than test chicks bearing tumors induced by cell suspensions of filtrable original tumors (26.4 per cent). This finding confirms an observation by Des Ligneris (6). On the other hand, no significant differences were associated with the age of the original hosts or with the filtrability of their tumors.

Next, we investigated the relation between the occurrence of metastases in the test chicks and the occurrence of the same lesions in the original hosts which provided the tumors inoculated into the test chicks. The results are shown in Table 2.

### TABLE 1
INCIDENCE OF METASTASES IN TEST CHICKS DEVELOPING TUMORS

<table>
<thead>
<tr>
<th>Age Group of the Original Tumors</th>
<th>Filtrable Original Tumors</th>
<th>Nonfiltrable Original Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor Age in Days</td>
<td>Metastases</td>
<td>Proportion</td>
</tr>
<tr>
<td>Geometric Mean</td>
<td>Per Cent</td>
<td>Geometric Mean</td>
</tr>
<tr>
<td>≤ 20</td>
<td>10/31</td>
<td>32.3</td>
</tr>
<tr>
<td>21-40</td>
<td>10/32</td>
<td>31.3</td>
</tr>
<tr>
<td>&gt; 40</td>
<td>30/82</td>
<td>36.5</td>
</tr>
<tr>
<td>All Ages</td>
<td>29.6</td>
<td>29/91</td>
</tr>
</tbody>
</table>

### TABLE 2
INCIDENCE OF METASTASES IN TEST CHICKS WITH TUMORS INDUCED BY FILTRATES OR CELL SUSPENSIONS

<table>
<thead>
<tr>
<th>Age Group of the Original Tumors</th>
<th>Metastasizing Original Tumors</th>
<th>Nonmetastasizing Original Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor Age in Days</td>
<td>Metastases</td>
<td>Proportion</td>
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<td>Geometric Mean</td>
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</table>

There were fewer metastases in the test chicks when the inoculated tumor had not metastasized in the original host than when the original tumor had metastasized (29.3 per cent and 39.3 per cent, respectively). To evaluate the significance of this difference and the effect of age of the original tumors upon each group, dose-response curves were calculated as before.

This analysis showed that, when the original tumors had metastasized, an increase in the age of these tumors was significantly associated with a decrease in the incidence of metastases in the test chicks (from 52.9 to 25.0 per cent). With nonmetastasizing original tumors, the effect of the tumor age, although manifesting itself in the same general direction as in the former group, was not statistically significant (from 36.8 to 25.0 per cent). The difference between the incidence of
metastases in test chicks inoculated with metas-
tasising original tumors and in those inoculated with nonmetastasizing ones was still apparent when the effect of the age of the original tumors was taken into consideration; however, this difference was not statistically significant.

**DISCUSSION**

When filtrates, as well as cells, from progressively aging original sarcomas were inoculated into test chicks, the incidence of metastases in these young hosts was significantly decreased as the age of the original tumor increased. It is clear, therefore, that the influence of the age factor on the metastasizing power of the test tumors took place through a change in the virus itself. Furthermore, this finding indicates the persistence of an effect of the age factor from the original host upon many generations of the virus even in the new host. The situation is the same as previously hypothesized for the virus in the process of masking (10).

It is curious why, with the methods followed, the effect of the age of the tumor on the formation of metastases was not evidenced in the original hosts. Possibly the metastases, presumably present in these hosts since the primary tumors were young, were not affected by the age factor, which would act in a cumulative way as the tumor ages (10). Yet, the injection of the virus from progressively aging original tumors into other hosts allowed isolation of the effect of tumor age from the original host.

In agreement with the above interpretations, our experiments showed that the age of the tumors was of less consequence when these tumors did not metastasize in the original hosts. In such situations, the age factor is likely to have been significant since the initial stages of growth, thereby preventing early formation of metastases, and so further aging could add but little to the original effect. Likewise, although not statistically significant, the incidence of metastases in the test chicks was higher for metastasizing than for nonmetastasizing original tumors, which fact may indicate that the effect of the age factor was more pronounced in the case of tumors that did not metastasize in the original hosts.

The fact that significantly more metastases are observed in test chicks injected with filtrates than if injected with cells from an original tumor grown in young hosts (9) may indicate that, when freed from the aging cells of a previous host, the virus manifests a higher degree of vigor. The effect was not observed when the original tumors had grown in adult hosts in which the age factor presumably exerted a more intense and permanent influence on the virus.

The facts disclosed in this study supplement the discussion in the preceding paper (9) concerning the influence that qualitative changes in the virus, eventuating in masking, have on the properties of the malignant cell.

**SUMMARY**

The incidence of metastases from the Rous sarcoma in hosts inoculated in a given passage with filtrates and cells of the sarcoma was significantly decreased with an increase in the age of the tumors of the preceding generation from which the injected materials were obtained.

The finding is discussed in relation to the depressing action of the age factor on properties of the tumors through an effect on the causative virus.

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The Development of Metastases from the Rous Sarcoma in Relation to Some Characteristics of Its Causative Virus

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