On the Induction of Malignant Tumors in Pigeons by a Chicken Sarcoma Virus after Previous Adaptation of the Virus to Ducks*

PAULO R. F. BORGES† AND F. DURAN-REYNALS

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It was previously reported (5) that cells from duck variants of the Rous sarcoma were effective in inducing malignant growths in adult pigeons inoculated in the breast; this is in contrast to results obtained with the original chicken tumor (2).

The present study is concerned with the effect of filtrates from one of the duck variants, and also with characteristic features of the pigeon tumors—induced by either cells or filtrates—which are associated with a special state, masking, of the causative virus.

MATERIALS AND METHODS

The pigeons employed were of the varieties commonly found in the markets; the chickens and ducks were of the Plymouth Rock and Pekin breeds, respectively.

The duck tumor, variant 14 (d) 7 of the Rous sarcoma, was obtained by injecting a newly hatched duck with a cell suspension of the chicken tumor grown in a host 14 months old (4). It is the most malignant of all the variants of the Rous sarcoma, and, in contrast to the behavior of other variants (1), the causative virus kept its pathogenic power for adult chickens despite its adaptation to ducks.

Both cell suspensions and filtrates were injected into the breast of pigeons, ducklings, and chicks at doses of 1 or 2 cc. Filtrates were sometimes injected into the veins of ducklings and chicks. The same methods were followed when tumor tissue or bone marrow from tumor-bearing pigeons was used for further passages. The age of the birds at the time of injection will be indicated in the corresponding sections.

For histological studies, hematoxylin and eosin stain was routinely used. Special staining or impregnation methods were performed for elastic, collagenous, or reticular fibers, for iron-containing pigments, and also for the detection of acid-fast or ordinary bacteria and fungi. Blood smears were stained with the May Grtnwald and Giemsa solutions.

Transmissibility of duck sarcomas to pigeons by means of filtrates and cell suspensions.—The results of the inoculation of pigeons of different ages with these tumor materials are given in Table 1.

It is clear from the data that cell suspensions induced tumors in all the twelve pigeons which received injections, while filtrates were grossly effective in only nine of seventeen pigeons. The tumors regressed in five of the pigeons of the former group and in six of the latter group. However, the incidence of regression would have probably been higher in both groups if the tumor-bearing pigeons had not been killed for passage purposes, as was done in all but three cases in which the pigeons died with tumors.

Growth was faster after injection of cells than after that of filtrates, in the former case tumors being noticeable as early as 1 week after inoculation, as compared to 2 or 3 weeks in the latter case. In several birds the tumors attained sizes of 16 sq. cm. after intervals of as long as 5 months. However, regression of tumors of this large size was observed. The growths were soft, infiltrating, and often showed necrotic areas.

* This investigation was aided by grants from the Jane Coffin Childs Memorial Fund for Medical Research, the American Cancer Society, and the National Cancer Institute, National Institutes of Health, Public Health Service.
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Received for publication September 28, 1951.

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Metastases, in the liver and ribs, were observed only in three pigeons; two of them had been injected with cells and the other with filtrate. Hemorrhagic lesions, which so frequently develop in chicks and ducklings injected with tumor viruses (1, 2, 4, 6), were never observed in the pigeons.

Data concerning the transmissibility of the pigeon tumors are given in Table 2. In experiments 2, 3, 5, 7, 8, and 9 the tumors were induced by cells of the duck variant, whereas in experiments 1, 4, 6, and 10 they were induced by filtrates. In this second passage the age of the pigeons varied from 6 weeks to 5 months, and that of the ducklings and chicks from 5 to 10 days. Half the number of the ducklings and chicks was injected with 1 cc. of filtrate in the vein instead of the breast.

From the data in the table and some complementary observations the following are clear:

1. Filtrates from the pigeon tumors were inactive in other pigeons, and also in ducklings and chicks.

2. Cell suspensions from the ten pigeon tumors tested were equally inactive in inducing steadily growing tumors in other pigeons. However, temporary growth was obtained, at least in some cases, as shown by the frequent development of nodules in the injected site, and by the fact that in one bird that was killed a nodule of that sort proved to be a tumor. Other lesions of an inflammatory-like character were sometimes found in the breast muscle of the inoculated pigeons. These lesions will be described later.

3. Cell suspensions from four of the five pigeon tumors tested induced, in chicks and ducklings, steadily growing tumors which were frequently followed by widespread metastases and hemorrhagic lesions. Filtrates from one duckling tumor and from one chick tumor proved to be effective on other chicks and ducklings. The only pigeon tumor, in experiment 8, that failed to grow in chicks and ducklings was a tumor 10 weeks old in a host 20 months old.

**Microscopic studies.**—A previous investigation (1) disclosed two puzzling features in the 14 tumors. (d) 7 duck variant growing in pigeons which have been here studied in more detail. The first was the frequent presence in the tumor of formations resembling atypical inflammatory granulomas, and the second was the occurrence, in some cases, of a myeloid infiltration close to or distant from the tumor, so conspicuous as to make one suspect the existence of a leukotic process.

The granuloma-like lesions were especially present, in the primary tumors or their metastases, around areas showing regressive phenomena (Figs. 1 and 2). These areas were made up of necrobiotic or necrotic tissue surrounded by giant cells. The latter, apparently derived from the tumor cells, had in general an irregular shape and contained hyperchromatic nuclei, resembling foreign body giant cells. In a few cases recent hemorrhage or a slight granulocytic infiltration were present around the layer of giant cells. No foreign bodies, acid-fast bacilli, or any other micro-organisms could be detected on the histological sections. These lesions apparently were determined by two mechanisms:

**TABLE 2**

<table>
<thead>
<tr>
<th>Donor host</th>
<th>Age of Pigeon (Mos.)</th>
<th>Age of Tumor (Wks.)</th>
<th>Pigeons</th>
<th>Ducklings</th>
<th>Chicks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>0/5</td>
<td>2/2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>3</td>
<td>0/4</td>
<td>2/2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>4</td>
<td>0/4</td>
<td>0/3</td>
<td>0/5</td>
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<tr>
<td>4</td>
<td>10</td>
<td>5</td>
<td>0/4</td>
<td>2/2</td>
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<td>5</td>
<td>0/4</td>
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</tr>
<tr>
<td>6</td>
<td>2</td>
<td>9</td>
<td>0/9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>15</td>
<td>10</td>
<td>0/4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>10</td>
<td>0/1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>20</td>
<td>10</td>
<td>0/1</td>
<td>0/2</td>
<td>0/8</td>
</tr>
<tr>
<td>10</td>
<td>15</td>
<td>10</td>
<td>0/4</td>
<td>0/2</td>
<td>0/6</td>
</tr>
</tbody>
</table>

*Figures expressing the results indicate the number of animals developing tumors over the total of animals inoculated.

**TABLE 1**

<table>
<thead>
<tr>
<th>TUMOR MATERIAL INJECTED</th>
<th>NO. OF PIGEONS</th>
<th>AGE OF PIGEONS</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>PROGRESSIVE GROWTH</td>
</tr>
<tr>
<td>Exp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>C.S.</td>
<td>1</td>
<td>7 days</td>
</tr>
<tr>
<td>2</td>
<td>F.</td>
<td>3</td>
<td>7 days</td>
</tr>
<tr>
<td>3</td>
<td>C.S.</td>
<td>2</td>
<td>2 wks.</td>
</tr>
<tr>
<td>4</td>
<td>F.</td>
<td>3</td>
<td>2 wks.</td>
</tr>
<tr>
<td>5</td>
<td>C.S.</td>
<td>2</td>
<td>5 wks.</td>
</tr>
<tr>
<td>6</td>
<td>F.</td>
<td>3</td>
<td>6 mos.</td>
</tr>
<tr>
<td>7</td>
<td>C.S.</td>
<td>2</td>
<td>6 mos.</td>
</tr>
<tr>
<td>8</td>
<td>F.</td>
<td>3</td>
<td>6 mos.</td>
</tr>
<tr>
<td>9</td>
<td>C.S.</td>
<td>5</td>
<td>1–2 yrs.</td>
</tr>
<tr>
<td>10</td>
<td>F.</td>
<td>5</td>
<td>1–2 yrs.</td>
</tr>
</tbody>
</table>

* C.S. = cell suspension; F. = filtrate.
located especially around regions of degeneration in the Rous sarcoma growing in chickens (10).

Lesions somewhat comparable to the ones found in the tumors were also observed at the site of injection, in the breast muscles of three pigeons, when attempting a second cell passage of pigeon-grown tumors (Fig. 3). In these cases a granulomatous structure was much more evident, the layer of giant cells being surrounded by fibroblasts intermixed with round cells, a few granulocytes, and occasional red blood cells. No foreign body or micro-organism was found. Apparently, these lesions were the consequence of a nonspecific local tissue reaction against the tumor material inoculated and should be considered as a foreign body type of granuloma, in the same class as those produced by Levine (8) in the muscles of chicks injected with suspensions of powdered normal muscle of chickens.¹

To determine the meaning of the myeloid infiltration around the tumors, a systematic morphological study of the blood was carried out on 26 pigeons which had received successful or unsuccessful injections in the breast muscle of cell suspensions or filtrates from duck or pigeon tumors. Another six normal pigeons were similarly studied. Blood smears were taken at least once before the pigeons were injected and, in most cases, several times afterward, generally at 1—2-week intervals; a final blood examination was always made before the pigeons were killed. In no case were changes in the blood observed that made one suspect that a leukemic condition existed.

In an additional experiment, 1 cc. of either heparrinized blood or a suspension of bone marrow cells of tumor-bearing pigeons was injected into the breast muscles of eight pigeons; for a control, 1 cc. of a similar suspension of cells from a normal duckling was injected into three pigeons. No alterations worth recording were observed in any of the inoculated animals.

**DISCUSSION**

Since filtrates of the duck variant 14 (d) 7 of the Rous sarcoma were effective in adult pigeons and since it was known (4) that filtrates of the same tumor were also effective in adult chickens, a situation was created in which the same virus was capable of inducing sarcomas in mature individuals from three different animal species.

This indicates that, in the process of variation of the original Rous virus, which resulted in its adaptation to ducks, the virus not only kept its pathogenic power for the original species, the chicken, but acquired at the same time a pathogenic power for still another species, the pigeon. Whether the young age of the donor ducks was responsible for the inability of the virus to vary, as is the case in the chicken-duck sequence (3), or whether the virus was inherently incapable of further variation remains to be determined.

The tumor virus became masked in the pigeon tumors, as shown by the ineffectiveness of their filtrates in pigeons, ducklings, and chicks, and became unmasked upon growth of the tumors in ducklings and chicks, as shown by effectiveness of tumor filtrates in other ducklings and chicks. Another manifestation of the masking-unmasking sequence was the absence, in tumor-bearing pigeons, of hemorrhagic lesions—the lesions expressing the effect of a free virus (6)—and the frequent presence of such lesions in the ducklings and chicks in which the pigeon tumor was transplanted. These phenomena are essentially the same as those observed in the original Rous virus which becomes masked in tumors in old chickens and is unmasked in chick tumors (6).²

With the Rous sarcoma, virus masking was significantly associated with signs of depression of the growths such as low metastasizing power and low transmissibility by cells, events frequently followed by tumor regression (6). The same phenomena have been observed in the pigeon tumors, for metastases were observed in only three out of ten tumors that grew progressively, and regression was frequent in the first generation, in eleven out of 21 tumors, and was constant, in the second generation, in the birds in which initial growth was observed.³ Also, the frequent presence of granuloma-like lesions in tumors in old chickens and is unmasked in chick tumors (6).²

¹ The following observations made on separate pigeons, not included in the tables, are worth mentioning. Three out of nine pigeons, injected intravenously with 1 cc. of filtrate of one of the pigeon tumors, developed, in the liver and lung, lesions which grossly resembled tumors. Microscopically, however, the lesions were typical of tuberculosis, and appropriate stains revealed the presence of acid-fast bacilli. A curious situation was found in one of the pigeons bearing a large tumor in the breast induced by cells from the 14 (d) 7 sarcoma. In this bird, in addition to atypical metastasis in the ribs, were nodules in the lungs and liver (Fig. 4) which could be interpreted as tuberculous granulomas or tumor metastases showing the granuloma-like lesions above described; acid-fast bacilli were not present. Whether the pigeons showing the tuberculous lesions were affected by the disease before inoculation of the tumor material or whether the latter revived a latent tuberculous infection cannot be decided. The observations illustrate possible causes of error when working on pigeon tumors.

² This observation, as well as others pertaining to the behavior of the Rous sarcoma in old chickens, is part of an investigation carried out by Dr. P. M. Freire and ourselves, the results of which will soon be published. The main points of this investigation have been summarized in a review (6).

³ It may be significant in this respect that the only pigeon tumor that failed to grow in ducklings and chicks, in experi-
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like formations and the infiltration associated with the tumors were probably indicative of failing tumor growth.

SUMMARY

Filtrates from a variant of the Rous sarcoma, effective in both chickens and ducks, induced tumors in nine out of seventeen pigeons inoculated. Cell suspensions from the same variant induced tumors in all the twelve pigeons inoculated.

From the total of 21 tumors produced by either cells or filtrates, metastases were observed in only three cases, while regression occurred in eleven cases. The tumors could not be maintained by further cell passages in pigeons.

In all the ten pigeon tumors studied in this respect, the virus was found to be masked, as shown by the ineffectiveness of its filtrates in pigeons, chicks, and ducklings. The virus became unmasked in chicks and ducklings, as shown by the effectiveness of filtrates from the tumors induced in these hosts by cell suspensions of the pigeon tumors.

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2. ———. The Infection of Turkeys and Guinea Fowls by the Rous Sarcoma Virus and the Accompanying Variations of the Virus. Ibid., 3:569–77, 1943.
and 2.46 gm. ± 0.49 gm. in the control female group (Table 1).

In the course of the 27-day experiment the male controls gained 5.8 gm., while the female controls gained 4.6 gm. (see Table 2). The regrowth of hair from the shaved areas occurred at a slow rate in the controls of both sexes. Most of the controls presented the usual cachectic appearance near the termination of the experiment. Two of the females and one male in the control groups died on D + 26.

Experimental mice injected with somatotrophin.— Tumor transplants failed to grow in three of the females and two of the males injected with somatotrophin.

The average tumor weight was 4.99 gm. ± 0.90 gm. for the females and 5.75 gm. ± 1.07 gm. for the males (see Table 1). The increase in tumor weight in the hormone-treated mice is quite striking when compared to that of their respective controls (Chart 2). The increase in weight of the tumors was significant at the 5 per cent level for the males and at the 2 per cent level for the females.

The effect of somatotrophin on the body weight of the mice was marked. The males gained 15.3 gm., and the females gained 12.5 gm. When one subtracts the gain of weight of the respective controls from that of the experimental groups, it is seen that somatotrophin was responsible for a 9.5-gm. increase in the males and a 7.9-gm. increase in the females (Table 2 and Chart 1). Hence, it is obvious from these results that somatotrophin induced a body weight increase aside from and in spite of the rapid tumor weight increase. No significant positive correlation between the tumor weights and corrected body weights (Chart 3) could be demonstrated.

Growth of hair occurred at a much faster rate in the hormone-injected animals than in the controls, and after 27 days the experimental groups had nearly complete hair regrowth over the shaved areas, whereas the controls were still nearly barren.

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>No. of mice</th>
<th>Weight on D+1 (gm.)</th>
<th>Weight on D+57 (gm.)</th>
<th>Difference between D+1 and D+57 minus the tumor weight (gm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control male</td>
<td>21</td>
<td>22.6 ± 0.75*</td>
<td>28.4 ± 0.88</td>
<td>+12.5</td>
</tr>
<tr>
<td>Control female</td>
<td>15</td>
<td>22.1 ± 0.75</td>
<td>28.7 ± 0.88</td>
<td>+12.6</td>
</tr>
<tr>
<td>Experimental male</td>
<td>20</td>
<td>23.2 ± 0.92</td>
<td>28.5 ± 1.30</td>
<td>+15.3</td>
</tr>
<tr>
<td>Experimental female</td>
<td>21</td>
<td>23.7 ± 0.91</td>
<td>36.2 ± 1.92</td>
<td>+12.5</td>
</tr>
</tbody>
</table>

* Standard error of the mean.
† The values in parentheses are P values of Fisher obtained when the experimental groups are compared with their respective controls.

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**Chart 2**—Showing the effect of growth hormone on the growth of a mammary adenocarcinoma in CSH mice.

**Chart 3**—The relation of tumor weight to body weight without the tumor in CSH mice bearing a transplantable mammary adenocarcinoma, injected with pituitary growth hormone.
in the areas which had been previously shaved.

Of great interest was the observation that the experimental group with the large tumors appeared in better health than the controls; and in none of them were signs of severe cachexia observed. One male in the hormone-treated groups with a tumor weighing 17.28 gm. appeared to be in good health on D + 27. The better appearance of the mice in the somatotrophin groups was unquestionably due in part to the better condition of their pelage.

**DISCUSSION**

Bischoff and Maxwell (1) reported an increased rate of growth of Carcinoma 256 in rats and a small increased rate of growth of Sarcoma 180 in mice following administration of crude growth-promoting extracts of the pituitary. Sugiura and Benedict (7) also reported that extracts of the anterior pituitary had a stimulating effect on the growth of neoplasms in mice. However, Schulman and Greenberg (6), working with transplanted mammary adenocarcinoma in strain A mice, found that a commercial growth hormone preparation had no appreciable effect on tumor growth. It may have been, however, that in the latter case the hormone was not effective throughout the experiment, since all animals suffered a weight loss after the first week of therapy.

**TABLE 3**

<table>
<thead>
<tr>
<th>Group</th>
<th>Tumor weight as a weight per cent of the body weight</th>
<th>Total minus body tumor weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control male</td>
<td>10.4 ± 1.55*</td>
<td>12.7 ± 2.3</td>
</tr>
<tr>
<td>Control female</td>
<td>9.3 ± 2.02</td>
<td>11.0 ± 2.7</td>
</tr>
<tr>
<td>Experimental male</td>
<td>13.9 ± 1.94†</td>
<td>17.5 ± 3.2</td>
</tr>
<tr>
<td>Experimental female</td>
<td>13.1 ± 2.10 (C.R. = 1.31)</td>
<td>16.2 ± 3.0 (C.R. = 1.3)</td>
</tr>
</tbody>
</table>

* Standard error of the mean.
† C.R. = critical ratio obtained when the experimental groups are compared with their respective controls.

In this experiment the weight increase of the somatotrophin-injected group over the increase of the controls was significant at the 1 per cent level in both sexes (Table 2 and Chart 1), and, hence, the hormone was effective. The increased tumor weight when compared to that of the respective controls was significant at the 5 per cent level for the males and at the 2 per cent level for the females.

When the tumor weights of the various groups are expressed as a percentage of the body weights (with or without the tumor), and the means of these percentages compared, no statistically significant increase in the tumor weights of the experimental groups was observed (Table 3). However, this might be expected in view of the fact that the hormone increases both the body and tumor weight.

**CONCLUSIONS**

1. The administration of pituitary growth hormone (somatotrophin) to C3H mice bearing a transplantable mammary adenocarcinoma resulted in a significant increase in body weight of both males and females as compared to the increase of body weight in the controls.

2. In both sexes the tumor weight at the end of 27 days of administration of pituitary somatotrophin was significantly greater than that in the control mice.

**REFERENCES**

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