Growth and Regression of the Rous Sarcoma as a Function of the Age of the Host*

P. M. Freire,† Estelle Bryan, and F. Duran-Reynals

(From the Department of Microbiology, Yale University School of Medicine, New Haven, Conn.)

Although there is extensive information concerning regression of the Rous sarcoma, the subject was restudied by us when it became clear that regression of the tumor was closely conditioned by the age factor—a factor which, as previously shown (11, 12), exerts such important effects on the tumor as masking its causative virus and suppressing its transmissibility by cells and its power to induce metastases. This paper reports the results of the study, together with other pertinent data.

Regression followed by varying degrees of immunity in several transplanted chicken tumors has been observed to occur spontaneously (2, 3, 5–7, 9, 16–19) or as a result of various treatments (4, 14, 15). No such regressions have been noted in young hosts, Gye and Purdy (18) observing only one case out of 6,000 young chickens bearing tumors. It has also been reported (1, 8, 14) that extirpation of a growing tumor is not followed by immunity.

MATERIALS AND METHODS

The present study is based on 192 adult chickens and 1,464 chicks of the Plymouth Rock breed. At the time of the first injection, with extracts or cells of the Rous sarcoma, the age of the chickens varied from 6 to 32 months, with a predominance of birds about 10 months old, while the age of the chicks was 15 days.

In this, as well as for the successive injections, the donor tumor was grown in chicks 10–20 days old. In every case the material inoculated was active, as shown by control injections. Saline was used for preparing the cell suspensions and the extracts, the latter being injected either unfiltered or after filtration through a Berkfeld N candle.

The amount of tumor material first inoculated and the sites injected varied widely. Thus, 102 adult chickens received 12–24 intradermal injections of 0.2 ml. of filtrates diluted at 1:40–1:40,000, while all the chicks received from four to six subcutaneous injections of 0.2 ml. of unfiltered extracts at 1:400. Of the rest of the adult chickens, 65 were injected with 1.5–4 ml. of filtrates at a 1:400 dilution, and 25 were injected with 1–2 ml. of cell suspension at 1:5, in the breast muscles.

In the case of adult chickens, some of the birds which did not develop tumors, or in which the tumors regressed after the first inoculation, were again challenged 1.5–7 months later, by the injection into the right breast muscles of 2–5 ml. of tumor cell suspension at a 1:5 dilution. The birds that resisted this second inoculation were again similarly challenged, and the procedure was repeated up to 8 times at intervals of from 3 weeks to 10 months when the experiment was discontinued.

In the case of chicks, some of the birds that did not develop tumors after the first inoculation were reinjected 55 days later—i.e., when the birds were 80 days old, with 1 ml. of unfiltered tumor extract at a 1:20 dilution into each side of the breast. The few birds that did not develop tumors were given a third injection at 1 ml. of cell suspension at 1:5 in each side of the breast, 33 days after the second inoculation—i.e., when the birds were 88 days old. The only survivor was injected a fourth time, 45 days later, therefore, at the age of 4 months, as done in the third injection.

RESULTS

Of the 192 adult chickens first inoculated, 27 (14 per cent) did not respond to the tumor injection, and eighteen of them served for the successive reinoculations. The remaining 165 chickens developed one or several tumors. Of these, the tumors in 140 (87 per cent) grew progressively until the death of the host, while in the remaining 25 birds (13 per cent) the tumors underwent regression. Eighteen of the birds were used for reinoculation. It is to be noted that, since 98 of the chickens were killed 8–12 weeks after inoculation, some of the tumors in these hosts might also have regressed in the course of time.

Of the 1,464 chicks first inoculated, 136 (9 per cent) did not respond to the tumor, and 48 of them served for successive reinoculations. The remaining 1,328 birds (91 per cent) developed one or several progressively growing tumors without any regressions being observed.

It is seen that the percentage of adult chickens not responding to the first inoculation was higher than that of young chickens, but the statistical significance of the difference is doubtful (x^2 =
3.836; \( P = 0.05 \)). Also, the incidence of tumors was higher when cell suspensions were injected as compared to filtrates, but here again the differences were not significant (\( \chi^2 = 2.595; P = 0.27 \)).

Another point is that there were no significant differences in the incidence of regressions, depending on the route of inoculation—intradermal or intramuscular (\( \chi^2 = 0.284; P = 0.85 \)). This may be at variance with results by other workers (5), but the experimental conditions were not identical in the two studies.

The results of reinoculating both chicks and chickens are given in Chart 1 in which the symbols representing the reactions of each individual bird throughout the successive inoculations are arranged vertically along straight lines, with each bird keeping its position in the horizontal group.

From data in the chart, the following is clear: Irrespective of their age, the birds which did not respond to the first tumor inoculation did not show any increased resistance to the second inoculation, since tumors developed in 89 per cent and 85 per cent in adult and young hosts, respectively; and, past the fourth inoculation, only one adult chicken proved to be immune to the tumor.

On the contrary, of the birds in which tumors regressed after the first inoculation—and this event took place exclusively in adult hosts—only 39 per cent and 7.6 per cent responded to the second and third inoculations, respectively. This is in contrast to the 96 per cent of responses to the first inoculation. The eleven remaining birds (a twelfth bird died accidentally) proved to be immune to all the successive reinoculations.

In summary, a solid immunity against the tumor developed only in aging hosts in which the tumor regressed after an initial growth. Irresponsiveness to the tumor first inoculated was largely independent of the age factor and in no way implied permanent resistance to the tumor.

### SOME ADDITIONAL OBSERVATIONS

The tumors regressing after the first inoculation ranged in size from a few square millimeters to 60 square centimeters. Apparently, the mass of reabsorbed tumor tissue did not determine the degree of resulting immunity, for we had examples of solid immunity following regression of the smallest tumors and of tumor development on further inoculation, following regression of some of the largest tumors.

The inoculations of tumor cells into the immune chickens brought about sharp reactions which subsided after several days. Histological study showed the ordinary signs of inflammation, with conspicuous infiltration by lymphoid-like cells, and pronounced muscle necrosis.

The eleven chickens that resisted at least four inoculations of tumor died or were killed at ages varying from 1½ to 4½ years. Eight of them bore no tumors or lesions worth mentioning. Two of the birds that died had tumors in the ovary which had
induced metastases either in the liver or the intestine; these tumors proved to be tubular adenomas. The last chicken had in the left breast a large, firm not viscid tumor, with extensive necrosis. This growth proved to be a fibrosarcoma different from the Rous sarcoma. However, in view of the location of the lesion one can presume that this tumor was a late and much changed growth resulting from the fourth and last inoculation of tumor cells.

DISCUSSION

If one does not try to be precise about the nature of the age factor, one can regard the regression of sarcomas as the culmination of previous effects of the factor on the tumor, which lead to masking of its causative virus and suppression of its transmissibility by cells and its metastasizing power (11, 12).

However, the age factor may well be of a complex nature, and, with the available data, one cannot decide whether tumor regression is or is not caused by the same component of the factor which is responsible for the previous effects on tumors. Neither can one decide whether the effect of the age factor on tumor regression is exerted through an action on the virus or directly on the tumor cell. However, since we know (12) that at least one effect of the age factor on the tumor, e.g., suppression of metastasis formation, is exerted through a change in the virus, one can admit as a possibility that the same is true in the case of tumor regression.

Whatever the nature of the age factor, it is clear that in avian tumors, as in infection in general, this factor is very important for the development of an effective resistance against the causative agents. This is to be contrasted with a state of irresponsiveness of the hosts to the tumor, which is largely independent of the age factor. Such state—the cause of which is entirely unknown—does not imply at all that the hosts are either resistant or more apt to develop resistance to the sarcoma.

Finally, the fact that epithelial malignancy developed in birds immune to the Rous sarcoma implies the existence of important antigenic differences between these two types of tumors.

SUMMARY

Regression of actively growing Rous sarcomas occurred in at least 15 per cent of 198 adult or old chickens, whereas it never occurred in 1,464 chicks 15 days old when injected.

Tumor regression was generally followed by resistance manifested either by lack of growth, or by a still higher rate of regressions following further inoculations. However, two of eleven birds immune to the sarcoma developed epithelial tumors of the ovary.

Irrespective of their age—in 9 per cent of chicks and 13 per cent of adult chickens—a number of hosts failed to respond to a first tumor inoculation but responded to reinoculation just as normal chickens do. This state of nonresponsiveness, therefore, is different from the efficient immune reactions linked with the age factor.

REFERENCES

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