Lymphoid Tumor Transfers from *Xenopus laevis laevis* to Alien Subspecies and Species, Including *Rana piniens*.

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**SUMMARY**

The lymphosarcoma of *Xenopus laevis laevis* was found to be readily transferable to other *Xenopus laevis* subspecies, to another *Xenopus* species, and to other anuran amphibian species. Furthermore, the presence of spontaneous renal adenocarcinoma in *Rana piniens* did not affect lymphosarcoma formation, since some *Rana piniens* kidneys bore both the sarcoma and the carcinoma. These results are discussed in terms of our present knowledge of the two tumors.

Recent experiments have shown that, when spontaneous or chemically induced lymphoid tumors in the South African clawed toad, *Xenopus laevis*, are transferred to other animals of the same species, lymphoid tumors develop both at the implantation site and in the visceral organs, particularly the liver, spleen, and kidneys (4). Furthermore, tumor transfers between *Xenopus* and the crested newt, *Triturus cristatus*, have shown that, in such xenografts at least, this lymphoid tumor is not transplantable at the cellular level but the resultant tumors arise by host cell transformation (2, 6).

The experiments described in this article may be divided into two main groups. First, tumor transfers from *Xenopus laevis laevis* to other *Xenopus laevis* subspecies, to another *Xenopus* species, and to two locally available anuran species; and, second, lymphoid tumor transfers to American leopard frogs (*Rana piniens*) from two populations with a comparatively high (Vermont frogs) or low (Wisconsin frogs) incidence of the renal adenocarcinoma previously described by Lucké (9). The latter group of experiments was carried out to discover whether the incidence of adenocarcinoma would be increased, whether lymphosarcomas would be induced as in other lymphoid tumor homografts or xenografts, or whether the presence of a spontaneous adenocarcinoma would preclude the formation of such an induced lymphosarcoma.

**MATERIALS AND METHODS**

The lymphosarcoma of liver used in these experiments originated from the methylcholanthrene-induced lymphosarcoma ILA (4) or the benzpyrene-induced lymphosarcoma ILD (5). The normal liver was taken from the immature offspring produced by mating two *Xenopus laevis laevis* obtained from South Africa. The transfer procedure was as follows: the donor animal was killed after anesthesia in 5 per cent urethan in water, then normal liver or liver tumor was removed and placed in one-tenth strength Niu and Twitty solution (8); the recipient host was anesthetized in the urethan solution and a single tissue fragment introduced into the dorsal lymph sac through a small cut in the skin of the back.

Tissues from *Xenopus laevis laevis* were transferred as follows:

1. Lymphosarcoma to immature *Xenopus laevis petersi* and *Xenopus laevis victorianus*.
2. Lymphosarcoma to immature *Xenopus fraseri* and, subsequently, back-transferred from *X. fraseri* to *X. laevis laevis*.
3. Lymphosarcoma to young frogs (*Rana esculenta*) and adult male toads (*Bufo bufo bufo*).
4. Series KK/LL: lymphosarcoma of liver to large and small Vermont and Wisconsin *Rana piniens*.
   - Series 20/21: normal liver to large and small Vermont and Wisconsin *Rana piniens*.
   - Series TT2/3: lymphosarcoma of liver was stored at −30°C for 7 days and then implanted in large Vermont and Wisconsin *Rana piniens*.

The *X. l. petersi*, *X. l. victorianus*, and *X. fraseri* had been bred and reared in our laboratory and were kept in water at about 23°C and fed twice weekly with beef liver or *Tubifex tubifex*. The *Rana esculenta* and *Bufo bufo bufo* were caught in the Geneva region, kept at room temperature in vivaria, and fed with house-flies or mealworms.

*Rana piniens* were obtained from the E. Steinhilber company of Oshkosh, Wisconsin, and from the J. M. Hazen company of Alburg, Vermont. The frogs from each population were of mixed sexes and in two size groups: small (50–60 mm., rostrum-cloaca length) and large (80 mm.). The *Rana piniens* were fed twice weekly with mealworms, and seven to nine were kept in each aquarium tank. The tanks measured 45 × 23 × 24 cm. and were sloped to give a dry bottom at one end and water at the other, the water being changed daily. The mean air...
temperature of the Rana pipiens room was 21.5° C. (range, 16°—25° C.).

The liver, spleen, and kidneys of animals which were killed, or found dead or dying were fixed in Zenker’s fluid, together with other visceral organs of abnormal appearance or persistent growths at the implantation site. Sections cut in paraffin at 7—10 μ were stained with Mayer’s acid haemalum and eosin. The experimental period for the experiments involving Rana pipiens was 100 days; that in the other experiments was 30—50 days.

RESULTS AND DISCUSSION

It is clear from Table 1 that lymphoid tumors in Xenopus laevis laevis are as readily transferable to other Xenopus laevis subspecies, to Xenopus fraseri, to Rana esculenta, and to Bufo bufo bufo as to other Xenopus laevis laevis (4) or Triturus species (2, 6). Although spontaneous lymphosarcomas have been found in Xenopus fraseri and a X. l. victorianus—X. l. laevis hybrid, no such neoplasms have been found in X. l. petersi or the other two anuran species (1).

The results of the transfers from Xenopus to Rana pipiens are very interesting in the light of our present knowledge of the Xenopus lymphosarcoma and the Rana pipiens adenocarcinoma. Tumors similar to those occurring spontaneously in Xenopus laevis laevis may be induced with methylcholanthrene (3) or benzpyrene (5), and 96 per cent of the Xenopus given homografts of lymphosarcoma ILA subsequently developed tumors. Moreover, as we have discussed, the Xenopus lymphoid tumor is readily transferable into other anuran and urodele species. The renal adenocarcinoma of Rana pipiens is found to occur spontaneously in about 2 per cent of the frogs collected in the Lake Champlain region of northern Vermont (10); but Rafferty (12) has found that the incidence of spontaneous tumors is promoted by high environmental temperature and large body size (i.e., old age) to 35—50 per cent in frogs maintained in the laboratory for about 35 weeks. The tumor appears to be race-specific, since the renal adenocarcinoma when transplanted will not induce similar tumors in other races of Rana pipiens (16) or in alien species of amphibia, fish, or reptiles (11). There is a certain amount of evidence that a virus is involved (7, 10), but this is not universally accepted (15), and Rafferty (13) has recently shown that, although the rate of tumor formation in frogs given injections of cell-free tumor extracts was accelerated for several months, the untreated control groups eventually reached the same level of tumor incidence as the injected experimental animals.

Nine of the 108 Rana pipiens given cellular grafts of Xenopus lymphoid tumor or normal liver died during the experimental period, but the remaining 99 frogs were killed between 90 and 100 days after implantation.

Table 2 represents a summary of tumor incidence and shows that all the Vermont frogs and most of the Wisconsin frogs given Xenopus lymphoid tumor implants subsequently developed lymphosarcomas. As in tumor homografts to Xenopus the lymphosarcomas affected the liver (Figs. 1, 2), spleen, and kidneys (Figs. 3, 4), and tumors frequently developed at the implantation site (dorsal lymph sac), invading the nearby skin and muscle. Although Table 2 suggests that the Wisconsin frogs formed lymphoid tumors less readily than those from Vermont, it should be noted that the implants given to the Wisconsin frogs contained less advanced lymphosarcomas than those given to the Vermont. In view of the host-cell transformation in xenografts between Xenopus and Triturus cristatus (2, 6), it would seem possible that the tumors in Rana pipiens given lymphoid tumor grafts were composed of Rana pipiens cells.

Lymphoid tumors developed in the Rana pipiens in the same manner as they develop in Xenopus. The earliest visible stage in the development of a lymphosarcoma of spleen or liver is the aggregation of small lymphocytes around a small germinial center (6). In later stages such lymphoid nodules become evenly distributed throughout the whole organ (Fig. 1) and increase in size until most of the normal tissue of the organ is destroyed. It is considered that, in Xenopus, tumors of the spleen, liver, and
perhaps, the kidneys arise de novo within the organs concerned, but that true, cellular metastasis occurs to other sites—such as the fat-bodies, mesentery, gonads, and alimentary canal (4, 6). In the present experiments with Rana pipiens, most of the hosts were killed before the tumonigenic agent which ordinarily must await graft rejection cytolysis for release.

A number of Rana pipiens in both Wisconsin and Vermont groups bore renal adenocarcinomas (Table 2), but

<table>
<thead>
<tr>
<th>Series</th>
<th>Donor tissue</th>
<th>Rana pipiens origin</th>
<th>No. treated</th>
<th>Tumor type</th>
<th>Number +</th>
<th>Sites affected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lymphosarcoma of liver</td>
<td>Vermont</td>
<td>35</td>
<td>L</td>
<td>35</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Lymphosarcoma of liver</td>
<td>Wisconsin</td>
<td>33</td>
<td>L</td>
<td>27</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Normal liver</td>
<td>Vermont</td>
<td>20</td>
<td>L</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Normal liver</td>
<td>Wisconsin</td>
<td>20</td>
<td>L</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Lymphosarcoma of liver</td>
<td>Vermont</td>
<td>4</td>
<td>L</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Lymphosarcoma of liver after 7 days at -30°C</td>
<td>Vermont</td>
<td>4</td>
<td>L</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Lymphosarcoma of liver</td>
<td>Vermont</td>
<td>4</td>
<td>L</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Lymphosarcoma of liver after 7 days at -30°C</td>
<td>Wisconsin</td>
<td>4</td>
<td>L</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

* L = lymphosarcoma, A = adenocarcinoma.
† Number of animals.
‡ Dorsal lymph sac.

Table 3 clearly indicates that there was no significant difference between those frogs given normal Xenopus tissues and those given lymphoid tumor implants. Therefore, although the implantation of Xenopus lymphoid tumor gave a very high incidence of lymphosarcoma in Rana pipiens, the incidence of renal adenocarcinoma was not significantly increased within the 100-day experimental period. None of the kidney carcinomas had metastasized to other organs.

The occurrence of seven renal adenocarcinomas in 61 Wisconsin Rana pipiens is rather surprising in view of the extremely low incidence observed by others (14). All but one of the Wisconsin kidney carcinomas (that in the small frog given Xenopus tumor—Table 3) were clearly visible with the naked eye, and there is no doubt that these tumors were of the same type as those found in Vermont frogs (Fig. 5). Although it is not known whether the in-

### TABLE 2

**TUMORS IN Rana pipiens AFTER LYMPHOSARCOMA TRANSFERS FROM Xenopus**

<table>
<thead>
<tr>
<th>Series</th>
<th>Donor tissue</th>
<th>Rana pipiens origin</th>
<th>No. treated</th>
<th>Tumor type</th>
<th>Number +</th>
<th>Sites affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>KK</td>
<td>Lymphosarcoma of liver</td>
<td>Vermont</td>
<td>35</td>
<td>L</td>
<td>35</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>Normal liver</td>
<td>Vermont</td>
<td>20</td>
<td>L</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>LL</td>
<td>Lymphosarcoma of liver</td>
<td>Wisconsin</td>
<td>33</td>
<td>L</td>
<td>27</td>
<td>6</td>
</tr>
<tr>
<td>21</td>
<td>Normal liver</td>
<td>Wisconsin</td>
<td>20</td>
<td>L</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>TT2</td>
<td>Lymphosarcoma of liver after 7 days at -30°C</td>
<td>Vermont</td>
<td>4</td>
<td>L</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>TT3</td>
<td>Lymphosarcoma of liver after 7 days at -30°C</td>
<td>Wisconsin</td>
<td>4</td>
<td>L</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

* L = lymphosarcoma, A = adenocarcinoma.
† Number of animals.
‡ Dorsal lymph sac.

### TABLE 3

**INCIDENCE OF ADENOCARCINOMA IN Rana pipiens**

<table>
<thead>
<tr>
<th>Experimental details</th>
<th>Rana pipiens population with renal adenocarcinoma/total number in group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vermont</td>
<td>Wisconsin</td>
</tr>
<tr>
<td>Small frogs, normal Xenopus liver</td>
<td>0/5</td>
</tr>
<tr>
<td>Small frogs, Xenopus lymphoid tumor</td>
<td>0/10</td>
</tr>
<tr>
<td>Large frogs, normal Xenopus liver</td>
<td>4/15</td>
</tr>
<tr>
<td>Large frogs, Xenopus lymphoid tumor</td>
<td>6/25</td>
</tr>
<tr>
<td>Large frogs, frozen Xenopus tumor</td>
<td>0/4</td>
</tr>
<tr>
<td>Untreated large frogs</td>
<td>3/6</td>
</tr>
<tr>
<td>Total:</td>
<td>13/65</td>
</tr>
</tbody>
</table>
cidence of renal adenocarcinoma is increased if Wisconsin frogs are maintained under Rafferty's tumor-promoting conditions, in our experiments the large size of the kidney carcinomas, the relatively low environmental temperature, and the short experimental period would suggest that the tumors were already present when the animals were obtained from the dealer.

Nine of the 68 frogs given Xenopus tumor implants were found to contain renal adenocarcinomas at the end of the experiment. In all nine cases the frogs also bore lymphosarcomas, and in seven the kidney contained both the epithelial adenocarcinoma and the mesenchymal lymphoid sarcoma (Figs. 6, 7), though in only one case were the tumors even slightly in contact with one another (Fig. 8). Therefore, it may be said that the formation of lymphosarcomas in Rana pipiens given Xenopus tumor implants was not affected by the presence of renal adenocarcinoma and that the kidney was able to contain both tumors. A further series of experiments is planned to take advantage of this unique opportunity of studying the possible interaction of these two tumors.

ACKNOWLEDGMENTS
The author is grateful to Profs. M. Fischberg, A. W. Blackler, and L. N. Ruben for their comments on the manuscript.

REFERENCES
3. ———. Methylocyclohexane-induced Lymphosarcomas in the Anuran Amphibian Xenopus laevis. Ibid., pp. 595-610.
FIG. 5.—Series CS21/14. Renal adenocarcinoma in a Wisconsin *Rana pipiens* given a Xenopus normal liver implant. X 50.

FIG. 6.—KK/12. Vermont *Rana pipiens* kidney containing an adenocarcinoma (A) and two lymphosarcoma nodules (L). Much of the kidneys had been destroyed by the adenocarcinoma and the remaining kidney tissue contained many lymphoid tumor nodules. X 120.

FIG. 7.—KK/14. Vermont *Rana pipiens* kidney bearing adenocarcinoma (A) and lymphosarcoma (L). X 120.

FIG. 8.—KK/11. Vermont *Rana pipiens* kidney bearing an adenocarcinoma (A) and two lymphoid tumor nodules (L). One of the lymphosarcoma nodules is in contact with the adenocarcinoma epithelium, but neither tumor is invading the other. X 120.
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