A Mutation in the Mouse Mammary Tumor Virus*

PHYLLIS B. BLAIR

(Department of Zoology and its Cancer Research Genetics Laboratory, University of California, Berkeley, Calif.)

SUMMARY

Analysis of a change in average tumor age in an inbred strain of mice has yielded data which indicate that the mammary tumor virus can undergo stable alteration in activity. It was discovered that descendants of one female of the A/Crgl stock were developing mammary tumors at an average age 4 months earlier than that of the breeding females in the rest of the stock. The former mice were separated out as a subline, and it was found that they differed from the strain not only in average age of tumor development but also in average number of tumors developing per tumor-bearing mouse and in tumor incidence in virgin females. Differences in mean tumor age between groups of reciprocal hybrids (between the strain and the subline) indicated that extrachromosomal influences were involved. Alteration of the tumor ages by reciprocal foster-nursing indicated that this extrachromosomal influence was the mammary tumor virus transmitted in the milk. Serial transmission of each virus through two generations of genetically identical mice eliminated the possibility that a genetic change in the mouse was being expressed as a change in activity of the virus transmitted to the offspring. Attempts to express the difference in activity of the viruses by introduction into other strains were successful.

These experiments establish a mutation in the mouse mammary tumor virus.

The mammary tumor agent, or virus, an important factor in the development of mammary tumors in mice, has many properties shared by viruses (2, 6, 14, 19), and it is generally considered to be a virus. Differences in the mammary tumor agents possessed by various strains of mice have been reported (for review see "Discussion"), and the available evidence suggests that the agent exhibits both autonomy and variability, as would be expected if it is a virus.

Investigation of a change in average tumor age in an inbred strain of mice maintained in our laboratory has led to the documentation of an inherited change in the activity of the mammary tumor virus. The breeding females of the A/Crgl (formerly designated A/He) stock consistently develop mammary tumors at a mean age of about 12 months. In 1953 it was discovered that females in one branch of this stock were developing mammary tumors at about 8 months of age. This latter group has since been maintained separately as the A/Crgl/3 (formerly designated A/vi) subline. Eleven generations of this subline are described herein.

The data presented herein show that the observed difference in tumor age can be attributed to a difference in the mammary tumor viruses carried by the stocks and suggest the mutational origin of the new virus.

MATERIALS AND METHODS

Care of the animals.—All mice were housed in metal cages and given food (Purina Laboratory Chow at the beginning of the experiment, later changed to the Rockland Farms Diet) and water ad libitum. The females were permitted to breed throughout their entire reproductive period, unless otherwise noted in the individual experiments.

Fostering technique.—The method of fostering used permitted the mother to deliver her young normally while caged on a screen of parallel metal bars above the foster-mother's nest. Each newborn mouse was cleaned by its mother, but, when released, it either fell through the bars or was pulled through by the foster-mother. Thus, the
newborn mouse had no opportunity to suckle its own mother, and the milk-borne mammary tumor virus received by the pup was derived from the foster-mother.

Autopsy routine.—Autopsies were performed on all stock or experimental females, and the size and position of all mammary tumors were recorded. Histological sections were made of the mammary tumors from approximately every third female and of every questionable growth in the mammary gland regions. No tissue grossly identified as a mammary tumor proved on section to be otherwise. The tumors were mammary adenocarcinomas, mainly type B, as described by Heston et al. (16).

RESULTS

CHARACTERIZATION OF THE A/Crgl AND THE A/Crgl/3 STOCKS

Average tumor age.—The consistent difference in age of tumor development within the A strain and the A/3 subline is summarized in Table 1.

TABLE 1

<table>
<thead>
<tr>
<th>GENERATION</th>
<th>A STRAIN</th>
<th>A/3 STRAIN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tumor age</td>
<td>Tumor age</td>
</tr>
<tr>
<td></td>
<td>No. mice</td>
<td>Mean (mo.)</td>
</tr>
<tr>
<td>82-84</td>
<td>186</td>
<td>11.5</td>
</tr>
<tr>
<td>85-88</td>
<td>40</td>
<td>11.5</td>
</tr>
<tr>
<td>89</td>
<td>5</td>
<td>12.0</td>
</tr>
<tr>
<td>90</td>
<td>12</td>
<td>12.0</td>
</tr>
<tr>
<td>91</td>
<td>13</td>
<td>12.5</td>
</tr>
<tr>
<td>92</td>
<td>21</td>
<td>13.0</td>
</tr>
<tr>
<td>93</td>
<td>16</td>
<td>12.7</td>
</tr>
<tr>
<td>94</td>
<td>23</td>
<td>12.5</td>
</tr>
<tr>
<td>95</td>
<td>42</td>
<td>13.1</td>
</tr>
<tr>
<td>96</td>
<td>25</td>
<td>12.4</td>
</tr>
<tr>
<td>97</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>98</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>99</td>
<td>23</td>
<td></td>
</tr>
</tbody>
</table>

* Personal communication, 1957.

Nulliparous mice and mice dying before the first mammary tumor had appeared in their respective groups are not included in the data.

The mean age of tumor development was taken as the ages at autopsy of all tumor-bearing females.

Virus preparation for serological study.—The donor BALB/cCrgl mice were foster-nursed on either A (A/Crgl) or A/3 (A/Crgl/3) females. Subsequently they were permitted to breed and were sacrificed during the 3d week of their second lactation. Mammary gland pairs #2, 3, and 4 were dissected out and weighed. The glands were homogenized in saline by means of a Waring Blender for a total of 2 minutes. The homogenates were then spun in an International clinical centrifuge for 30 minutes at high speed (calculated at 1540 g). The pellets were discarded, and the supernatants were then spun for 90 minutes in the multispeed attachment of an International Model U centrifuge (maximum, 30,500 g). The supernatants were discarded, and the pellets were re-suspended in saline. The solutions were cleared by spinning for 15 minutes in the International clinical centrifuge.

Within the A stock no pattern of tumor age differences occurred in this laboratory prior to generation 89. The females comprising the A stock since generation 89 are the descendants of one female in generation 88. A litter-mate sister of that female gave rise to the line of mice with an early average tumor age which is now referred to as the A/3 subline.

The mean age of tumor development in the A strain is 12.7 months, whereas that of the A/3 subline is 8.6 months. For comparison with the experimental animals to be discussed, these mean tumor ages were calculated from the generations of mice which lived concurrently with the various experimental groups (generations 94–96 of A, generations 94–99 of A/3).
Number of tumors.—In addition to the difference in age of tumor development, the two stocks also differ in the number of tumors which develop per tumorous mouse. An accurate count of mammary tumors per mouse was made on a series of tumor-bearing mice whose mammary glands were removed, stained, and examined under the dissecting microscope. Nineteen A females had an average of 1.7 tumors, whereas 28 A/3 females had an average of 3.1 tumors.

Transplantation of tumors.—For the purpose of testing the genetic similarity between the two stocks, tumors from two A and two A/3 females were transplanted into young mice of both stocks. Progressive growth of the transplanted tumors occurred. The genetic similarity of the two stocks is further illustrated by the fact that in this laboratory strain A tumors maintained in the tumor transplant bank are routinely transplanted with equal success into hosts from either of the two stocks. There is general agreement that transplantation is a sensitive test of subline differences (6).

Tumor incidence in virgin females.—Although the tumor incidence in parous strain A females is high, few tumors develop in females maintained as virgins. The A strain and the A/3 subline were compared with regard to mammary tumor incidence in virgin females. None of 45 A virgin females developed a mammary tumor, whereas five (11 per cent) of 44 A/3 virgin females developed mammary tumors (Table 2, Section A).

INFLUENCE OF THE VIRUS UPON THE AGE OF TUMOR DEVELOPMENT

The following experiments were designed to investigate the factor responsible for the inherited change in mean tumor age. The preliminary assumption was made that the difference in mean tumor age is controlled by one of the three factors which have been shown to play major roles in the development of mammary tumors in the mouse—genetic susceptibility, hormonal stimulation, or the mammary tumor virus. Environmental factors can also affect tumor development, but in this case environmental differences were not present. The ultimate pathway of hormonal differences is through the genotype; therefore, experiments were planned to distinguish between differences in genetic susceptibility or in the mammary tumor virus. Preliminary results of most of the experiments described in this section have been reported briefly (9).

Examination of reciprocal hybrids between the two stocks permits differentiation between chromosomal and extrachromosomal factors. If there

<table>
<thead>
<tr>
<th>Section</th>
<th>Strain</th>
<th>Source of Virus</th>
<th>No. Mice</th>
<th>No. Tumors</th>
<th>Mean Age (mo.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A virgin</td>
<td>A</td>
<td>45</td>
<td>0</td>
<td>16.4</td>
</tr>
<tr>
<td></td>
<td>A/3 virgin</td>
<td>A/3</td>
<td>44</td>
<td>11</td>
<td>19.3</td>
</tr>
<tr>
<td>B</td>
<td>BALB/c</td>
<td>A</td>
<td>66</td>
<td>50</td>
<td>11.8</td>
</tr>
<tr>
<td></td>
<td>BALB/c</td>
<td>A/3</td>
<td>68</td>
<td>43</td>
<td>17.5</td>
</tr>
<tr>
<td>C</td>
<td>F₁(BALB/c X C57BL/c)</td>
<td>A</td>
<td>47</td>
<td>13</td>
<td>16.9</td>
</tr>
<tr>
<td></td>
<td>F₁(BALB/c X C57BL/c)</td>
<td>A/3</td>
<td>17</td>
<td>6</td>
<td>20.4</td>
</tr>
<tr>
<td>D</td>
<td>A</td>
<td>CSH</td>
<td>56</td>
<td>59</td>
<td>11.2</td>
</tr>
<tr>
<td></td>
<td>A/3</td>
<td>CSH</td>
<td>53</td>
<td>52</td>
<td>14.5</td>
</tr>
<tr>
<td>E</td>
<td>CSH</td>
<td>A</td>
<td>8</td>
<td>7</td>
<td>15.6</td>
</tr>
<tr>
<td></td>
<td>CSH</td>
<td>A/3</td>
<td>17</td>
<td>14</td>
<td>16.0</td>
</tr>
<tr>
<td>F*</td>
<td>CSH</td>
<td>A/2</td>
<td>49</td>
<td>23</td>
<td>19.5</td>
</tr>
<tr>
<td>G</td>
<td>A</td>
<td>A and CSH</td>
<td>26</td>
<td>11</td>
<td>14.2</td>
</tr>
</tbody>
</table>

* DeOme, unpublished data.
is a genetic difference between the two stocks, the two groups of genetically identical hybrids should have similar average tumor ages. On the other hand, if the two stocks do not differ genetically, but do differ in some maternal extrachromosomal factor, each hybrid group should have an average tumor age similar to that of the strain from which the maternal parent was derived. Preliminary results indicated that an extrachromosomal influence controlled mean tumor age in these hybrid mice.

Although the responsible maternal influence is probably the mammary tumor virus transmitted through the milk, it might be a prenatal maternal influence. To distinguish between these possibilities, A females were foster-nursed on A/3 females and vice versa.

The genotype of the mouse can greatly influence the propagation and transmission of the virus (16). Therefore, it is necessary to consider the possibility that the observed change may actually have occurred in the genotype of the mouse but is being expressed as a difference in the quality or quantity of virus transmitted to the young. It is thus necessary to follow the viruses from both stocks for more than one generation in mice known to be genetically identical.

Chart 1 outlines the passage of each virus through the various experimental groups, and

---

**TABLE 3**

INCIDENCE OF MAMMARY CARCINOMA IN BREEDING FEMALE MICE

<table>
<thead>
<tr>
<th>EXPERIMENT NO.*</th>
<th>No.</th>
<th>TUMOR-BEARING MICE</th>
<th>TUMOR-FREE MICE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Per cent</td>
<td>Mean age (mo.)</td>
</tr>
<tr>
<td>1a</td>
<td>94</td>
<td>90</td>
<td>12.7</td>
</tr>
<tr>
<td>1b</td>
<td>123</td>
<td>90</td>
<td>8.6</td>
</tr>
<tr>
<td>2a</td>
<td>17</td>
<td>47</td>
<td>15.5</td>
</tr>
<tr>
<td>2b</td>
<td>35</td>
<td>77</td>
<td>10.1</td>
</tr>
<tr>
<td>3a</td>
<td>63</td>
<td>82</td>
<td>15.1</td>
</tr>
<tr>
<td>3b</td>
<td>63</td>
<td>81</td>
<td>9.4</td>
</tr>
<tr>
<td>4b</td>
<td>29</td>
<td>98</td>
<td>8.8</td>
</tr>
<tr>
<td>5a</td>
<td>40</td>
<td>65</td>
<td>12.5</td>
</tr>
<tr>
<td>5b</td>
<td>38</td>
<td>90</td>
<td>9.6</td>
</tr>
<tr>
<td>6a</td>
<td>21</td>
<td>48</td>
<td>12.7</td>
</tr>
<tr>
<td>6b</td>
<td>34</td>
<td>65</td>
<td>9.5</td>
</tr>
</tbody>
</table>

* See Chart 1.
Table 3 summarizes the tumor incidence and mean age of tumor development in each group. The mean tumor ages of the A strain and the A/3 subline are recorded as Experiment 1 in Chart 1 and Table 3. In the following sections each of the experimental groups is described in detail.

**Reciprocal hybrids (Experiment 3).**—Reciprocal F₁ hybrids were produced by mating A females with A/3 males (A × A/3) and by mating A/3 females with A males (A/3 × A). Thirty-four hybrid mice with A mothers had an average age of tumor development of 13.1 months; 51 hybrid mice with A/3 mothers had an average age of tumor development of 9.4 months (Chart 1, Experiment 3). Thus, the mean age of tumor development in each hybrid group is similar to that of the strain of the maternal parent, and can be ascribed to an extrachromosomal maternal influence.

**Reciprocal fostering (Experiment 2).**—The mean age of tumor development was determined in two groups of reciprocally fostered females. Eight A/3 females fostered on A females developed mammary tumors at an average age of 15.5 months, whereas 27 A females fostered on A/3 females developed mammary tumors at an average age of 10.1 months (Chart 1, Experiment 2). The mean age of tumor development in each group was similar to that of the stock of the foster mother, thus eliminating possible prenatal extrachromosomal influences and suggesting that the observed difference in tumor age was the result of a change in the activity of the mammary tumor virus.

**Serial transmission (Experiments 5 & 6).**—For the purpose of following the activity of the mammary tumor virus through further generations, genetically identical A/3 mice were fostered on females from each group of the reciprocal hybrids. The two groups of fostered females were bred with sibling males, and the mean age of tumor development in each group was determined (Chart 1, Experiment 5). In addition, the offspring of these two groups of fostered females were collected, and their mean tumor ages were determined (Chart 1, Experiment 6).

In the group of A/3 mice fostered on hybrids with A maternal parents (Experiment 5a), 26 mice had an average tumor age of 12.3 months, and nine offspring of these mice (Experiment 6a) developed mammary tumors at an average age of 12.7 months. On the other hand, in the group of A/3 mice fostered on hybrids with A/3 maternal parents (Experiment 5b), 54 females developed mammary tumors at an average age of 9.6 months, and 22 offspring of these mice (Experiment 6b) had an average tumor age of 9.5 months.

A group of F₁ females was collected from the group of hybrids with A/3 mothers (Experiment 4b). Twenty-seven females developed mammary tumors at an average age of 8.8 months. Thus, the average age of tumor development in this group was similar to that of females of the A/3 stock.

Experiments 5 and 6 indicate that the difference in activity between the two viruses remains stable after passage through two generations of mice known to be identical in genotype. We can therefore conclude that the observed difference in average age of tumor development in the two stocks of the A strain was the result of a difference in the mammary tumor viruses carried by these two stocks. The effect of each of the two viruses upon age of mammary tumor development has been expressed graphically in Chart 2.

**Statistical analysis.**—It is improbable that chance alone would account for the constant correlation of tumor age with the source of the mammary tumor virus reported herein (Chart 1). Further statistical analysis is not necessary to indicate the significance of the results. However, the two groups within each experiment were compared statistically by means of the "t" test. Within each of the experiments, the difference in average tumor age between the group with A virus and that with A/3 virus is highly significant (P = <0.01).
An analysis by the $\chi^2$ method of the difference in tumor incidence between the two groups in each of the experiments was also done. In some experiments (2, 3, and 5) a difference in tumor incidence was found ($P = <0.05$); in others (1 and 6) the difference was not significant. The lack of difference in final tumor incidence between the A strain and the A/3 subline (which are the largest groups) suggests that the observed smaller final tumor incidence in experimental groups receiving A virus than in those receiving A/3 virus may not be due to the viral difference.

**Effect of the Two Viruses When Introduced into Other Strains of Mice**

**A and A/3 viruses in BALB/c mice.**—Mice of the BALB/cCrI strain were infected with either the A or the A/3 virus, as part of the control series of another experiment dealing with the serological behavior of the A and the A/3 viruses. The test animals were BALB/c females, ranging in age at the time of inoculation from 17 to 24 days. They were weaned at 5 weeks of age and were bred for two litters. The offspring were removed within 24 hours after birth. All tumorous females were sacrificed. The experiment was terminated when the tumor-free females were 18 months of age.

Chart 3 presents in graphic form the cumulative percentage of tumor-bearing mice in these two groups. The average age of tumor development in the group possessing A/3 virus was 8.3 months; in the group with A virus, the average tumor age was 11.8 months (Table 2, Section B). This difference is highly significant by the “t” test ($P = <0.01$). A significant difference in tumor incidence was not observed; 33 of 66 mice (50 per cent) with A virus developed tumors, whereas 43 of 68 mice (63 per cent) with A/3 virus became tumorous.

Thus, the difference in activity between the A virus and the A/3 virus was observable in another genetic environment. In both the A strain and the BALB/c strain, the A/3 virus caused an earlier age of tumor development than did the A virus.

**A and A/3 viruses in hybrid mice.**—The two viruses have also been compared in a third genetic environment. BALB/cCrI females were fostered on either A or A/3 females. The BALB/c females were then mated with C57Bl/CrI males. The resulting female offspring were maintained as virgins until death.

The tumor incidence in both groups was low (Table 2, Section C). However, significantly more tumors appeared in the group which had received A/3 virus than in the group which had received A virus.

In this genetic environment the difference in activity of the A and the A/3 viruses was expressed in tumor incidence. The difference in average age of tumor development was not significant.

**Comparison of the Two Viruses with the Mammary Tumor Viruses Possessed by Other Strains of Mice in the Laboratory**

An alternative hypothesis to the mutational derivation of the A/3 virus is that these mice had been accidentally infected with the mammary tumor virus from another strain of mice. For this reason, the activity of the A and the A/3 viruses has been compared with the activity of the C3H/CrI and the A/CrI/2 viruses which were present in the laboratory at the time when the A/3 strain was recognized (the A/CrI/2 strain...
is derived from the A/Jax line transferred from Bar Harbor to our laboratory in 1936).

Comparison with the C3H virus.—Table 2, Section D, records the tumor incidence and mean tumor age of 56 A females and of 53 A/3 females fostered on females of the C3H strain. The mice in these two groups did not differ significantly with respect to mean age of tumor development or tumor incidence. However, the mean age of tumor development in both groups was significantly different from that of the A/3 subline, indicating that the CSH and A/3 viruses are not identical.

Comparison with the A/Crgl/2 virus.—A comparison has been made between the activity of the A virus or the A/3 virus and that of the A/2 virus in CSH mice. CSH females were fostered on either A or A/3 females and were maintained as breeding females. Among eight CSH females infected with A virus seven developed mammary tumors at a mean age of 15.6 months, whereas among seventeen CSH females infected with A/3 virus fourteen developed tumors at a mean age of 10.7 months (Table 2, Section E). The difference in mean age of tumor development between these two groups is highly significant (P = < 0.01).

As part of another experiment the virus of the A/2 strain was introduced into 49 CSH females. Twenty-three of those 49 females (47 per cent) developed mammary tumors at a mean age of 19.5 months (Table 2, Section F). Both the A and the A/3 virus caused tumor development in these CSH mice at an earlier average age and with a higher incidence than did the A/2 virus, indicating that the A and the A/3 viruses are not identical with the A/2 virus.

Mixed infection of A and C3H viruses.—The low mean tumor age of the A/3 strain might have resulted from the possible additive effect of A virus and another accidentally introduced mammary tumor virus, such as that in the CSH strain. This possibility was tested in an experimental group of females which were infected with both viruses. Twenty-six A females were transferred to CSH nursing females at 1 week of age, thereby receiving both A and CSH viruses. Eleven (42 per cent) developed mammary tumors at an average age of 11.4 months, indicating no additive effect of the two viruses (Table 2, Section G).

DISCUSSION

There have been several reports of differences in average tumor age and/or tumor incidence between reciprocal hybrids of mammary tumor virus-carrying strains, wherein the hybrids resembled their maternal parents (3, 8, 22, 24, 25).

No differences have also been reported (15). Differences in virus activity were inferred from experiments in which the virus from foreign strains was introduced into test stains by means of foster-nursing (7, 17, 18, 21), or by means of direct injection (11–13, 21). These experiments involved a single generation of mice. A difference between the activity of the A and the CSH virus was seen in F₁, F₂, and F₄ virgin A × C3H hybrids by Bittner (6).

Several workers have reported variations in the behavior of an inbred strain with regard to its mammary tumor incidence or age of tumor onset (4, 5, 10, 20, 23). In most cases the data do not permit any conclusions as to whether the observed changes were the result of alterations in the genotype of the mouse or of the virus.

A decrease in tumor incidence in a subline of DBA mice was reported by Burrows (10) and in the STOLI strain by MacDowell and Richter (20). Bittner (5) reported a rise in tumor incidence and a decrease in age of tumor development over an extended period of time in the A strain.

Bittner (4) reported that, by foster-nursing strain A mice on a virus-free strain, he was able to decrease the tumor incidence from 96 to 4.5 per cent, but that a subline derived from the 8th generation of the fostered mice developed 100 per cent tumors. He interpreted this as a mutation from an inactive to an active virus or as de novo origin of the virus.

Murray and Warner (23) reported the development of a nontumor line from the descendants of one Marsh albino female whose litter-mate sisters produced tumor lines. This change resulted from the inactivation or elimination of the virus; giving this low-tumor line the virus from another strain (DBA) resulted in a high incidence of tumors. A similar occurrence in a line of RIII mice has been reported by Andervont (1). In our laboratory, current information suggests the inactivation or disappearance of the virus in one branch of our CSH/Crgl line, resulting in a low-tumor subline (CSH/Crgl/2). In reciprocal hybrids, high-tumor incidence is restored if the maternal parent is CSH, but not if the maternal parent is CSH/2.

The experiments presented herein indicate that the mammary tumor virus possesses both autonomy and variability. The virus of the A strain and that of the A/3 subline differ in the effect each has on the age at which an infected mouse develops a mammary tumor, and the two viruses retain their individuality even after passage

* K. B. DeOme, unpublished data.

* E. B. Barnawell and K. B. DeOme, unpublished data.
through two generations of mice which are genetically identical in each generation. The stability of the change in activity is further shown by the fact that the A/3 subline after eleven generations still has an average age of tumor development which is 4 months earlier than that of the A strain from which the A/3 subline arose.

The data indicate that the A/3 strain of virus arose as a mutant and was then subjected to selection. Several other possible origins of the new virus can be postulated, such as nonhomogeneity of the original line, accidental infection, or the phenomenon referred to by virologists as "unmasking." The long history of inbreeding in strain A mice permits elimination of the first possibility. In addition, the new strain of virus has been compared with and found to differ from the mammary tumor viruses carried in other strains of mice present in the laboratory, indicating that it did not arise by accidental infection. Furthermore, such a stable change in activity as we see in this case exceeds the limitations of the concept of "unmasking." We therefore consider this to be an established case of a mutation in the mouse mammary tumor virus.

ACKNOWLEDGMENTS

I am greatly indebted to Professor K. B. DeOme and to Professor Howard A. Bern for their advice on the conduct of this study and for their help in the preparation of this manuscript. I am also indebted to the staff of the Cancer Research Genetics Laboratory for their technical assistance.

REFERENCES

A Mutation in the Mouse Mammary Tumor Virus

Phyllis B. Blair

Cancer Res 1960;20:635-642.

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/20/5_Part_1/635

Sign up to receive free email-alerts related to this article or journal.

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.