Primary Granulocytic Leukemia in the Rat

William Curry Moloney

Department of Medicine, Peter Bent Brigham Hospital, and Children's Cancer Research Foundation, Harvard Medical School, Boston, Massachusetts 02115

SUMMARY

Granulocytic leukemia is a rare disease in the rat; it seldom occurs spontaneously although chemical agents and ionizing radiation may increase the incidence in some rat strains. The disease resembles granulocytic leukemia in man and the leukemic cells possess a number of interesting enzymatic and other features. Granulocytic leukemia is readily transplantable and the leukemic cells grow well in vitro. This disease presents an excellent opportunity for investigation of granulocytic proliferation and differentiation; it may provide a unique test system for evaluation of chemotherapy and immunotherapy and serve as an animal model for acute granulocytic leukemia in man.

INTRODUCTION

GL in the rat resembles, in many respects, the disease in man. While rarely occurring spontaneously, the incidence may be somewhat increased by exposure to ionizing radiation and chemical agents. During the past 14 years, in experiments on leukemogenesis, noninbred and inbred rats were followed throughout their life spans. Among 2564 rats, 334 primary leukemias were encountered. Of these, only 11 were of the granulocytic variety. Observations on 9 rats with GL were previously reported (19). In this communication, 2 additional cases are described along with a review of the clinical, hematological, and other aspects of primary GL in the rat.

MATERIALS AND METHODS

Noninbred Wistar, inbred W/Fu, and Fischer rats were purchased from commercial suppliers for use in these experiments. Rats were housed 2 in a cage and fed a Purina laboratory chow diet with water ad libitum. When rats became ill, they were treated with penicillin and other members of the rat colony were given Terramycin via their drinking water. This resulted in a marked reduction in epidemics of pulmonary infections and extended the life-span of these conventionally raised rats.

Animals were inspected weekly, and prior to treatment white blood cell counts and blood smears were obtained from tail blood. Leukocyte counts (using a Coulter counter) and blood smears were obtained 1 week following X-ray and MCA; thereafter, throughout the life-span, white cell counts and blood smears were obtained at monthly intervals. If a rat developed an abnormal leukocyte count or suspicious cells were discovered in the smear, or if an animal became sick, WBC counts and blood smears were carried out as frequently as indicated. Wright-Giemsa-stained blood smears were carefully examined for presence of abnormal cells and, as an additional aid to identification, leukocyte alkaline phosphatase, MPO, and a special esterase method using AS-D chloracetate as a substrate were used when indicated (16, 21, 22). Also, in selected cases, serum, ascitic fluid, and urine lysozyme levels were determined, using the lysoplate method of Osserman and Lawlor (28). Rats were sacrificed by ether anesthesia when in a terminal state in order to obtain adequate blood specimens and fresh tissues for histological, cytological, and histochemical studies. At autopsy, the size and weight of the spleen and liver were determined and presence of enlarged glands, tumors, and green tissues was especially noted. Tissues were generally fixed in 10% unbuffered formalin solution and stained with hematoxylin and eosin. At the time of sacrifice, imprint and paint brush smears were made on coverslips from the bone marrow, spleen, liver, enlarged lymph nodes, and tumors, if present. Smears were air dried and stained with Wright-Giemsa; histochemical studies were also carried out on these preparations.

To study the effect of radiation, rats of varying ages were exposed to total-body X-ray. Factors were: 250 kVp; 15 ms.; thorasi 1 filter (0.25 mm; 0.25 Cu; 1 Al) 50 cm TSO; half-value layer, 2.00 mm Cu; Total dose, 50 to 450 rads. Rate, 30 to 32 rads/min. MCA was administered in 5- to 10-mg daily doses in sesame oil by stomach tube. Splenectomies were carried out under ether anesthesia. Chromosome preparations were obtained directly from the bone marrow, spleen, or chloroma with modifications of earlier methods described by Nowell and Hungerford (25).

RESULTS

Following the initial report on 9 cases, 2 additional rats with CGL were encountered. Rat 361, a male noninbred...
Table 1
Clinical and laboratory features of GL in the rat

<table>
<thead>
<tr>
<th>Rat.</th>
<th>Sex</th>
<th>Strain</th>
<th>Treatment</th>
<th>Age treated (mo.)</th>
<th>Age at death (mo.)</th>
<th>Duration of leukemia (days)</th>
<th>WBC/mm^3</th>
<th>Peripheral blood differential</th>
<th>Spleen (g)</th>
<th>Liver</th>
<th>Bone marrow</th>
<th>Lymph nodes</th>
<th>Chloroma</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>466</td>
<td>F</td>
<td>W</td>
<td>450 R</td>
<td>3</td>
<td>12</td>
<td>3</td>
<td>42,000</td>
<td>N. seg Band, Lymphs</td>
<td>4</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>Bone Marrow</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mono, Meta, Myel, Pro myel</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Green</td>
</tr>
<tr>
<td>452</td>
<td>M</td>
<td>W</td>
<td>450 R</td>
<td>3</td>
<td>16</td>
<td>15</td>
<td>15,000</td>
<td>N. seg Band, Lymphs</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>?</td>
<td>No</td>
<td>AGL</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Myelo-blasts, Lymphs</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>451</td>
<td>F</td>
<td>W</td>
<td>450 R</td>
<td>3</td>
<td>12</td>
<td>29</td>
<td>56,000</td>
<td>N. seg Band, Lymphs</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>Green</td>
<td>Nodal SAGL</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Meta, Myel, Pro myel</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pelger-Huet cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>385</td>
<td>F</td>
<td>W</td>
<td>MCA</td>
<td>3</td>
<td>13</td>
<td>41</td>
<td>179,000</td>
<td>N. seg Band, Lymphs</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>Green</td>
<td>Green SAGL</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mono, Meta, Myel, Pro myel</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pelger-Huet</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>224</td>
<td>F</td>
<td>W</td>
<td>MCA</td>
<td>3</td>
<td>13</td>
<td>42</td>
<td>139,000</td>
<td>N. seg Band, Lymphs</td>
<td>++++, large</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>Green</td>
<td>Nodal SAGL</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mono, Meta, Myel, Pro myel</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>562</td>
<td>M</td>
<td>W</td>
<td>450 R</td>
<td>3</td>
<td>12</td>
<td>23</td>
<td>31,000</td>
<td>N. seg Band, Pro myel</td>
<td>++++, large</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>No</td>
<td>AGL</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lymphs, At. L, Myelo-blasts</td>
<td>31</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>470</td>
<td>F</td>
<td>W</td>
<td>450 R</td>
<td>3</td>
<td>16</td>
<td>?</td>
<td>82,000</td>
<td>N. seg Band, Lymphs</td>
<td>++++, large</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>No</td>
<td>SAGL Basophilic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mono, Meta, Myel, Pro myel</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>439</td>
<td>M</td>
<td>W</td>
<td>450 R</td>
<td>3</td>
<td>14</td>
<td>14</td>
<td>50,000</td>
<td>N. seg Band, Lymphs</td>
<td>++++, 6</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>No</td>
<td>AGL</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mono, Meta, Myel, Pro myel</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2307</td>
<td>M/W</td>
<td>M/C</td>
<td>150 R</td>
<td>3</td>
<td>14</td>
<td>?</td>
<td>116,000</td>
<td>N. seg Band, Lymphs</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>Green</td>
<td>Green nodes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mono, Myel, Lymphs</td>
<td>35</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Downloaded from cancerres.aacrjournals.org on April 20, 2017. © 1974 American Association for Cancer Research.
Primary Granulocytic Leukemia in the Rat

U, had been treated with 10 mg of MCA p.o. for 18 days at age 4 months. Two months following treatment, the WBC rose to 82,000/cu mm. During the next 2 months anemia and splenomegaly developed, and at age 8 months the rat was sacrificed. At this time, the WBC was 665,000/cu mm with a differential typical of CGL (Table 1). Nucleated red cells and marked polychromasia were also present in the peripheral blood smear. Imprint and paint brush smears of the bone marrow, spleen, liver, and lymph nodes revealed extensive infiltration of these organs with immature myeloid elements. Histochemical studies carried out on the peripheral blood and organ smears showed strongly positive MPO, leukocyte alkaline phosphatase, and esterase activity. Cytogenetic studies, attempted on the peripheral blood and marrow, were unsuccessful. At autopsy, the bone marrow was green, and it gave off a pink fluorescence on exposure to UV. All organs appeared grossly infiltrated and microscopic examination confirmed the presence of extensive myelocytic invasion.

Rat 2953, an untreated male noninbred Wistar, at age of 16 months, had an elevated WBC with increased numbers of mature and immature myeloid cells in the peripheral blood. The WBC increased steadily and at the time of sacrifice, when the rat was aged 20 months, the WBC was 185,000/cu mm with a peripheral blood smear typical of CGL (Table 1). Imprint smears of the marrow, spleen, liver, and lymph nodes revealed intensive infiltration with myeloid elements that were strongly positive for MPO. At autopsy, the liver, spleen, kidneys, and lymph nodes were grossly enlarged and appeared infiltrated. The bone marrow and retroperitoneal and mediastinal lymph nodes were green. Microscopic examination confirmed the extensive involvement of practically all tissues with immature myeloid cells. Cytogenetic studies on direct preparations from the peripheral blood showed no significant numerical (n = 42) or morphological abnormalities.

Incidence of GL

Untreated Rats. In these studies, 100 noninbred Wistar rats were followed throughout their life-spans and 3 leukemias were noted, 2 AMNL and 1 GL. When 437 untreated inbred W/Fu and Fischer rats were followed in a like manner, 87 leukemias occurred, none were of the granulocytic variety (Table 2).

Treated Rats. In experiments previously reported, noninbred Wistar rats were treated with total-body X-ray, MCA, or both agents (19). Among 445 animals, 14 leukemias developed; 9 of these were granulocytic. In a series of 1582 inbred W/Fu and Fischer rats treated with total-body X-ray, MCA, or splenectomy, 230 leukemias occurred, but only 1 was of the granulocytic type (Table 3).

In previous publications, it was pointed out that high-level (450 R) total-body X-ray or splenectomy markedly reduced the incidence of spontaneously occurring AMNL among inbred W/Fu and Fischer rats. Administration of MCA to young, inbred W/Fu rats greatly increased the incidence of AMNL (17, 20).
Clinical and Laboratory Features of GL.

It is obvious that GL occurs spontaneously in very low incidence, especially among inbred rats. Unlike the more commonly occurring AMNL, GL developed earlier and rats were much younger at time of death (average age at death, 13.4 months; range, 8 to 18 months) (Table 1).

Clinical features of GL were variable and clear distinctions between AGL, SGL, and CGL were not always possible. Of the 11 cases, 3 were classified as CGL because of greatly elevated leukocyte counts (116,000 to 665,000/cu mm) and the relative absence of myeloblasts and promyelocytes in the peripheral smears (Fig. 1). At postmortem, in all 3 cases of CGL the marrow was green, and in 2 animals green lymph nodes were also noted. Of the other 8 cases, 4 were considered to be AGL and 4 were SAGL. However, this was an arbitrary classification based mainly on the numbers of myeloblasts and promyelocytes in the peripheral blood and tissues at autopsy (Figs. 2 and 3). The course of AGL and SAGL was short, varying from 2 days to 6 weeks. Leukocyte counts ranged from 15,000 to 179,000/cu mm and the peripheral blood at times contained relatively few myeloblasts or promyelocytes. Of the 8 AGL and SAGL cases, 4 demonstrated green bone marrow and/or lymph nodes at postmortem. One case, classified as SAGL, was an unusual instance of basophilic leukemia. This rat had a terminal WBC of 82,000/cu mm with 90% basophils of varying maturity (Fig. 4). Imprint smears and histological sections at sacrifice revealed diffuse infiltration of the liver, spleen, marrow, lymph nodes, and other organs with abnormal basophils. In contrast to human GL, with the 1 exceptional basophilic case, no increased numbers of eosinophilic or basophilic granulocytes were noted. While the Pelger-Huet anomaly was noted in 3 cases of SAGL, no Auer rods were observed in any of these GL’s in the rat. In addition to the abnormal leukocytic population, nucleated red cells, along with marked polychromasia, were frequently present in the peripheral blood smears (Fig. 2).

As pointed out previously, in addition to distinctive neutrophilic granules, the leukemic cells contained very active leukocyte alkaline phosphatase, not only in mature granulocytes but also in the promyelocytes and in some myeloblasts (16). This finding is in sharp contrast to the well-established lack of leukocyte alkaline phosphatase in granulocytes of human CGL. MPO and a special esterase were also present in mature and immature granulocytes. The presence of excessive extracellular amounts of all 3 of these cytoplasmic enzymes was especially noteworthy in chloroleukemic tissues.

DISCUSSION

These studies confirm that GL is extremely rare in the rat, especially among inbred strains. Since the 1st publication in 1936 by Wilens and Sproul (38) on GL occurring in inbred Osborne-Mendel rats, there have been 2 other accounts of spontaneous GL among inbred rats, Saxton et al. in 1948 (31) and a recent report by Wrathmell and Alexander (39). The latter authors describe a spontaneous leukemia in an August inbred rat strain and report the successful passage of this leukemia to inbred August rats. There have been only 6 reports of spontaneous GL in noninbred rats.

In view of the well-established leukemogenic effect of ionizing radiation in mice and man, the relatively slight
leukemogenic effect of total-body X-ray, $^{227}$Ac and $^{89}$Sr in noninbred rats has been noteworthy. Even more striking has been the lack of GL among inbred W/Fu and Fischer rats following total body X-ray. The marked reduction of incidence of spontaneously occurring AMNL in W/Fu and Fischer inbred rats following total-body X-ray has been pointed out in previous articles (17). While few in number, nevertheless, the chloroleukemias induced by Zipf et al. in SD (42) and by Jones et al. in Holtzman (13) rats have provided readily transplantable leukemias useful for the study of enzymatic, metabolic, and other activities of leukemic granulocytes. Unfortunately, the studies recently reported by Gong (6), in which an extremely high incidence of GL was produced by total-body X-ray following extensive bleeding of SD rats, was not confirmed by Maloney et al. (15). Similar experiments carried out in this laboratory during the past 3 years have also failed to substantiate Gong's findings (W. C. Moloney, unpublished results).

In 1937, DeGennaro and DiGrazio (3) first reported the induction of GL in an albino rat by the topical application of benzpyrene. Subsequently, a number of chemical agents have been used to induce leukemia in the rat. The most useful has been MCA first reported by Shay, et al. (32, 33). While high-dose, long-term administration of MCA may induce chloroleukemia up to a 20% incidence among noninbred Wistar rats (34), MCA has not been effective in producing GL when fed to inbred W/Fu and Fischer rats (17, 18). However, during experiments on the induction of breast cancer, Kim and Furth noted 2 cases of CGL in MCA-treated inbred W/Fu rats (U. Kim and J. Furth, personal communication). The GL in the W/Fu rat was designated LW12 and was transferred to the Mason Research Institute in 1961 where it was serially passaged to inbred W/Fu rats until 1973; at that time, the LW12 cells were placed in the frozen state. In January 1974, Greenberger et al., during the course of studies on the viral aspects of leukemia in the rat, obtained the LW12 cells from the Mason Research Institute. The cells were reconstituted and injected into inbred W/Fu rats with the production of acute GL in 3 to 4 weeks. Subsequently, Greenberger has successfully grown the LW12 cells in vitro and has continued studies on the viral aspects of leukemogenesis in the rat.

Since the studies of Huggins and Sugiyama (11) in 1966, it has been known that DMBA induces a high incidence of leukemia in noninbred Long-Evans rats. DMBA is toxic to inbred W/Fu and Fischer rats; however, Ioachim et al. (12) have reported the induction of GL in Long-Evans and inbred W/Fu rats. More recently, Gal et al. (4) described the induction of GL in noninbred Wistar and inbred WOP rats. Both groups of investigators have successfully transplanted the DMBA-induced GL and have grown the leukemic cells in vitro. Hoelzer and Harriss (10) have also reported on the cellular and other characteristics of GL induced by ethynitrosourea in an inbred BD IX rat.

Nearly all the studies on the activities of chloroleukemic cells have been carried out on transplanted material, mainly the Shay and Jones chloroleukemia. Handler and Handler (8) have extensively studied many biological aspects of Shay chloroleukemia cells and recently have reviewed their research in this field. Moloney et al. (21) described the histochemical aspects of leukocyte alkaline phosphatase in the Shay chloroma: Yunis et al. (1, 40, 41) have studied glycogen phosphorylase and synthetase as well as the biochemical features of leukocyte alkaline phosphatase in Shay chloroma cells. The presence of MPO has been a distinguishing feature of chloroleukemic cells and the overabundance of this enzyme was noted by Shay et al. (12, 14, 32) and other investigators. An esterase requiring a special AS-D naphthol chloroacetate substrate has been described in chloroleukemic cells from the Shay, Jones, and other chloromas in the rat (22). In 1971, McCaffrey, working in Baltimore's laboratory, found large amounts of RNA-dependent DNA polymerase in Shay chloroma cells (R. McCaffrey, unpublished results); more recently, Greenberger made similar observations on Jones chloroma cells (J. S. Greenberger, unpublished results). Among the most interesting observations on chloroleukemic rats have been the studies on lysozyme. First discovered by Goldbach et al. in 1963 (5) in chloroma cell extracts, this enzyme was found in serum, in ascitic fluid, and in the urine of Shay chloroma bearing rats by Rosenthal and Moloney (30) in 1967. Osserman et al. (26) have extensively studied the physical, biochemical, and other properties of lysozyme. They have carried out investigations on the role of the kidney in the excretion of lysozyme and have described the nephrotoxic effects of this enzyme in humans and in the rat (27). Studies along similar lines have been carried out in this laboratory by Rosenthal and Greenberger (7, 29).

A striking feature of chloroma is the green color noted in the tumors, lymph nodes, bone marrow, and other organs. Similar green tumors and tissues are found in GL in man. Various studies have been carried out on the nature of the green pigment, and Nichol et al. (23) have described it as a heme-prosthetic group linked to a peptide moiety.

The cytogenetic aspects of chloroleukemia have been studied in the Shay GL by Nowell and Hungerford (25) and in the $^{89}$Sr-induced chloroma by Jones et al. (13). In this laboratory, cytogenetic studies have been carried out on de novo chloroleukemias as well as serial studies on the Mound, Shay, and Jones GL. No consistent numerical or morphological changes have been noted: however, in view of the recent developments in banding techniques, cytogenetic studies will need to be reevaluated.

One of the most intriguing facets of the problem of leukemogenesis in the rat has been the inability to transmit GL with cell-free filtrates. Weinstein and Moloney demonstrated the presence of C particles in the Shay GL (2, 37) and others have confirmed this finding.8 Recently, Greenberger and Aaronson identified numerous C-type particles in cultured Jones GL. To date, however, GL has not been passaged by filtrates containing these C-type viruses, and studies are continuing on the mechanism of leukemogenesis in the rat.8


Primary Granulocytic Leukemia in the Rat

NOVEMBER 1974

3053
Over the past several years, successful in vitro culture of Shay and Jones GL has been accomplished by a number of investigators. In 1970, Yunis and Warren were able to maintain long-term cultures of Shay and Jones GL in vitro (A. Yunis and J. Warren, unpublished results). Ioachim et al. (12) have grown DMBA-induced chloroleukemia cells in vitro, and Greenberger et al. (7) have carried out prolonged in vitro cultures of Jones and LW12 GL. Shay GL has also been grown successfully in diffusion chambers implanted in the rat and Vilpo and Rytomaa (35) have described the proliferation kinetics and other characteristics of Shay GL cells in diffusion chambers (36).

With the availability of several lines of chloroleukemia in inbred rat strains, experimental approaches are now possible using these GL as models for chemotherapy and immunotherapy. Moreover, successful in vitro culture should furnish large quantities of leukemic cells for biochemical, metabolic, kinetic, cyto genetic, and viral studies.

ACKNOWLEDGMENTS

The author is grateful to Vincent King for his many contributions to these studies, also to Dr. Anthony Boschetti for most of the pathology and to Dr. Arthur T. Skarin for the photomicrographs.

REFERENCES


Fig. 1. Peripheral blood smear from a rat with CGL showing segmented neutrophils. Wright stain, × 1100.

Fig. 2. A peripheral blood smear from a rat with transplanted chloroleukemia showing characteristic promyelocytes, myeloblasts, and nucleated red cells. Wright stain, × 2200.

Fig. 3. Peripheral blood smear from a rat with SAGL showing myelocytes and promyelocytes. Wright stain, × 1900.

Fig. 4. Peripheral blood smear from a rat with basophilic leukemia. Wright stain, × 2000.
Primary Granulocytic Leukemia in the Rat

William Curry Moloney

Cancer Res 1974;34:3049-3057.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/34/11/3049

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

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

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