Electrocardiographic and Pathological Studies of the Heart in Experimental Guinea Pig Leukemia

Dino A. Belletti, Ludwik Gross, Yolande Dreyfuss, Theodore Ehrenreich, Dorothy Feldman, and Lorraine A. Moore

Cancer Research Unit, Veterans Administration Hospital, Bronx, New York 10468 (D. A. B., L. G., Y. D., T. E., D. F., L. A. M.), and Hoffman-La Roche Inc., Department of Experimental Pathology, Nutley, New Jersey 07110 (D. F.)

SUMMARY

Electrocardiographic tracings were performed on 26 strain 2 guinea pigs in which leukemia was induced by inoculation of L2C leukemic cells. All inoculated animals developed leukemia. In terminal phases of this disease significant electrocardiographic changes were observed in 20 of the 26 animals; in 5 animals the electrocardiograms were normal and in 1 guinea pig the changes were borderline. The most significant changes consisted of the onset of a Q-wave or a T-wave inversion or both. Pathological examination of the heart removed in the terminal phase of the disease revealed infiltration of the endocardium and epicardium and in the capillaries of the myocardium. Such areas of infiltration, when viewed with the electron microscope, revealed the presence of leukemic cells within the lumen of capillaries and in areas immediately surrounding the capillaries. Infiltration of myocardial fibers was not observed. The electrocardiographic changes observed in guinea pig leukemia may be related to the leukemic infiltration of myocardial capillaries and the resulting anoxia.

INTRODUCTION

Abnormal cardiac function has been observed frequently in patients with leukemia. The cause of abnormal function may be due to several factors, such as leukemic infiltration of the heart (8, 15, 17), hemorrhage in its wall (14, 17), hypoxia resulting from severe anemia (10, 16), leukemic pericarditis (2, 4, 16, 22), heart block (3, 6, 7, 16), etc. The frequency of clinical signs of cardiac involvement, including electrocardiographic changes in patients with leukemia, has been reported to vary from 14 to 37% (1, 6, 8, 11, 12, 14, 17, 18, 21).

The purpose of this study was to determine whether electrocardiographic changes were present in animals with experimentally induced leukemia. We had a choice in this laboratory to study leukemia induced in either mice, rats, or guinea pigs. The guinea pig was chosen because the relatively large size of this animal makes it easier to carry out the electrocardiographic tracings. We have at our disposal a leukemic strain (L2C) that induces stem-cell leukemia following inoculation of leukemic cell suspensions into young adult guinea pigs of strain 2 inbred line. This experimental model appeared to be suitable for this study, particularly in view of the fact that leukemia induced in these animals is consistently accompanied by a generalized blood involvement with peripheral WBC frequently exceeding 300,000/cu mm in the terminal phase of the disease.

Accordingly, a study was undertaken to determine the incidence, extent, and area of cardiac involvement by leukemic cell infiltration and the corresponding electrographic changes in strain 2 guinea pigs with generalized leukemia induced by inoculation of leukemic L2C cell suspensions.

MATERIALS AND METHODS

Animals. Strain 2 guinea pigs originally obtained from Horton's Laboratory Animals Inc. in Los Gatos, Calif., in 1968 have been bred by brother-to-sister mating in our laboratory since that time. Young healthy animals from our colony of this inbred line have been used in this study. The guinea pigs used in our study were 8 to 10 weeks old at the onset of the experiments and weighed about 350 to 500 g each.

L2C Strain of Leukemia. The L2C leukemia strain had its origin in a case of spontaneous leukemia that developed in one of the guinea pigs bred at the National Cancer Institute in Bethesda, Md., over 20 years ago (5) and has been transplanted by cell graft, since that time, in young guinea pigs of the same line. We obtained this leukemic strain in 1968 from Dr. C. W. Jungeblut, who was then at Lenox Hill Hospital in New York City, and we have carried this leukemic strain by cell transfer in strain 2 guinea pigs in our laboratory (13).

Preparation of Leukemic Cell Suspensions. A guinea pig with advanced leukemia, induced with a L2C leukemic cell inoculation, was sacrificed by ether inhalation. After the skin was shaved, the abdominal cavity was exposed. Fragments of spleen, mesenteric tumor, and in some instances fragments of a peripheral lymph node also were removed aseptically, weighed, cut with scissors, and ground in a mortar with sufficient sterile 0.9% NaCl solution added to obtain a cell suspension of 10% concentration. The cell suspension was then passed through a sterile voile cloth and serial dilutions were prepared. In most instances 0.5 ml of a 10^{-3} dilution was inoculated s.c.; in a few instances 10^{-3} or 10^{-4} dilutions were used. All inoculated animals developed leukemia.
**RESULTS**

**Electrocardiographic Tracings in Normal Guinea Pigs**

The heart rate in normal young healthy guinea pigs varied from 260 to 320 beats/min. PR interval varied from 0.05 to 0.08 sec. The QRS duration varied from 0.02 to 0.03 sec. The QRS electrical axis varied from −10 to 100°. No Q-waves were noted in Leads 1, 2, and 3 or in Lead AVF. In 1 guinea pig a Q-wave was noted in Lead V1; this was considered to be a normal variation. The T-wave was upright in Leads 1, 2, and 3 and either upright or inverted in precordial Lead V5. No ST changes were noted in the electrocardiograms of normal healthy guinea pigs (Fig. 1).

**Electrocardiographic Tracings in Leukemic Guinea Pigs**

In the 1st group consisting of 12 guinea pigs, electrocardiograms were taken at 2- to 3-day intervals following inoculation with leukemic cell suspensions. The 1st electrocardiographic changes occurred 14 days after inoculation; they became more frequent as the disease progressed (Figs. 2 and 2a). In this group, 4 guinea pigs developed significant Q-wave and T-wave changes, 4 other guinea pigs developed only T-wave changes, and 1 guinea pig developed a flat T-wave in Lead 1. The remaining guinea pigs had normal electrocardiograms (Table 1).

In the 2nd group, 14 guinea pigs were inoculated with leukemic cells suspensions; in this group electrocardiograms were taken only in the terminal phase of the disease. Twelve guinea pigs in this group had abnormal electrocardiograms, described in Table 2, and 2 guinea pigs had normal electrocardiograms.

All 12 animals in the 1st group and 14 animals in the 2nd group, including those that had normal electrocardiograms, had extensive leukemic cell infiltration of the endocardium and epicardium, revealed on pathological examination after they were sacrificed in terminal phase of the disease.

In summary, of 26 guinea pigs with generalized leukemia examined in terminal phase of leukemia, 20 (77%) had abnormal electrocardiograms, 1 had a borderline electrocardiogram, and 5 had normal electrocardiograms.

**Pathological Findings**

**General Description.** The hearts were not appreciably enlarged or deformed. The principal site of leukemic infiltration was at the base of the heart. Small, dark brown, 1- to 2-mm lesions were noted on the surfaces of the auricles and the great vessels at their junction with the heart. In many instances, the small lymph nodes adjacent to the great vessels were enlarged. Small punctate lesions, appearing to be leukemic infiltrations, were seen on the epicardium and endocardium of the right and left ventricles. There were no other anatomic sites of leukemic involvement. Pericardial cavity effusion or leukemic infiltration of the pericardial sac were not noted. Hemorrhage was not observed in any of the anatomic sites in the heart.

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**Electrocardiographic Tracings.** Electrocardiograms were taken of 7 young healthy guinea pigs as an initial preliminary study; these animals were not used for further experimentation. Prior to inoculation, electrocardiograms were taken of another group consisting of 12 animals. Following a base-line electrocardiogram, each of the 12 guinea pigs in the 2nd group received s.c. inoculations of leukemic cells. At 2- to 3-day intervals, a 7-lead electrocardiogram, WBC, and differential blood cell examination were made until the terminal phase of the induced disease was reached (Table 1). Finally, in the 3rd group, consisting of 14 animals, no electrocardiograms were taken prior to inoculation; these 14 animals received s.c. inoculations of leukemic cells. All inoculated animals developed leukemia within 2 to 3 weeks after inoculation and were sacrificed at the terminal stage of the disease, after a time interval varying from 15 to 44 days after inoculation. Electrocardiograms were taken in animals of this group only at the terminal phase of the disease (Table 2).

The electrocardiograms were taken under light ether anesthesia. In each guinea pig 6 limb leads and 1 precordial lead were taken as a base line (Fig. 3). The 1st precordial lead corresponds to a point in between the V1 and V2 human chest lead. The site in the guinea pig was usually at the lower end of the sternum in the center. This point was marked with indelible ink, enabling us to apply the chest electrode at the same site during subsequent electrocardiograms.

The electrocardiograms were considered abnormal if a significant T-wave change or appearance of a significant Q-wave appeared as compared with the original base-line study. Since the normal variations in the electrocardiograms of guinea pigs have not been established, only significant changes in the electrocardiograms were considered abnormal. The electrocardiograms were analyzed for heart rate, PR interval duration, QRS axis, the presence or absence of Q-wave, and whether the T-wave was upright or inverted.

**Pathological Examination.** At autopsy, the hearts were removed and fixed in Bouin's solution. Multiple serial horizontal sections were made, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The degree and localization of the leukemic cell infiltration of the myocardium, epicardium, and endocardium were studied with light microscopy.

**Electron Microscopy.** Specimens were fixed in 4% phosphate-buffered glutaraldehyde followed by 1% phosphate-buffered osmic acid. They were then processed as previously described (9). Tissue blocks were sectioned with a Porter-Blum microtome using a diamond knife. Sections were carbon coated, stained with uranyl acetate and lead citrate (20), and examined in a Philips 300 electron microscope.

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* The electrocardiographic terms used are: L1, Lead 1; L2, Lead 2; L3, Lead 3; AVR, augmented chest lead, right upper limb; AVL, augmented chest lead, left upper limb; AVF, augmented chest lead, both lower limbs; V1, unipolar precordial Lead 1; V2, unipolar precordial Lead 2; Q, R, S, T, standard wave designations in electrocardiographic tracings; Inv. T, inverted T-wave.
**Table 1**

**Electrocardiographic changes in advanced guinea pig leukemia**

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Abnormal changes</th>
<th>Terminal WBC/cu mm³</th>
<th>Base of heart</th>
<th>Left ventricle</th>
<th>Right ventricle</th>
<th>Epicardium</th>
<th>Endocardium</th>
<th>Myocardium</th>
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<tbody>
<tr>
<td>158</td>
<td>M</td>
<td>Abnormal</td>
<td>Q-wave in V₁</td>
<td>99,000</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>+</td>
<td>±</td>
<td>±</td>
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<tr>
<td>161</td>
<td>M</td>
<td>Abnormal</td>
<td>Inv. T-wave in V₁</td>
<td>484,000</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>162</td>
<td>M</td>
<td>Abnormal</td>
<td>Q-wave in L₁ + ALV</td>
<td>152,000</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>77</td>
<td>F</td>
<td>Borderline</td>
<td>Flat T-wave in L₁</td>
<td>261,000</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>+</td>
<td>±</td>
<td>±</td>
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<tr>
<td>83</td>
<td>F</td>
<td>Abnormal</td>
<td>Q-wave in L₁</td>
<td>156,800</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>+</td>
<td>±</td>
<td>±</td>
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<td>86</td>
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<td>Inv. T-wave in V₁</td>
<td>329,000</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>118</td>
<td>F</td>
<td>Normal</td>
<td>Inv. T-wave in V₁</td>
<td>124,000</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>+</td>
<td>±</td>
<td>±</td>
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<tr>
<td>121</td>
<td>F</td>
<td>Abnormal</td>
<td>Inv. T-wave in V₁</td>
<td>156,400</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>+</td>
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<td>±</td>
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<td>120</td>
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<td>Inv. T-wave in L₁</td>
<td>275,000</td>
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<td>±</td>
<td>+</td>
<td>±</td>
<td>±</td>
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<td>126</td>
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<td>Inv. T-wave in L₁</td>
<td>305,000</td>
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<td>±</td>
<td>±</td>
<td>+</td>
<td>±</td>
<td>±</td>
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<td>123</td>
<td>F</td>
<td>Abnormal</td>
<td>Inv. T-wave in L₁</td>
<td>118,000</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>+</td>
<td>±</td>
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<td>220</td>
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<td>+</td>
<td>+</td>
<td>±</td>
<td>+</td>
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* Electrocardiogram taken in terminal phase of leukemia immediately prior to sacrifice of the animals. All guinea pigs in this group also had electrocardiograms taken when in good health, before leukemic cell inoculation and all had normal electrocardiograms at that time.

* Blood taken from earlobe.

**Table 2**

**Electrocardiographic changes in advanced guinea pig leukemia**

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Abnormal changes</th>
<th>Terminal WBC/cu mm³</th>
<th>Base of heart</th>
<th>Left ventricle</th>
<th>Right ventricle</th>
<th>Epicardium</th>
<th>Endocardium</th>
<th>Myocardium</th>
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<tbody>
<tr>
<td>116</td>
<td>M</td>
<td>Abnormal</td>
<td>Q-wave in L₁ + V₁</td>
<td>61,200</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>+</td>
<td>±</td>
<td>±</td>
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<tr>
<td>22</td>
<td>F</td>
<td>Abnormal</td>
<td>Q-wave in V₁</td>
<td>141,000</td>
<td>++</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>±</td>
<td>±</td>
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<tr>
<td>32</td>
<td>F</td>
<td>Abnormal</td>
<td>Inv. T-wave in L₁</td>
<td>170,000</td>
<td>+++</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>118</td>
<td>M</td>
<td>Abnormal</td>
<td>Q-wave in L₁</td>
<td>228,000</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>128</td>
<td>M</td>
<td>Abnormal</td>
<td>Inv. T-wave in V₁</td>
<td>110,000</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
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<tr>
<td>123</td>
<td>M</td>
<td>Normal</td>
<td>Inv. T-wave in L₁</td>
<td>135,000</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
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<td>136</td>
<td>M</td>
<td>Abnormal</td>
<td>Q-wave in L₁</td>
<td>155,000</td>
<td>±</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>±</td>
<td>±</td>
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<tr>
<td>68</td>
<td>F</td>
<td>Abnormal</td>
<td>Q-wave in V₁</td>
<td>150,000</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
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<tr>
<td>127</td>
<td>M</td>
<td>Normal</td>
<td>Inv. T-wave in L₁</td>
<td>105,600</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>+</td>
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<tr>
<td>148</td>
<td>M</td>
<td>Abnormal</td>
<td>Inv. T-wave in L₁</td>
<td>136,000</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>±</td>
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<tr>
<td>146</td>
<td>M</td>
<td>Abnormal</td>
<td>Q-wave in V₁</td>
<td>372,000</td>
<td>+++</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>±</td>
<td>±</td>
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<tr>
<td>170</td>
<td>M</td>
<td>Abnormal</td>
<td>Q-wave in L₁</td>
<td>375,000</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>±</td>
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<tr>
<td>177</td>
<td>M</td>
<td>Abnormal</td>
<td>Q-wave in L₁</td>
<td>406,000</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>+</td>
<td>±</td>
<td>±</td>
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<td>134,000</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
</tbody>
</table>

* Electrocardiograms taken in terminal phase of leukemia immediately prior to sacrifice of the animals.

* Blood taken from earlobe.

**Light Microscopy.** Leukemic cells in clusters and occasionally in large sheets were noted in the lesions of the epicardium at the base of the heart and around the great vessels (Figs. 4 and 5). Lymph nodes often showed extensive leukemic invasion. In a few animals, the lymph nodes were completely replaced by leukemic cells (Fig. 4) and could represent the source of the leukemic infiltration of the base of the heart. Leukemic infiltration in the other anatomic sites of the heart described above was relatively slight. Both ventricles were involved; there were small clus-
ters of leukemic cells infiltrating the epicardium and endocardium. These leukemic deposits were generally small but occasionally they infringed on the underlying myocardium (Fig. 6). Infiltration of the myocardium appeared to result from direct extension of the epicardial or endocardial infiltration. However, in appropriately thin histological sections and on high power, the leukemic cells were found to be in dilated capillaries situated between myocardial fibers (Fig. 7). Thus, the leukemic cells appeared to be separated from the myofibrils by capillary endothelium and only an occasional cell was noted outside the capillary wall. Microscopic hemorrhage was not noted.

**Electron Microscopy.** Electron microscopic examination of sections from tissue blocks containing leukemic infiltration revealed the presence of leukemic cells within the lumen of capillaries and immediately surrounding capillaries. Fig. 8 demonstrates a leukemic cell within a capillary, proximal to the myocardium. In Fig. 9, a leukemic cell appears outside a capillary. In higher magnification of the outlined area (Fig. 9a), a guinea pig leukemia virus particle is shown (arrow). Fig. 10 illustrates a group of guinea pig leukemia virus particles (arrow) within a leukemic cell adjacent to heart muscle.

**DISCUSSION**

In this study, all guinea pigs inoculated with leukemic cells developed generalized leukemia. Cardiac involvement in the form of leukemic cell infiltration was present to some degree in all animals, involving usually the endocardium, epicardium, and occasionally also the myocardium. A larger number of leukemic cells was observed in the epicardium than in the endocardium.

The infiltration with leukemic cells was more pronounced in the auricles than in the ventricles. This observation was in contrast with the recent report of Urban et al. (19) who observed that in lambs in which leukemia was induced following inoculation of blood from leukemic cattle, the ventricles had a heavier infiltration with leukemic cells as compared with the auricles. In the group of guinea pigs used in experiments where reported we did not observe pericardial effusion in the leukemic animals. However, among other leukemic animals, not included in this study, we have seen 2 or 3 guinea pigs with pericardial effusion.

The electrocardiograms showed a significant abnormality in 20 of 26 (77%) of the leukemic guinea pigs. The earliest electrocardiographic changes were noticed on the 14th day after inoculation with leukemic cells; however, in some animals significant electrocardiographic changes were not noted until the terminal phase of the disease, i.e., up to 45 days after inoculation. There was a correlation between the onset of the electrocardiographic changes and the peripheral WBC elevation; in those animals in which there were electrocardiographic changes, the WBC were markedly elevated. The significant electrocardiographic changes were represented by the onset of a Q-wave or a T-wave inversion or both. There was no significant change in the heart rate. No ST changes were noted. No evidence of atrioventricular block and interventricular conduction disturbance was noted.

There was striking invasion of myocardial capillaries by leukemic cells. The pathological changes in these vessels may explain some of the electrocardiographic changes noted during the terminal phase of the disease. Very few leukemic cells actually infiltrated the muscle fibers of the myocardium. The great majority of these cells were within the capillaries of the endocardium, myocardium, and epicardium.

It is possible that the electrocardiographic changes noted during the terminal phase of the disease were most probably related to anoxic changes secondary to either complete or partial occlusion of the capillaries of the heart or that they resulted from compression of the myocardial cells by overdistended capillaries.

**REFERENCES**

Fig. 1. A typical normal tracing in a young healthy guinea pig.
Fig. 2. An electrocardiogram taken from a guinea pig in terminal phase of leukemia, revealing a Q-wave and a T-wave inversion in Lead 1. Such changes suggest leukemic involvement of the anterolateral region of the left ventricle.
Fig. 2a. Higher magnification of Fig. 2, representing the original electrocardiogram taken from a guinea pig in the terminal phase of leukemia, revealing a Q-wave in Lead 1 and a T-wave inversion in Lead 1.
Fig. 3. An anesthetized guinea pig with the electrodes in place.
Fig. 4. Transverse coronal section of heart with a large, thick-walled, slightly dilated left ventricle and a much smaller right ventricle. Above the left auricle there is an enlarged, leukemic lymph node. Small leukemic deposits are present in epicardium and endocardium (arrows). H & E, × 5.
Fig. 5. Multiple, horizontal, transverse heart sections. Top left, base of heart and large vessels, bottom right, apex. Large leukemic infiltration present at base of heart and small leukemic deposits present elsewhere in epicardium and endocardium (arrows). H & E, × 3.
Fig. 6. Low-power view of left ventricular wall showing extensive leukemic infiltration of epicardium with moderate involvement of the underlying myocardium. H & E, × 150.
Fig. 7. Detail of Fig. 6 illustrating the leukemic cell infiltration in the myocardium. Leukemic cells are present within capillaries and are separated from myocardial fibers by the capillary wall and endothelium (arrow). Myocardial fibers are compressed where the leukemic cells distend the capillaries (upper). A few cells are present outside the capillary wall (thick arrow). H & E, × 350.
Fig. 8. Electron micrograph of heart muscle from a leukemic guinea pig. Also shown is a leukemic cell within the lumen of a capillary. × 21,200.
Figs. 9 and 9a. A leukemic cell in an intercellular space proximal to cardiac muscle. Also shown is a leukemic virus particle (arrow) is shown. Fig. 9, × 22,640; Fig. 9a, × 58,000.
Fig. 10. A leukemic cell, containing a group of leukemic virus particles (arrow), is situated adjacent to heart muscle. × 71,000.
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