Cadmium pollution has been a source of increasing concern in recent years (1). Cadmium is eliminated slowly and accumulates with age. The effects of cadmium exposure in man are not well known, but it may be involved in tumor formation and cardiovascular disease. Studies on experimental mammals serve both to empirically evaluate potentially harmful effects of cadmium and to elucidate the underlying mechanisms. The mammalian testis is differentially sensitive to Cd\textsuperscript{2+}, and necrosis follows administration of sublethal doses (2). Inbred strains of mice differ markedly in their sensitivity to Cd\textsuperscript{2+}-induced testicular damage (3, 4). The purpose of this study was to determine the genetic basis for these interstrain differences. Such a study is a prerequisite for biochemical or physiological investigations attempting to detect the basis of these interstrain differences. Also, if the inheritance is found to be simple, the gene or genes responsible for the physiological strain differences may be useful markers in a variety of other genetic studies.

In this paper we demonstrate that resistance to Cd\textsuperscript{2+}-induced testicular necrosis in inbred mice is controlled by a single autosomal recessive gene. Evidence for this conclusion derives from (a) a survey of inbred strains and F\textsubscript{1} hybrids, (b) segregation and linkage studies utilizing backcross and F\textsubscript{2} generations, and (c) analyses of 23 partially inbred recombinant inbred (RI) lines derived from the F\textsubscript{2} generation of a C57BL/6J × DBA/2J cross. In addition, we tested several congenic resistant strains in an effort to detect linkage between the Cd\textsuperscript{2+} sensitivity gene and other marker genes.

**Materials and Methods.** All mice were obtained from either the Production Department or the research colonies of The Jackson Laboratory. This study included males from 10 to 12 wk old of 45 inbred strains and six F\textsubscript{1} hybrids. Eighteen strains were previously tested by Gunn, Gould and Anderson (3). In an effort to detect the linkage of the gene affecting Cd\textsuperscript{2+} susceptibility, we also tested 13 congenic resistant strains of the type in which a gene from a susceptible strain has been placed on the genetic background of a resistant strain (C57BL/10Sn). If the gene conferring susceptibility to Cd\textsuperscript{2+} were closely linked to one of the selected markers, it would probably be carried along with the marker, thus revealing linkage.

Male C57BL/6J × DBA/2J F\textsubscript{1} hybrids (B6D2F\textsubscript{1}) were mated to either C57BL/6J females or B6D2F\textsubscript{1} females to produce backcross and F\textsubscript{2} progeny, respectively.

In an additional test for single gene inheritance, we used recombinant inbred (RI) lines (5) derived by brother–sister mating from the F\textsubscript{2} generation of a B6D2F\textsubscript{1} hybrid. These are denoted by BXD and a numeral suffix, e.g., BXD-2.

Mice were injected subcutaneously with a single dose of 0.03 mM/kg of body weight of CdCl\textsubscript{2} (Fisher) in a volume of approximately 0.1 ml. Forty-eight hours later the mice were asphyxiated with CO\textsubscript{2}; the testes were removed and fixed in 10% neutral formalin. After 2 days in fixative, each testis was cut along either the longitudinal or transversal axis for gross pathological evaluation. Samples of both resistant and susceptible testes were sectioned and stained with hematoxylin and eosin for microscopic evaluation. This procedure was followed for all specimens for which the gross diagnosis was considered doubtful.

**Results.** In general, there was no difficulty
in classifying individual mice as either resistant or susceptible on the basis of gross examination of fixed, sliced testes. Testes from resistant strains appeared uniformly white or cream-colored, while testes from susceptible strains were generally darker and exhibited easily visible black hemorrhagic areas. Microscopically, the findings were extensive edema, hyperemia, hemorrhages, necrosis, and destruction of spermatogenic tissue.

The results of testing the 18 inbred strains previously analyzed by Gunn, Gould, and Anderson (3) confirmed their findings in detail. Strains AKR/J, CBA/J, C57BR/ cdJ, C57L/J, C58/J, DBA/1J, DBA/2J, RF/J, SWR/J, and 129/J proved to be susceptible, whereas strains A/J, A/HeJ, BALB/cJ, C3H/HeJ, C3HeB/FcJ, C57BL/6J, and C57BL/10J proved to be resistant. Among the SJL/J mice, 14 were susceptible and 6 were resistant. This was the only case with variability within an inbred strain and this had been noted previously (3). Eighteen additional strains were classified as susceptible: AU/SsJ, BDF/J, BUB/BnJ, CBA/CaJ, CBA/H-TGJ, C57BL/KsJ, CE/J, LP/J, LT/ReJ, MA/J, NZB/BnJ, P/J, PL/J, ROP/Gn, RIIt/J, SEA/GnJ, SM/J, ST/bJ, WC/ReJ, and WH/ReJ. Seven other strains were found to be resistant: HRS/J, I/LnJ, LG/J, PRO/Re, SEC/1ReJ, WB/ReJ, and WK/ReJ. The strain distribution pattern reflects known relationships among inbred strains (6). For example, sublines of the same strains were either both resistant, e.g., A/HeJ and A/J, C3H/HeJ and C3HeB/FcJ, C57BL/6J, and C57BL/10J, or both susceptible, e.g., DBA/1J and DBA/2J.

The clear distinction between resistant and susceptible strains, with the exception of SJL/J, suggested that a single major gene might account for the difference. To further evaluate this hypothesis several F1 hybrids were tested. The results are presented in Table I. The six standard F1 hybrids available from the Production Department of The Jackson Laboratory provided all of the desired combinations: (i) susceptible × susceptible (AKD2F1), (ii) resistant × susceptible (LAF1, C3D2F1, and B6D2F1) and (iii) resistant × resistant (CAF1, B6AF1). The results demonstrated dominance or partial dominance of susceptibility over resistance, since all the F1 hybrids involving a susceptible parent, either male or female, were susceptible. These results exclude X- or Y-linked inheritance. To critically analyze the single gene hypothesis, backcross (C57BL/6J × B6D2F1) and F2 mice from the B6D2F1 were tested. The ratio of susceptible to resistant mice in the F2 generation (22:10) and in the backcross to the resistant parent (10:14) conformed to the expected Mendelian ratios of 3:1 and 1:1, respectively. No significant association between the segregating coat color genes, dilute (d) and brown (b), and cadmium susceptibility was observed in the F2 generation.

Since all of the congeneric resistant strains resembled the resistant C57BL/10Sn partner, it can be concluded that the gene conferring cadmium susceptibility is neither identical to nor closely linked to the genes listed in Table II.

The results of testing 23 BXD RI lines are presented in Table III. BXD- lines 5, 6, 16, 27, and 29 appeared to be genetically

<table>
<thead>
<tr>
<th>F1 hybrid designation</th>
<th>Female parent</th>
<th>Male parent</th>
<th>Mating type</th>
<th>No. of positive/total</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKD2F1</td>
<td>AKR/J</td>
<td>DBA/2J</td>
<td>S × S</td>
<td>4/4</td>
</tr>
<tr>
<td>LAF1</td>
<td>C57L/J</td>
<td>A/J</td>
<td>S × R</td>
<td>4/4</td>
</tr>
<tr>
<td>B6D2F1</td>
<td>C57BL/6J</td>
<td>DBA/2J</td>
<td>R × S</td>
<td>10/10</td>
</tr>
<tr>
<td>C3D2F1</td>
<td>C3H/HeJ</td>
<td>DBA/2J</td>
<td>R × S</td>
<td>4/4</td>
</tr>
<tr>
<td>B6AF1</td>
<td>C57BL/6J</td>
<td>A/J</td>
<td>R × R</td>
<td>0/4</td>
</tr>
<tr>
<td>CAF1</td>
<td>BALB/cJ</td>
<td>A/J</td>
<td>R × R</td>
<td>0/4</td>
</tr>
</tbody>
</table>

* S denotes a susceptible strain; R, a resistant strain.
TABLE II. Results of Testing C57BL/10Sn Congenic-Resistant Strains for Cd²⁺-Induced Testicular Damage.

<table>
<thead>
<tr>
<th>Strain abbr</th>
<th>&quot;Donor&quot; strain</th>
<th>Known introduced gene(s)</th>
<th>No. of backcross generations</th>
<th>No. of positive/total</th>
</tr>
</thead>
<tbody>
<tr>
<td>B10.D2/o</td>
<td>DBA/2J</td>
<td>H⁻, ²⁴, ⁵⁻</td>
<td>5</td>
<td>0/4</td>
</tr>
<tr>
<td>B10.D2(55N)</td>
<td>DBA/2J</td>
<td>H⁻⁻¹¹ (not H⁻⁻¹¹⁺)</td>
<td>9</td>
<td>0/4</td>
</tr>
<tr>
<td>B10.D8(57N)</td>
<td>DBA/2J</td>
<td>H⁻⁻⁰</td>
<td>7</td>
<td>0/4</td>
</tr>
<tr>
<td>B10.D2(38N)</td>
<td>DBA/2J</td>
<td>H⁻⁻⁰⁻, M⁻⁻¹⁺, M⁺⁻¹⁻</td>
<td>8</td>
<td>0/6</td>
</tr>
<tr>
<td>B10.129(5M)/o</td>
<td>129/J</td>
<td>H⁻⁻¹, H⁻⁻⁰⁻, M⁻⁻¹⁺</td>
<td>10</td>
<td>0/4</td>
</tr>
<tr>
<td>B10.129(6M)</td>
<td>129/J</td>
<td>H⁻⁻¹⁻⁻⁻⁻, M⁻⁻¹⁻⁻⁻⁻</td>
<td>6</td>
<td>0/4</td>
</tr>
<tr>
<td>B10.129(7Ma)²</td>
<td>129/J</td>
<td>?</td>
<td>10</td>
<td>0/4</td>
</tr>
<tr>
<td>B10.129(7Mb)²</td>
<td>129/J</td>
<td>?</td>
<td>10</td>
<td>0/4</td>
</tr>
<tr>
<td>B10.129(7Mc)²</td>
<td>129/J</td>
<td>?</td>
<td>10</td>
<td>0/4</td>
</tr>
</tbody>
</table>
| B10.129(9M) | 129/J         | H⁻⁻¹⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻ нарко family may be more general. Additional evidence is as follows: strains LT/Re, SEA/GnJ, and PRO/Re were derived from crosses of BALB/c × C58, BALB/c × P, and C57BL/6 × 129 (i.e., resistant × susceptible), respectively. In each case, these derived strains resemble one or the other of the progenitor strains, i.e., in two cases the susceptible strain and in one case the resistant strain.

The strain distribution pattern for cdm is unlike that of any other known polymorphism. Thus, cdm apparently is not another manifestation of some other known gene. Whether the cdm allele is polymorphic in feral mice or is a mutation that occurred in domestic mice is not known. The limited distribution of the cdm gene among inbred strains suggests that the latter possibility may be correct. BALB/cJ, A/J, A/HeJ, C3H/HeJ, and C3HeB/FeJ are all descendants of the Bagg-albino stock (7). HRS/J and SEC/1Re are descended from BALB/c. Thus, the occurrence of the cdm allele in the Baggb-albino stock could account for all of the cdm bearing strains except C57BL/6J, C57BL/10J, LG/J, and WB/Re. The facts that cdm is limited in distribution and recessive suggest that cdm is a mutant from wild
type and that susceptibility is the normal state. This interpretation is supported by the observation that other mammals (e.g., rat, rabbit, and hamster) are sensitive to Cd\(^{2+}\)-induced testicular damage (8). Resistance to Cd\(^{2+}\)-induced testicular damage probably should not be considered an adaptive trait. In this regard, preliminary studies suggest that mice of the $cdm/ cdm$ genotype may be more susceptible to acute Cd\(^{2+}\) toxicity than $cdm/+$ and $+/+$ mice.

The mechanisms by which $cdm$ homozygotes are protected from Cd\(^{2+}\)-induced testicular damage are unknown. Lucis and Lucis (9) made the important observation that two Cd\(^{2+}\) resistant strains (C57BL/6J and BALB/cJ) had less testicular uptake of \(^{109}\)Cd\(^{2+}\) than two susceptible strains (CBA/J and DBA/1J). Although it would be hazardous to draw conclusions from a correlation based on only four points, the magnitude of the difference (fourfold) is sufficient to explain the difference between the strains with respect to testicular necrosis. Since only low tracer doses of \(^{109}\)Cd were used in the uptake studies, it is unlikely that the difference in uptake was the consequence of differential testicular damage rather than the cause. It is not known whether the differential testicular uptake of Cd\(^{2+}\) in $+$ and $cdm$ strains is an intrinsic property of the testis or merely a reflection of altered Cd\(^{2+}\) circulation. Cd\(^{2+}\) is reported to be chiefly bound to a low molecular weight, high methionine containing protein called "metallothionein" (10). Shaik and Lucis (11) isolated two major Cd\(^{2+}\) binding proteins from rat liver. The primary effect of the $cdm$ gene might be a quantitative or qualitative alteration of the cadmium binding proteins of one or more tissues.

The reason for the differential sensitivity of testes to Cd\(^{2+}\) is uncertain. The concentration of injected Cd\(^{2+}\) is much lower in the testes than in the liver, kidney, or at the site of cadmium injection (12). The high degree of vascularization of the testis may render it particularly sensitive (13). It is generally thought that the deleterious effects of cadmium are due to competitive interactions with zinc. Cadmium is known to inhibit various enzymes including carbonic anhydrase. Cadmium induced testicular interstitial cell tumors in mice and rats and chronic cadmium exposure may cause a number of human diseases. Studies aimed at elucidating the mechanisms of Cd\(^{2+}\) effects in experimental mammals should help to evaluate and predict the biological hazards of environmental cadmium pollution.

The BXD recombinant inbred lines should be useful in discovering pleiotropic effects of the $cdm$ gene, e.g., distribution of \(^{109}\)Cd to testes and other organs and the LD\(_{50}\) of $+$ and $cdm$ mice of both sexes. Comparison of the strain distribution pattern of the $cdm$ locus in the BXD lines with the strain distribution pattern of other mapped loci may reveal the chromosomal location of the $cdm$ locus. These lines are presently being typed for gene loci determining isoenzymes, cell surface antigens, and other characteristics. Once the $cdm$ gene is mapped it in turn could
be useful as a genetic marker. The strain
distribution pattern of cdm among standard
inbred strains furnishes additional biological
definition for these strains and also provides
additional data on which to base inferences
about probable interstrain relationships (6).

Summary. Resistance to cadmium-induced
testicular necrosis is determined by a single
autosomal recessive gene (cdm) in inbred
mice. Forty-five inbred strains, six F₁ hybrids,
14 congenic resistant strains, and 23 recombi-
nant strains were tested for cadmium-
induced testicular damage. The cdm gene is
not closely linked with several gene loci.

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oratory is fully accredited by the American Associa-
tion for Accreditation of Laboratory Animal Care.

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