Adrenal Cortical Function in High- and Low-Leukemia Strains of Mice*

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SUMMARY

The following functional differences have been demonstrated in normal mice of the high leukemia strain AKR, when compared with mice of the low leukemia strain C3H: (a) relative thymus hyperplasia, (b) increased sensitivity to lymphocytolytic effects of cortisone, and (c) unresponsiveness of lymphoid organs to adrenalectomy or injected ACTH. It is concluded that the adrenal in AKR mice is hypofunctional with respect to production of lymphocytolytic corticosteroids.

Mice of the high-leukemia strain, AKR, have an average incidence of spontaneous leukemia of 85–95 per cent. Experiments may therefore be performed on normal mice of this strain, at any age from birth to the onset of the leukemia age period, with the 90 per cent certainty that such mice would later have developed leukemia. Thus, mice of this strain present unique opportunities for the study of the preleukemic state and of the factors contributing to the development of lymphoid leukemia.

The importance of the adrenal and, in particular, of the glucocorticosteroids, in the pathogenesis of lymphoid leukemia, has been demonstrated by a number of workers. Law (5) showed that adrenalectomy increased the incidence of lymphoid leukemia in C58 strain mice. Kaplan, Marder, and Brown (4) found that adrenalectomy enhanced the induction of lymphoid leukemia by total-body irradiation. Glucocorticosteroids have been shown to delay (7) or decrease (8) the incidence of spontaneous leukemia, and to counteract the leukemogenic effects of whole-body irradiation (4, 7).

Arnesen (1) pointed out that the adrenal cortex in strain AKR mice contains little lipide-staining material, when compared with normal mice of the low-leukemia strain WLO. In view of the associated relative thymus hyperplasia in AKR mice, he suggested (8) that the adrenal, in the normal AKR mouse, may be hypofunctional.

We have confirmed Arnesen's observation that frozen sections of AKR adrenals contain little lipide-staining material in the cortex, when compared with C3H adrenals.

This paper reports the results of a series of biological tests, which also suggest that the adrenal is hypofunctional in normal mice of the high-leukemia strain AKR.

MATERIALS AND METHODS

Mice.—Mice used were those of the high leukemia strain AKR and the low-leukemia strain C3H. The AKR mice were originally obtained from Dr. Jacob Furth and have been maintained in an inbred state in this Institute. The current incidence of spontaneous lymphoid leukemia in this colony is 95 per cent. The C3H mice originated in the Imperial Cancer Research Fund Laboratories, London, and have been maintained in an inbred state in this Institute. The current incidence of spontaneous lymphoid leukemia in this colony is 0.5–1.0 per cent, for mice under 1 year of age.

Both colonies were housed under identical conditions in adjacent animal rooms. The mice were housed in metal tins with sawdust bedding. Diet consisted of Purina chow and water ad libitum, supplemented with carrots twice weekly and greens periodically.

Males and females were housed separately after weaning, and the female mice described in this paper were all virgin.

All mice included in this series were subse-
quently examined at autopsy and found to be free of significant disease.

Adrenalectomy.—Bilateral adrenalectomy was performed as a one-step operation, under ether anesthesia. The midline dorsal incision was closed with Michel clips. Postoperatively, the mice were maintained on 1 per cent saline, in lieu of drinking water. No supportive corticosteroid therapy was employed. Room temperature was maintained at 75°F. Both strains of mice withstood the initial effects of adrenalectomy well. The postoperative mortality in the present experiments, covering the first week after operation, was 1 per cent. However, adrenalectomized mice of both strains, maintained on this regimen for longer periods, showed a 40 per cent mortality from adrenal insufficiency, between the 6th and 80th weeks after operation.

At autopsy, the completeness of adrenalectomy was checked in all mice, using X4 magnification.

Cortisone and ACTH.—Cortisone acetate (Merck, Schering), strength 25 mg/ml, was diluted before injection in sterile 1 per cent saline, to give a final injected volume of 0.2 ml. per mouse.

ACTH (sheep) (Commonwealth Serum Laboratories, Melbourne) was similarly diluted with sterile 1 per cent saline to give a final injected volume of 0.2 ml. per mouse.

Injections were made subcutaneously in the dorsal region of the sacrum, using a No. 26 needle.

White cell counts.—White cell counts were performed on tail blood, obtained by pricking the tail veins with a No. 11 scalpel blade. Human white cell diluting pipettes were filled to the 0.1 mark and diluted with 3 per cent acetic acid to give a final dilution of 1:100. Fuchs-Rosenthal counting chambers with a well depth of 0.2 mm. were used throughout, and a volume of 2 cu. mm. was counted.

Differential counts were performed on slide preparations of tail blood stained with Wright's stain. Two hundred cells were counted per slide, with a standard battlement pattern. Monocytes and intermediate forms between monocytes and lymphocytes were pooled and included in the "lymphocyte" count.

Lymphoid organ weights.—Immediately after the performance of white cell counts, the mice were killed with ether and weighed on a beam balance (accuracy ± 0.5 gm.). The lymphoid organs were dissected free of fat and weighed on a torsion balance (capacity, 250 mg., accuracy, ± 0.5 mg.).

Statistical analysis.—Standard deviations were calculated according to the formula:

\[ SD = \sqrt{\frac{\Sigma \text{devs from mean}^2}{n-1}} \]

Analysis for statistically significant differences between the various groups was made using the Student "t" series method.

RESULTS

Relative thymus hyperplasia.—Normal mice of strains AKR and C3H, between 1 and 25 weeks of age, were weighed, and the weights of the lymphoid organs determined. Charts 1-4 show the results obtained for both strains and sexes.

The body growth rates for AKR and C3H mice were almost identical between 1 and 25 weeks. In AKR males there was a higher body weight around 12 weeks, in agreement with the observations of Arnesen (2). However, in the present series, this was only slight in degree and not statistically significant.

In AKR females, body weight slightly exceeded that of C3H mice from the 12th-25th week, but again these differences were not statistically significant.

When the thymus weights for the two strains are considered, it may be seen that the AKR thymuses greatly exceeded in weight those of C3H strain mice, in both sexes. This difference was highly significant statistically at all ages after the first week. After this time the male AKR thymuses averaged twice the weight of the corresponding C3H thymuses, and the female AKR thymuses averaged 2-3 times those of the corresponding C3H females.

Several features of the thymus growth curves are worthy of comment. Whereas there was little sex difference in the weights of the C3H thymuses, the AKR thymuses showed a pronounced sex difference, females being much heavier than males. In the first 3 weeks of life, the AKR thymuses grew at twice the rate of the C3H thymuses, despite the identical body growth rates in the two strains.

Age involution commenced in male and female thymuses of C3H mice at 4 weeks for the males and 5 weeks for the females. In AKR mice, age involution was delayed until 5-6 weeks for males and 7-9 weeks for females.

Finally, the female AKR thymuses showed a strikingly wide variation in thymus weight between 9 and 25 weeks. Some thymuses weighed twice that of other mice (150 mg. versus 75 mg.). The significance of this variability, in relation to the subsequent onset of leukemia in these mice, is under study.

Effects of cortisone on lymphoid tissues.—Normal, 5-week-old male AKR and C3H mice were matched for body weight. These mice were divided into four groups each. Cortisone, in varying doses,
1.0, 0.5, and 0.1 mg., was then injected subcutaneously into groups of these mice. Control mice received 0.2 ml. of saline. Twenty-four hours later, white cell counts were performed on all mice, and the lymphoid organs were weighed. Three such experiments were performed, with similar results, and the results have been pooled in Table 1.

The lymphoid organs weighed were the thymus, the six main subcutaneous lymph nodes (four axillary, two inguinal), the mesenteric node, and the spleen. No method could be devised for weighting, accurately, the Peyer’s patches, although the changes observed in them were qualitatively the same as in the other lymphoid organs.

It will be noted that the thymus was the most sensitive organ to the lymphocytolytic effects of cortisone. The other lymphoid organs, in order of sensitivity, were subcutaneous lymph nodes, mesenteric node, and spleen.

At each dose level of cortisone, there was a greater weight loss in all the lymphoid tissues of the AKR, than in the C3H mice. This was most marked in the thymus. For cortisone doses, 0.1, 0.5, and 1.0 mg., there was a thymus weight loss of 21, 62, and 56 mg. in the AKR, but only 16, 26, and 28 mg. in the C3H mice.

![Chart 1](chart1.png)  ![Chart 3](chart3.png)

**Chart 1.** Mean body weights, with standard deviation, of 193 AKR and 138 C3H male mice of various ages.

**Chart 3.** Mean thymus weights, with standard deviations, of 166 AKR and 225 C3H male mice of various ages.

![Chart 2](chart2.png)  ![Chart 4](chart4.png)

**Chart 2.** Mean body weights, with standard deviations, of 175 AKR and 188 C3H female mice of various ages.

**Chart 4.** Mean thymus weights, with standard deviations, of 142 AKR and 124 C3H female mice of various ages.
The weight loss in the lymphoid organs of the AKR mice was also greater than in the C3H, if calculated on a percentage loss basis. For example, with cortisone doses of 0.1, 0.5, and 1.0 mg., there was a 24, 48, and 60 per cent weight loss in the AKR lymph nodes, but only a weight loss of 3, 27, and 39 per cent in the C3H lymph nodes.

The lymphocyte levels in the peripheral blood of mice given injections of cortisone also demonstrated the greater sensitivity of AKR mice to cortisone. Although the peripheral lymphocyte levels were not as sensitive an indicator as the thymus of cortisone action at low dose levels, with the highest dose they showed a greater percentage depression than did any of the lymphoid organs.

These results suggest that the relative thymus hyperplasia in AKR mice is not due to any inherent insensitivity on the part of AKR lymphoid tissues to the lymphocytolytic effects of adrenal corticoids.

**Effect of adrenalectomy on lymphoid tissues.**—

Five-week-old AKR and C3H mice, of both sexes were placed in paired groups, on a body weight basis. The mice in one group of each pair were subjected to bilateral adrenalectomy, the mice in the other group, to sham operation.

Five days after operation, white cell counts were performed on the tail blood of all the mice. The mice were then killed, and the body weight and the weight of the lymphoid organs determined. The effect of adrenalectomy on lymphoid organ weights, at times other than 5 days postoperative-

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**Table 1**

**Effect of Various Doses of Cortisone on Lymphoid Organs**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment</th>
<th>Dose (mg.)</th>
<th>No. mice</th>
<th>Mean body wt. (gm.)*</th>
<th>Mean spleen wt. (mg.)</th>
<th>Mean lymph node wt. (mg.)</th>
<th>Mean mesenteric node wt. (mg.)</th>
<th>Mean thymus wt. (mg.)</th>
<th>Mean total lymphoid wt. (mg.)</th>
<th>Mean lymphocytes in peripheral blood</th>
<th>Mean polymorphs in peripheral blood</th>
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</thead>
<tbody>
<tr>
<td>AKR</td>
<td>Sal.</td>
<td></td>
<td>14</td>
<td>13.6 ± 1.0</td>
<td>44 ± 8</td>
<td>25 ± 2</td>
<td>58 ± 5</td>
<td>88 ± 8</td>
<td>100 ± 13</td>
<td>8700 ± 900</td>
<td>2800 ± 500</td>
</tr>
<tr>
<td>AKR</td>
<td>Cort. 0.1</td>
<td></td>
<td>14</td>
<td>13.5 ± 1.2</td>
<td>45 ± 13</td>
<td>19 ± 5↑</td>
<td>25 ± 5↑</td>
<td>67 ± 18↑</td>
<td>156 ± 19↑</td>
<td>8800 ± 1600</td>
<td>3500 ± 900</td>
</tr>
<tr>
<td>AKR</td>
<td>Cort. 0.5</td>
<td></td>
<td>14</td>
<td>13.3 ± 0.8</td>
<td>53 ± 13</td>
<td>13 ± 2↑</td>
<td>19 ± 4↑</td>
<td>96 ± 6↑</td>
<td>91 ± 15↑</td>
<td>1800 ± 400</td>
<td>3800 ± 100</td>
</tr>
<tr>
<td>AKR</td>
<td>Cort. 1.0</td>
<td></td>
<td>14</td>
<td>13.7 ± 0.9</td>
<td>27 ± 9↑</td>
<td>10 ± 3↑</td>
<td>16 ± 4↑</td>
<td>32 ± 7↑</td>
<td>85 ± 18↑</td>
<td>980 ± 400</td>
<td>1500 ± 900</td>
</tr>
<tr>
<td>CSH</td>
<td>Sal.</td>
<td></td>
<td>14</td>
<td>12.9 ± 0.8</td>
<td>54 ± 17</td>
<td>34 ± 9</td>
<td>32 ± 4</td>
<td>46 ± 13</td>
<td>168 ± 28</td>
<td>7300 ± 1600</td>
<td>3000 ± 1100</td>
</tr>
<tr>
<td>CSH</td>
<td>Cort. 0.1</td>
<td></td>
<td>14</td>
<td>13.0 ± 1.2</td>
<td>51 ± 14</td>
<td>33 ± 5</td>
<td>31 ± 7</td>
<td>90 ± 8</td>
<td>145 ± 23</td>
<td>6700 ± 1100</td>
<td>3100 ± 600</td>
</tr>
<tr>
<td>CSH</td>
<td>Cort. 0.5</td>
<td></td>
<td>14</td>
<td>13.9 ± 0.6</td>
<td>42 ± 13</td>
<td>25 ± 9↑</td>
<td>26 ± 5↑</td>
<td>20 ± 2</td>
<td>115 ± 20↑</td>
<td>3000 ± 1800</td>
<td>3800 ± 500</td>
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<tr>
<td>CSH</td>
<td>Cort. 1.0</td>
<td></td>
<td>14</td>
<td>13.0 ± 1.0</td>
<td>33 ± 9↑</td>
<td>21 ± 5↑</td>
<td>21 ± 4↑</td>
<td>18 ± 4</td>
<td>95 ± 10↑</td>
<td>1600 ± 400</td>
<td>3000 ± 900</td>
</tr>
</tbody>
</table>

* ± standard deviation.  
† Results significantly different, P < 0.01.
seem to parallel more closely the general state of inanition of the adrenalectomized mouse than fluctuations in lymphoid tissue activity.

It should be pointed out that, although the thymus in C3H mice increased in weight after adrenalectomy, it still did not approach that of normal or adrenalectomized AKR mice. Differences in adrenal function can, therefore, be only a partial explanation of the thymus weight differences between the two strains.

Further, since both male and female AKR mice responded similarly to adrenalectomy, the adrenal appears to play little part in determining the large sex difference in thymus weights in this strain.

Effect of ACTH on lymphoid tissues.—The response of AKR and C3H mice to injected ACTH was next investigated, with lymphoid atrophy used as the index of adrenal stimulation.

### TABLE 2
Effect of Adrenalectomy on Lymphoid Organs

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sex</th>
<th>Operation</th>
<th>No. mice</th>
<th>Mean body wt. (gm.)*</th>
<th>Mean spleen wt. (mg.)</th>
<th>Mean mesenteric node wt. (mg.)</th>
<th>Mean thymus wt. (mg.)</th>
<th>Mean total lymphoid wt. (mg.)</th>
<th>Mean lymphocytes in peripheral blood</th>
<th>Mean polymorphs in peripheral blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKR</td>
<td>M</td>
<td>A</td>
<td>18</td>
<td>16.6 ± 1.4†</td>
<td>55 ± 6</td>
<td>114 ± 13</td>
<td>257 ± 26†</td>
<td>16,300 ± 5100†</td>
<td>6,800 ± 1500</td>
<td></td>
</tr>
<tr>
<td>AKR</td>
<td>M</td>
<td>S</td>
<td>16</td>
<td>18.4 ± 2.0</td>
<td>59 ± 5</td>
<td>115 ± 15</td>
<td>284 ± 29</td>
<td>15,500 ± 1800†</td>
<td>5700 ± 2500</td>
<td></td>
</tr>
<tr>
<td>AKR</td>
<td>F</td>
<td>A</td>
<td>11</td>
<td>14.5 ± 1.9</td>
<td>55 ± 5</td>
<td>121 ± 17</td>
<td>256 ± 29</td>
<td>16,500 ± 8500†</td>
<td>4,800 ± 1800</td>
<td></td>
</tr>
<tr>
<td>AKR</td>
<td>F</td>
<td>S</td>
<td>11</td>
<td>15.8 ± 1.4</td>
<td>41 ± 6</td>
<td>121 ± 15</td>
<td>292 ± 37</td>
<td>10,800 ± 1300†</td>
<td>3,000 ± 900</td>
<td></td>
</tr>
<tr>
<td>C3H</td>
<td>M</td>
<td>A</td>
<td>15</td>
<td>17.0 ± 1.8</td>
<td>59 ± 4†</td>
<td>72 ± 7†</td>
<td>87 ± 16†</td>
<td>12,100 ± 1100†</td>
<td>4,500 ± 500</td>
<td></td>
</tr>
<tr>
<td>C3H</td>
<td>M</td>
<td>S</td>
<td>12</td>
<td>17.0 ± 1.2</td>
<td>47 ± 9</td>
<td>58 ± 8</td>
<td>245 ± 19</td>
<td>8,500 ± 1100†</td>
<td>4,100 ± 1200</td>
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<tr>
<td>C3H</td>
<td>F</td>
<td>A</td>
<td>12</td>
<td>15.3 ± 1.3†</td>
<td>57 ± 7†</td>
<td>74 ± 8†</td>
<td>282 ± 22†</td>
<td>15,500 ± 9000†</td>
<td>4,400 ± 1700</td>
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<tr>
<td>C3H</td>
<td>F</td>
<td>S</td>
<td>12</td>
<td>14.8 ± 1.1</td>
<td>45 ± 6</td>
<td>63 ± 11</td>
<td>254 ± 24</td>
<td>7,500 ± 700</td>
<td>3,800 ± 600</td>
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</tr>
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</table>

* ± standard deviation.
† Results significantly different, P < 0.01.

A = adrenalectomy.
S = sham-operated.

### TABLE 3
Effect of ACTH on Lymphoid Organs

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sex</th>
<th>Treatment</th>
<th>No. mice</th>
<th>Mean body wt. (gm.)*</th>
<th>Mean spleen wt. (mg.)</th>
<th>Mean mesenteric node wt. (mg.)</th>
<th>Mean thymus wt. (mg.)</th>
<th>Mean total lymphoid wt. (mg.)</th>
<th>Mean lymphocytes in peripheral blood</th>
<th>Mean polymorphs in peripheral blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKR</td>
<td>M</td>
<td>ACTH</td>
<td>11</td>
<td>25.0 ± 0.9</td>
<td>51 ± 6</td>
<td>65 ± 11</td>
<td>89 ± 10</td>
<td>283 ± 28</td>
<td>13,000 ± 1400†</td>
<td>3,400 ± 800</td>
</tr>
<tr>
<td>AKR</td>
<td>M</td>
<td>Saline</td>
<td>10</td>
<td>25.0 ± 0.6</td>
<td>40 ± 7</td>
<td>54 ± 14</td>
<td>100 ± 15</td>
<td>268 ± 24</td>
<td>5,900 ± 900</td>
<td>2,800 ± 800</td>
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<tr>
<td>AKR</td>
<td>F</td>
<td>ACTH</td>
<td>14</td>
<td>19.0 ± 1.7</td>
<td>55 ± 11</td>
<td>123 ± 18</td>
<td>287 ± 19</td>
<td>11,000 ± 3600†</td>
<td>2,800 ± 1100</td>
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<tr>
<td>AKR</td>
<td>F</td>
<td>Saline</td>
<td>13</td>
<td>18.0 ± 1.3</td>
<td>56 ± 9</td>
<td>132 ± 15</td>
<td>292 ± 18</td>
<td>10,500 ± 1800†</td>
<td>2,800 ± 700</td>
<td></td>
</tr>
<tr>
<td>C3H</td>
<td>M</td>
<td>ACTH</td>
<td>12</td>
<td>21.2 ± 1.7</td>
<td>49 ± 11</td>
<td>50 ± 8†</td>
<td>28 ± 9†</td>
<td>214 ± 55</td>
<td>11,800 ± 1000†</td>
<td>3,800 ± 1600</td>
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<tr>
<td>C3H</td>
<td>M</td>
<td>Saline</td>
<td>12</td>
<td>20.5 ± 1.1</td>
<td>52 ± 10</td>
<td>50 ± 6</td>
<td>47 ± 11</td>
<td>245 ± 34</td>
<td>9,400 ± 800</td>
<td>4,000 ± 1500</td>
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<tr>
<td>C3H</td>
<td>F</td>
<td>ACTH</td>
<td>14</td>
<td>16.7 ± 0.8</td>
<td>44 ± 9</td>
<td>49 ± 10</td>
<td>64 ± 7†</td>
<td>242 ± 15†</td>
<td>10,300 ± 1600†</td>
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<td>C3H</td>
<td>F</td>
<td>Saline</td>
<td>14</td>
<td>16.6 ± 1.0</td>
<td>49 ± 11</td>
<td>55 ± 9</td>
<td>82 ± 7</td>
<td>277 ± 13</td>
<td>9,800 ± 1800</td>
<td>2,400 ± 900</td>
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</table>

* ± standard deviation.
† Results significantly different, P < 0.01.
then injected subcutaneously with 2 I.U. of ACTH in 0.2 ml. of 1 per cent saline. Control groups were injected with saline alone.

Twenty-four hours later white cell counts were performed on all mice and the weights of the lymphoid organs determined.

Table 3 shows that, in C3H mice, significant atrophy of the lymphoid organs, particularly of the thymus, followed the injection of ACTH.

By contrast, no lymphoid organ atrophy occurred in AKR mice following the injection of ACTH. A slight fall in the thymus weight of mice in the experimental group did occur, but this was not significant statistically.

In all mice receiving injections of ACTH slight generalized edema was observed. This had the effect of masking possible lymphoid atrophy in organs weighed. Control groups were given injections daily of 0.2 ml. saline.

Table 4 shows that, in response to multiple injections of ACTH, definite atrophy of the thymus occurred in AKR mice. In the C3H mice, this thymus weight loss was more pronounced, and a significant weight loss also occurred in the subcutaneous lymph nodes.

These results suggest that the AKR adrenal cortex is capable of stimulation by injected ACTH but that its responsiveness to stimulation is considerably less than that of C3H adrenals.

**DISCUSSION**

This paper has described a number of functional differences between normal mice of the high leukemia strain, AKR, and corresponding mice of the table 3 shows that, in C3H mice, significant atrophy of the lymphoid organs, particularly the thymus, followed the injection of ACTH.

By contrast, no lymphoid organ atrophy occurred in AKR mice following the injection of ACTH. A slight fall in the thymus weight of mice in the experimental group did occur, but this was not significant statistically.

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**TABLE 4**

**EFFECT OF MULTIPLE INJECTIONS OF ACTH ON LYMPHOID ORGANS**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment</th>
<th>Dosage</th>
<th>No. mice</th>
<th>Mean body wt. (gm.)</th>
<th>Mean spleen wt. (mg.)</th>
<th>Mean lymph node wt. (mg.)</th>
<th>Mean mesenteric node wt. (mg.)</th>
<th>Mean thymus wt. (mg.)</th>
<th>Mean total lymphoid wt. (mg.)</th>
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</thead>
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<td>AKR</td>
<td>ACTH</td>
<td>2 I.U. daily for 5 days</td>
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<td>20.3±1.0</td>
<td>60±18</td>
<td>55±7</td>
<td>50±9</td>
<td>61±13†</td>
<td>204±26†</td>
</tr>
<tr>
<td>AKR</td>
<td>Saline</td>
<td>0.2 ml. daily</td>
<td>11</td>
<td>21.0±1.0</td>
<td>74±7</td>
<td>41±7</td>
<td>34±9</td>
<td>101±11</td>
<td>250±21</td>
</tr>
<tr>
<td>C3H</td>
<td>ACTH</td>
<td>2 I.U. daily for 5 days</td>
<td>10</td>
<td>19.5±1.4</td>
<td>80±9</td>
<td>39±7†</td>
<td>47±7</td>
<td>98±9†</td>
<td>194±22†</td>
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<tr>
<td>C3H</td>
<td>Saline</td>
<td>0.2 ml. daily</td>
<td>10</td>
<td>19.7±0.9</td>
<td>90±17</td>
<td>48±4</td>
<td>57±10</td>
<td>54±8</td>
<td>249±29</td>
</tr>
</tbody>
</table>

* ± standard deviation.† Results significantly different, P < 0.01.

the spleen and lymph nodes of the C3H mice and of causing an actual weight increase in the AKR lymphoid organs.

In both strains, mice injected with ACTH showed a slight lymphocytosis at 24 hours. This was more prominent in the male mice. The interpretation of this finding is uncertain. The ACTH preparation used was sheep in origin, and the lymphocytosis observed is similar to that seen following the injection of some foreign proteins into mice. The lymphocytosis could, however, conceivably be a specific effect or side effect of ACTH itself.

In a second series of experiments, the effect of multiple injections of ACTH on lymphoid organ weights was investigated.

Five-week-old, male AKR and C3H mice were arranged in paired groups on a body weight basis. These mice were then injected subcutaneously with 2 I.U. ACTH, daily for 5 days. On the 5th day the mice were killed and their lymphoid

low-leukemia strain C3H. These are: (a) relative thymus hyperplasia, (b) increased sensitivity to the lymphocytolytic activity of cortisone, (c) failure of lymphoid organs to increase in weight following adrenalectomy, and (d) failure of lymphoid organs to atrophy following a single injection of ACTH.

Taken individually, none of these findings is necessarily indicative of adrenal hypofunction. However, their association together makes it likely that the adrenal in the AKR mouse is distinctly hypofunctional, at least with respect to lymphocytolytic corticosteroid production.

In two of the experiments, there was a suggestion that the AKR adrenal may actually secrete a lymphoid-stimulating steroid. These were the slight lymphoid atrophy following adrenalectomy, and the lymphoid weight increase following the injection of ACTH. However, other interpretations of these findings are possible, as has already been indicated. Attempts to demonstrate a stimu-
latory effect of AKR adrenal cortex extracts on lymphoid tissue have, to date, proved inconclusive.

The present experiments have given little indication whether the adrenal hypofunction is a primary one or secondary to other endocrine disturbances. The effect of repeated injections of ACTH in producing thymus atrophy in AKR mice can be equally interpreted as indicating either a primary adrenal hyporesponsiveness or a depressed functional activity in the adrenal secondary to pituitary hyposecretion of ACTH.

It is of interest to note that body growth is normal in the AKR mouse and that weight loss occurs following adrenalectomy. This suggests that the production of other adrenal steroids may be normal in the AKR.

In the complex process of lymphocyte homeostasis, the adrenal cortex plays a major role. Glucocorticosteroids not only destroy lymphoid cells in situ but also suppress mitosis in the primitive lymphoid cells (3). An inadequate production of these steroids in an animal must tend to create an imbalance, favoring lymphoid proliferation. If abnormally active lymphopoiesis is a prerequisite in the pathogenesis of lymphoid leukemia, then adrenal hypofunction could be an important factor in this process.

Experimental evidence has, indeed, been obtained that adrenalectomy increases the incidence of lymphoid leukemia in mice (3) and, conversely, that adrenal glucocorticosteroids delay or decrease the incidence of spontaneous (4, 7, 8), or radiation-induced (4) lymphoid leukemia.

The finding of adrenal hypofunction in normal AKR mice, which are later destined to develop a high incidence of spontaneous leukemia, fits well with these earlier observations.

It is not suggested here that adrenal hypofunction is the primary mediator of the leukemogenic process in the spontaneous disease in AKR mice. It seems likely, however, that it constitutes at least an important accessory influence facilitating the emergence of neoplastic lymphoid cells, evoked by the primary etiological agent—whether this be virus, gene, or hormone.

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Adrenal Cortical Function in High- and Low-Leukemia Strains of Mice

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