The Combined Effects of Cortisone and Roentgen Radiation upon Natural and Induced Resistance to Homoiotransplantation of Mouse Leukemia, Line I₅

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The complex interrelationship of genetic and nongenetic factors determining spontaneous, induced, and transplantable rodent leukemias has been ably reviewed by Richter and MacDowell (24), by Furth (5), and by Kirschbaum (19). It is pointed out (5, 12, 24) that the genetic constitution of the host limits the transmissibility of leukemic cells to the mouse strain of origin and to F₁ hybrids. X-radiation to date has constituted a unique means for rendering an otherwise resistant host of foreign species (21) or strain (22) receptive to the transplantation of mouse leukemic tissue. When it was learned in this laboratory (37) that the preparation of resistant mice by prior treatment with an adrenocortical steroid hormone, cortisone, likewise may make possible homoiotransplantation of mouse leukemia, experimental studies were continued to learn the effect of cortisone and x-radiation, singly and in combination, upon the transmissibility of lymphoid leukemia, line I₅, to mice of resistant strains. The evidence to be presented in this report indicates (a) that cortisone and x-radiation when employed singly may make naturally resistant mice susceptible to a foreign line of leukemia; (b) that prior treatment by these two agents in combination results in a synergistic effect to render resistant mice susceptible to the test line of leukemia; (c) that mice actively immunized by an injection of leukemic cells may acquire by specific immunization a protective mechanism more resistant to the alternative effects of cortisone and x-radiation than the innate resistance naturally provided by genetic constitution.

MATERIALS AND METHODS

Leukemic cells.—Leukemic cells representative of lymphoid leukemia, line I₅, were employed, because the factors that determine the success of the transplantations of this line of leukemia have been carefully assessed by MacDowell and his associates (14, 16). This line of leukemia originated spontaneously in an inbred strain of mouse designated as C58 (17). It has been maintained by serial transmission and studied over many years in the laboratory of one of the authors (E. C. MacD.). For control purposes, nonleukemic splenic tissue from strain C58 mice was employed.

Mice.—Five strains of mice were utilized in this investigation: (a) the naturally susceptible C58 strain of inbred mouse served as host to maintain by serial transmission line I₅ leukemic cells and to test for transmissibility the cellular suspensions from test resistant mice; (b) the Bagg albino (BALB), an inbred strain from MacDowell's colony; (c) Strong A, an inbred strain; (d) a stock strain of Swiss albino mouse, the CFW strain; and, finally, (e) an inbred strain, Storms-Little (STOLI).

Preparation of cellular suspension.—Splenic tissue provided cells for transplantation and for immunization. Immediately upon removal the whole spleen was minced with scissors, suspended in 2.8 ml. of 0.85 per cent NaCl per gram of tissue and filtered through four layers of Brunswick gauze to remove large clumps of cells. The cells in suspension were counted with the aid of a Levy-Hausser counting chamber to provide by dilution a constant number of cells for each injection. The suspension was kept during irradiation in paper cartons measuring 8.5 cm. in diameter by 10 cm. in height. The dosage ranged from 100 r to 400 r, 800 r being used most commonly. The roentgen rays were generated by a current of 15 m. amp. having a 250 kv. peak. They were filtered through 0.5 mm. of Cu and 1.0 mm. of Pb.

Preparation of test mice.—Test mice of the resistant strains were prepared by treatment with cortisone and/or x-radiation prior to injection with leukemic cells. X-radiation was administered 24 hours before the injection of leukemic cells as a single massive dose to the whole body in a target skin distance of 80 cm. The mice in groups of three were kept during irradiation in paper cartons measuring 8.5 cm. in diameter by 10 cm. in height. The dosage ranged from 100 r to 400 r, 800 r being used most commonly. The roentgen rays were generated by a current of 15 m. amp. having a 230 kv. peak. They were filtered through 0.5 mm. of Cu and 1.0 mm. of Pb.

Line I₅: The subscript b indicates freedom from a virus which had contaminated this line for a period of years (35).
of Al. The half value layer was 1.35 mm. of Cu. The size of the field was 14.0 cm. in diameter. The output, as determined by a Victoreen r-meter, was 41.5 r/minute in air.

Cortisone acetate (Cortone-Merck) brand of 11-dehydro-17-hydroxycorticosterone-11-acetate, 25 mg/ml, was employed directly or by diluting it in saline to provide a concentration of 1.0 mg/0.1 ml, for injection subcutaneously. The dosage was a single injection of either 0.35 mg. or 2.0 mg., or of seven successive injections, 0.05 mg. daily, for a total of 0.35 mg.

Examination of mice.—Each mouse was examined daily to determine whether its spleen or lymph nodes were palpable. When enlarged, these are readily palpated by the experienced examiner. At autopsy the diagnosis of leukemia was further established by transfer of pooled splenic tissue to susceptible C58 strain mice.

RESULTS

Effect of x-radiation upon the transmissibility of line 14 leukemia.—Pilot control experiments (Series A) with stock Swiss albino mice were carried out to determine the approximate dosage of irradiation to be used. Whole-body x-radiation in a dosage range of from 100 r to 400 r was given, followed 24 hours later by the injection of leukemic cells. The mice were observed daily over a period of 30 days; the results are presented in Table 1.

The results of these experiments led to a second series of experiments (Series B) in which a single dose of 300 r was given to two inbred strains of mice, BALB and A, and to the Swiss albino stock strain. The data are presented in Table 2.

It is evident from Table 2 that x-radiation of the whole body in a single dose of 300 r served effectively to break down the resistance of the host as evidenced by the growth of leukemic homoiografts in mice of two unrelated inbred strains and of one

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tbody>
<tr>
<td><strong>The Effect of Graded Massive Doses of Roentgen Radiation upon the Susceptibility of Stock Swiss Mice, CFW Strain, to Mouse Leukemia, Line 14</strong></td>
</tr>
<tr>
<td>Whole-body Radiation (r units)</td>
</tr>
<tr>
<td>100</td>
</tr>
<tr>
<td>200</td>
</tr>
<tr>
<td>300</td>
</tr>
<tr>
<td>400</td>
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<tr>
<td>None</td>
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</tbody>
</table>
| * Transfer numbers 10 and 11.

The denominator signifies the number of test mice; the numerator, the number of survivors.

All the mice in the control group (Table 1) that received only x-radiation survived for 30 days, except for a single mouse in the group that was given 400 r. The second control group, consisting of the animals that received leukemic cells without irradiation, all survived. In contrast to these findings, death resulted in all 28 recipient mice when leukemic cells were injected within 24 hours of a single dose of x-radiation (either 300 r or 400 r). Moreover, leukemic cells in combination with lesser doses of x-radiation resulted in death of six of the ten animals that received 200 r and two of the ten that received 100 r. The diagnosis of leukemia in each dead test mouse was established at autopsy by its greatly enlarged spleen and in selected cases by the production of leukemia after injection of spleen cell suspensions in C58 mice.

stock strain. In contrast to death from leukemia of the 60 test mice, the 60 mice in the two control groups survived the injection of leukemic cells, or treatment by x-radiation, 300 r, when given singly. The results of these two experiments provided ample evidence for the ability of roentgen radiation to overcome the natural innate resistance of the test mice to line 14 leukemia and thereby confirmed the findings of earlier workers that roentgen radiation makes possible cross-strain transplantation of mouse leukemia to resistant strains of mice.

These findings led to experiments to learn whether a totally dissimilar agent, cortisone acetate, which has been shown to alter host resistance to a variety of infectious agents (1, 3, 4, 6, 7, 9—11, 19, 20, 23, 25—32, 35, 36, 38, 39), may operate similarly to render resistant mice susceptible to the test line of leukemic cell.

Effect of cortisone upon the transmissibility of Line 14 leukemia.—It was the known adverse effect of cortisone upon host resistance that led to its employment (97) for the treatment of normally resistant mice in attempts to homoiotransplant a line of leukemic cells of established characteristics. Two series of experiments (Series C and D) were carried out. These two series differed in the number of injections of cortisone that were employed. Moreover, the second series of experiments (Series D) was planned with the dual purpose of establishing by confirmation the results of the first series

<table>
<thead>
<tr>
<th>TABLE 2</th>
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<tbody>
<tr>
<td><strong>The Effect of a Single Dose of Roentgen Radiation, 300 r, upon the Susceptibility of Three Resistant Inbred Strains of Mice to Mouse Leukemia, Line 14</strong></td>
</tr>
<tr>
<td>Strain of Test Mice Employed</td>
</tr>
<tr>
<td>Roentgen Radiation</td>
</tr>
<tr>
<td>BALB</td>
</tr>
<tr>
<td>Strong A</td>
</tr>
<tr>
<td>Swiss albino, CFW strain</td>
</tr>
</tbody>
</table>

* Transfer numbers 10, 11, 15.

† The denominator signifies the number of test mice; the numerator, the number of survivors.

‡ Average time to death measured in days.
and to serve as a control in measuring the effects of cortisone and x-radiation in combination upon the host's resistance to the transplantability of the leukemic cells. The findings of the experiments in Series C are presented in Table 3.

The data in Table 3 for Series C show that death from leukemia resulted for all BALB test mice and for a third of the stock Swiss albino mice which had been prepared by treatment with cortisone prior to the injection of the test dose of leukemic cells. Contrariwise, the A mice survived the injections of cortisone and leukemic cells, as did the control group of mice that received cortisone only. These results were interpreted to mean that cortisone in

<table>
<thead>
<tr>
<th>STRAIN OF</th>
<th>CORTISONE*</th>
<th>Leukemic cells</th>
<th>Cortisone</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEST MICE</td>
<td>and</td>
<td>only</td>
<td>only</td>
</tr>
<tr>
<td>BALB</td>
<td>0/12</td>
<td>9/10 S</td>
<td>10/10 S</td>
</tr>
<tr>
<td>Strong A</td>
<td>10/10 S</td>
<td>10/10 S</td>
<td>10/10 S</td>
</tr>
<tr>
<td>Swiss albino</td>
<td>15/18</td>
<td>11/6 S</td>
<td>20/30 S</td>
</tr>
<tr>
<td>CFW strain</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The cortisone acetate, 0.05 mg., was injected subcutaneously on 7 successive days for a total dose of 0.35 mg.
† The denominator signifies the number of test mice; the numerator, the number of survivors.
‡ Average time to death measured in days; "S" signifies 100 per cent survival.

The salient finding of this series of experiments was the enhancing or synergistic effect brought about by the concomitant utilization of cortisone and x-radiation as agents for altering natural resistance. This effect is clearly shown by the data that relate to each of the three test resistant strains of mice (see Groups 1 and 2). It will be noted in Chart 1 that all 90 test mice died of leukemia and that the average interval to death ranged from 3.0 to 5.2 days, in contrast to an average interval to death of from 10.1 to 15.5 days for the mice that succumbed to leukemia following treatment by either cortisone or x-radiation alone.

Tests in confirmation of the diagnosis of leukemia, line 1B, for this series of experiments were carried out by the selection at random of spleens from moribund mice for transplantation both to mice of the susceptible C58 strain and to mice of the same resistant strain from which each spleen had been excised. It was found that the injection of the harvested leukemic cells resulted in death from leukemia for all C58 mice and in no evidence for disease in mice of resistant strains.

The ability of artificially induced active immunity
to modify the alternative effects of cortisone and x-radiation upon host resistance.—When it was learned from the foregoing experiments that cortisone and x-radiation, singly and in combination, exerted a detrimental effect upon host resistance, it seemed probable that the complex, naturally occurring mechanism commonly referred to as natural or innate resistance had been affected adversely. The rapid development in treated mice of leukemia which caused death in from 3 to 5 days made unlikely a significant role for actively acquired immunity. Nevertheless, experiments were designed (Series E) to assess the effect of artificially induced active immunization. The results were contrary to expectations.

Groups of three resistant strains of mice (BALB, A, and stock Swiss) were immunized over a period of 3 weeks by three injections intraperitoneally of line I b leukemic splenic tissue from C58 mice. The dosage employed consisted of 1.0 gm. of minced splenic tissue. It thereby became possible to superimpose an artificially induced active immunity upon the pre-existing natural or innate resistance. The mice in the control groups received the same amount of splenic tissue from normal C58 mice. The test and control mice were subjected to cortisone or whole-body radiation, or both, 7 days after the third immunizing dose of tissue had been injected. The data that relate to the dosages of cortisone and x-radiation, the number

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**TABLE 4**

<table>
<thead>
<tr>
<th>EXPERIMENTAL GROUPS*</th>
<th>X-ray (r units)</th>
<th>Cortisone (mg.)</th>
<th>Strain of Mice</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>800</td>
<td>2.0</td>
<td>BALB</td>
<td>0/15</td>
</tr>
<tr>
<td>Group 2</td>
<td>800</td>
<td>0.35</td>
<td>STOLI</td>
<td>0/10</td>
</tr>
<tr>
<td>Group 3</td>
<td>200</td>
<td>None</td>
<td>Swiss Albinoid</td>
<td>0/20</td>
</tr>
<tr>
<td>Group 4</td>
<td>None</td>
<td>2.0</td>
<td></td>
<td>5.5</td>
</tr>
<tr>
<td>Group 5</td>
<td>None</td>
<td>0.35</td>
<td></td>
<td>4.2</td>
</tr>
</tbody>
</table>

* Transfer numbers 10, 11, 38, 33.  
† The denominator signifies the number of mice; the numerator, the number of survivors.  
‡ Average time to death measured in days; "S" signifies 100 per cent survival.

**CHART 1**—The effects of cortisone acetate and roentgen radiation employed in combination and independently upon the susceptibility of three resistant strains of mice to mouse leukemia, line I b.
of mice, and the findings are contained in Table 5.

It may be noted in Table 5 and in Chart 2 that of the 45 BALB mice which had been immunized with leukemic splenic tissue, 38 survived. These results are totally different from the groups of BALB mice that received for immunization normal splenic tissue, since death from leukemia resulted for 44 of 45 in this group. The protective effect of active immunization against the otherwise detrimental effect of cortisone and x-radiation was similarly in evidence both for A mice and for the Swiss stock strain.

Table 5

THE EFFECTS OF CORTISONE ACETATE AND ROENTGEN RADIATION EMPLOYED IN COMBINATION AND INDEPENDENTLY UPON THE SUSCEPTIBILITY OF THREE RESISTANT STRAINS OF MICE TO MOUSE LEUKEMIA, LINE Lb, AFTER PREVIOUS IMMUNIZATION WITH HOMOLOGOUS CELLS

<table>
<thead>
<tr>
<th>Treatment and Dose</th>
<th>Leukemic Splenic Tissue</th>
<th>Normal Splenic Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB</td>
<td>200 0.35</td>
<td>11/15† 0/15</td>
</tr>
<tr>
<td></td>
<td>500 0.35</td>
<td>15/15 1/15</td>
</tr>
<tr>
<td></td>
<td>200 0.35</td>
<td>12/15 0/15</td>
</tr>
<tr>
<td>A</td>
<td>500 0.35</td>
<td>10/10 10/10</td>
</tr>
<tr>
<td></td>
<td>900 0.35</td>
<td>10/10 0/10</td>
</tr>
<tr>
<td></td>
<td>300 0.35</td>
<td>27/30 9/15</td>
</tr>
<tr>
<td>Swiss albino</td>
<td>300 0.35</td>
<td>20/20 18/15</td>
</tr>
<tr>
<td>CFW strain</td>
<td>300 0.35</td>
<td>20/20 18/15</td>
</tr>
</tbody>
</table>

*The denominator signifies number of test mice; the numerator, the number of survivors.
†Transfer numbers 25, 30, 35, and 36 were employed for the test challenge dose of line Lb leukemic cells.

It may be noted that of the 145 recipients which had been immunized with leukemic tissue, 125 survived. This finding was in contrast to the recipients which had been immunized with normal splenic tissue, for only 25 survived of the 125 mice in that category. Moreover, 23 of these survivors were in the two groups that received only 0.35 mg. of cortisone. Thus, the results that were obtained from the use of x-radiation and cortisone in combination (Table 5) were different from the findings observed (Tables 1–4) when the test animals had not been actively immunized.

DISCUSSION

The experiments reported in the present paper demonstrate that either of two extrinsic agents, cortisone or x-radiation, may induce susceptibility in a mouse naturally resistant, as assessed by the success of homoiotransplantation of leukemic cells. Moreover, when these two agents were given in combination before the test injection, an enhanced response resulted. The study was carried out by employing line Lb lymphoid leukemia in four resistant strains of mice. The x-radiation and the administration of cortisone preceded the transplantation of leukemic cells by 24 hours or less. For cortisone, it more commonly was 2 hours. The influence of other time intervals in preparation for transfer of leukemic cells has not yet been determined. It was concluded that the effects of cortisone and x-radiation singly and in combination alter the host receptivity of the treated mouse without apparent effect upon the leukemic cells. This interpretation came from the demonstration that line Lb leukemic cells from otherwise resistant mice resulted in death from leukemia for all C58 mice and in no evidence for disease in mice of resistant strains.

Genetic constitution is commonly accepted as the determinant that controls the transplantability of leukemic cells (5, 12). X-radiation is the single extrinsic factor that is outstanding in its ability to overcome the species specificity that characterizes the successful transmission of leukemia to normal animals (8, 18). This recognized capacity of ionizing radiation to reverse the response of naturally resistant inbred mice to homoiotransplantation was readily confirmed (Tables 1, 2, 4). Whole-body x-radiation, in a dosage of 300 or 400 r, broke down the resistance of stock Swiss albino mice and of two inbred strains, BALB and A. This deleterious effect was less apparent when 100 r (Table 1) or 200 r were employed (Tables 1, 2, 4). A review of other data does not reveal a difference in mouse strain response to irradiation.

Cortisone likewise successfully converted resistant mice to a state of susceptibility to homoiotransplantation with line Lb leukemic cells (Tables 8, 4). However, it may be seen that a strain difference in response to the alternative effects of cortisone was made apparent. Mice of the BALB strain showed a greater response to the effects of cortisone than did the other inbred strains. The difference was evidenced for each test group of BALB mice by more deaths and a shorter time to death than was observed in comparable test groups of mice of other strains. Contrariwise, the findings suggest that A mice are the least affected by cortisone. These differential findings in mouse strain response to cortisone were likewise apparent when mice which had been actively immunized to line Lb were tested (Table 5). When leukemic cells were injected into actively immune mice, no deaths resulted among the A mice and only three of fifteen among the stock Swiss mice, in contrast to death for fourteen of fifteen BALB mice. Any differences in strain response were obscured by the use of x-radiation and cortisone in combination, since all test mice died and the time to death was markedly shortened.
The adverse effect of cortisone upon host resistance to a variety of infectious agents (1, 3, 4, 6, 7, 9–11, 19, 20, 25, 26, 28–32, 35, 36, 38, 39) is manifest by an increase in the severity of the infectious process, or in death. Ionizing irradiation likewise may alter host response to its detriment (27, 33). The action of the two agents upon the host are similar in the impairment of the inflammatory response (6, 19, 20, 23, 35) and in the production of antibodies (2, 18). Further similarities were brought out by the present findings showing that either x-radiation or cortisone may successfully overcome the innate resistance of inbred mice to homoiotransplantation of lymphoid leukemia, line Lb. Moreover, the synergistic effect of the two agents, as measured by the success of transplantation and the shortened survival time to death, was comparable to the enhancing effect of the two agents upon a variety of bacterial, mycotic, and viral infections (4, 7, 26, 32).

Evidence in support of the immunological specificity of leukemic cells was established indirectly when cortisone and x-radiation, singly and in combination, were found to exert little effect upon the resistance of mice which had been prepared prior to treatment by active immunization with leukemic cells. For example, 38 survived of the 45 mice which had been immunized with leukemic splenic tissue, in contrast to death from leukemia of 44 of the 45 mice which had been immunized with normal splenic tissue. Three experiments on three mouse strains demonstrated conclusively the protective effect of active immunization against the otherwise detrimental effect of cortisone and x-radiation (Charts 1 and 2). These same experiments differentiated sharply between natural innate or genetic resistance, and acquired or induced resistance. It is evident that the methods employed in the present study make it possible to investigate the mechanisms responsible for these differences in a variety of host-cell parasite relationships by the utilization of tumor cells and microbiological agents.

**SUMMARY**

Two extrinsic agents, cortisone and ionizing radiation, were found to convert the naturally resistant status conferred by the genetic constitution of the test inbred mice into a state of susceptibility. Mice representative of three resistant inbred strains, BALB, A, and STOLI, and of the stock Swiss albino CFW strain, by the administration of cortisone and x-radiation singly and in combination, were made susceptible to the homoiotrans-
plantation of lymphoid leukemia, line Ib, when tested within 24 hours. The two agents operated synergistically to potentiate the alternative effect that may result from the employment of either agent singly. Contrariwise, mice made resistant by actively induced immunization with leukemic cells were little affected by the administration of cortisone and radiation singly or in combination.

REFERENCES
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