On the Possibility of Cure of Malignant Lymphoid Tumors.

I. Treatment of Autochthonous Lymphoid Tumors in C57BL Mice with Massive Doses of Lymphocytolytic Agents

By Henry S. Kaplan and C. Susan Nagareda

The malignant lymphomas and lymphatic leukemia are almost invariably fatal in man, although effective palliation, and in some instances significant prolongation of life, may be obtained by treatment with x-rays and/or chemotherapeutic agents. The inevitability of a fatal outcome, barring death from intercurrent illness or other unrelated causes, is particularly well documented when the disease has become generalized. Since the vast majority of malignant lymphomas are not diagnosed until dissemination has occurred, opportunities for prolonged observation of adequately treated, apparently localized cases have been few. Isolated reports of long-term survival without recrudescence in such localized instances have been discounted because of the occasional long-term survival of disseminated cases (in which, however, death usually results from the original disease).

Many serious students of this group of neoplasms have accepted the view that they arise from multiple, systemically-distributed foci and are thus disseminated from the outset. If this were true, the conclusion would be justified that cure of these conditions is never possible, even in their earliest stages, with currently available modalities of treatment. This point of view is implicit in the widely accepted practice of treating malignant lymphomas palliatively, rather than with curative intention, even in apparently localized and conceivably early instances.

Acceptable evidence of the curability of malignant lymphomas would require: (1) microscopic proof of the presence of the disease prior to treatment; (2) evidence of apparently complete regression of clinically manifest tumor after completion of treatment; (3) sustained freedom from all manifestations of the disease over a substantial fraction of the life span; and (4) whenever possible, autopsy proof of the complete absence of microscopic residues of the disease in individuals dying at long intervals after treatment.

These stringent criteria are exceedingly difficult to fulfill in human studies. Despite the reservations that must inevitably attend any extrapolation to man from work in laboratory animals, it seemed of interest to investigate this question in mice, in which it is possible to establish both the diagnosis and the full extent of disease at any desired time by killing control groups and

From the Department of Radiology, Stanford University School of Medicine, Stanford, Calif.

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subjecting them to autopsy and microscopic examination. Moreover, the relatively short life span of mice makes it possible to follow treated animals for the entire anticipated duration of life, to autopsy long-term survivors, and to establish by microscopic examination the fact that the tissues are free of tumor.

The neoplasm selected for investigation was the radiation-induced lymphoma of strain C57B1 mice. This neoplasm is a lymphocytic or lymphoblastic lymphosarcoma, which can be induced in virtually 100 per cent of these animals with appropriate doses of radiation.4 It was shown many years ago that the tumors apparently arise unicentrically, the site of predilection being the thymus,5 and that their development may be effectively prevented by thymectomy prior to, or even after, irradiation.6,7

The choice of therapeutic agents was based on the fact that x-ray treatment causes profound thymic involution and lymphocyte destruction8 and on the fact that hormonal agents such as cortisone, hydrocortisone, and testosterone, which also cause acute thymic involution,9 all significantly inhibit development of lymphomas when administered concurrently with irradiation and continued for a few weeks thereafter.10-12 Moreover, cortisone has exhibited therapeutic activity against transplantable mouse lymphomas,13 and both local and total-body x-irradiation have been employed therapeutically for transplantable lymphomas and leukemias in the mouse.14,15

**Materials and Methods**

Female strain C57B1 mice, bred in our laboratory, were weaned at 28 ± 3 days of age and distributed randomly among six experimental groups (table 1). Systemic irradiation was started when the mice were 60 ± 3 days of age; four doses of 200 r each were given at seven day intervals.9 The animals of Group I received a placebo of normal saline (0.03 cc.) intramuscularly at either 50 days or 100 days after the first X-ray exposure; thereafter, they were otherwise untreated. Group II was sacrificed at 50 or 100 days after the initial X-ray exposure; all of these mice were autopsied, and their tissues were examined grossly and histologically, with special attention to the presence of early thymic lymphoid tumors. Group III was given 1,000 r localized over the thymic region at 50 or 100 days. Mice of this group were anesthetized with sodium amytal and placed on a lead sheet 2.0 mm. thick. A second lead sheet of 3.4 mm. thickness with 1.2 cm. by 1.5 cm. windows cut out was placed 2.0 cm. above the first lead sheet, shielding the entire mouse except for the thymus area.9 The remaining three groups of mice (Groups IV, V, VI) were given single intramuscular injections of 0.3, 1.0, or 3.0 mg. of hydrocortisone1 at 1, 21, 50, or 100 days after the first X-ray exposure. All mice were then observed for periods up to 600 days for the development of lymphoid tumors. Moribund animals were sacrificed and autopsied; tissues were routinely processed for histologic examination whenever the diagnosis of lymphoma was not grossly obvious at autopsy. Deaths due to other types of reticuloendothelial neoplasms, which are histologically distinguishable from the typical lymphocytic or lymphoblastic lymphosarcoma,16,17 were recorded as negative for lymphoma and tabulated separately.

*Physical factors were: 120 KVP; 9 ma.; 0.25 mm. Cu + 1.0 mm. Al added filter; 30 cm. mouse-target distance; 32 r/min.; HVL = 0.39 mm. Cu.
†Physical factors were: 120 KVP; 7.5 ma.; no filtration; 30 cm. mouse-target distance; mean thymus dose rate of 107.4/r/min.
‡Hydrocortone (hydrocortisone acetate) was generously supplied by Dr. Seymour Alpert of Merck, Sharp & Dohme, Westpoint, Pa.
Table 1.—Final Lymphoid Tumor Incidence and Latent Period

<table>
<thead>
<tr>
<th>Group</th>
<th>Post irradiation treatment 1,2</th>
<th>Days post 1st X-ray</th>
<th>Net no. mice</th>
<th>Lymphomas no.</th>
<th>Mean latent period 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Placebo, saline</td>
<td>50 100</td>
<td>57 37</td>
<td>53 35 93 95</td>
<td>184 181</td>
</tr>
<tr>
<td>II</td>
<td>Sacrifice, histology</td>
<td>50 100</td>
<td>43 36</td>
<td>37 35 86</td>
<td>-</td>
</tr>
<tr>
<td>III</td>
<td>1,000 r, thymic region</td>
<td>50 100</td>
<td>34 36</td>
<td>14 19 53</td>
<td>241 208</td>
</tr>
<tr>
<td>IV</td>
<td>Hydrocortisone, 0.3 mg.</td>
<td>1 21 50</td>
<td>26 32 46</td>
<td>23 26 40 87</td>
<td>201 187 194</td>
</tr>
<tr>
<td>V</td>
<td>Hydrocortisone, 1.0 mg.</td>
<td>1 21 50</td>
<td>30 37 36</td>
<td>20 20 20 55</td>
<td>226 250 228</td>
</tr>
<tr>
<td>VI</td>
<td>Hydrocortisone, 3.0 mg.</td>
<td>21 50 100</td>
<td>33 52 36</td>
<td>15 19 16 44</td>
<td>241 285 268</td>
</tr>
</tbody>
</table>

1. Whole body X-ray: 200 r x 4 q 7 days.
2. Hydrocortisone acetate (Merck Sharp & Dome) single i.m. injection.
3. Days post first X-ray exposure.

RESULTS

The data are summarized in Table 1 and cumulative lymphoma incidence curves are presented in Figures 1 and 2. The irradiated, placebo-treated animals of Group I developed a final lymphoid tumor incidence of approximately 94 per cent. The earliest lymphoma deaths occurred at about 120 days, and the latest at about 250 days after the first inducing dose of x-irradiation. Examination of thymuses from mice that were sacrificed at either 50 or 100 days after irradiation (Group II) revealed that lymphoid tumors were already present in microscopic form in 37 of 43 (86 per cent) at 50 days and 35 of 36 (97 per cent) at 100 days. At 50 days the lymphoid tumors were almost invariably confined to the thymus and quite frequently limited to one lobe, while at 100 days about one third had invaded beyond the thymic capsule and/or become disseminated. The histologic appearance of early lymphoid tumors developing in the thymus has been briefly described elsewhere.

When x-ray therapy in single doses of 1,000 r, localized over the thymic region, was administered at either 50 or 100 days (Group III), a significant reduction in deaths due to lymphomas was observed, with final incidence levels of 41 and 53 per cent, respectively. The cumulative incidence curves (Figure 2) show that deaths due to lymphoid tumors occurred in Group III.
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animals treated at 100 days with almost the same frequency as in the control mice of Group I until about 165 days after irradiation, when the Group III curve abruptly reached a plateau, with only sporadic lymphoma deaths occurring thereafter to about 350 days. In contrast, lymphoid tumor deaths in mice of Group III treated at 50 days departed from the control cumulative curve immediately and reached a plateau slightly earlier.

Highly significant suppression of lymphoid tumor development also occurred in animals of Group VI treated with a single massive dose of hydrocortisone (3.0 mg.). The response was not significantly affected by the time of administration of the hydrocortisone; final lymphoma incidence levels in mice so treated at 21, 50, or 100 days after initial irradiation were all in the same range (37-45 per cent). Treatment with this large dose of hydrocortisone was also attempted on the first day of irradiation, but abandoned due to excessive mortality. Deaths due to lymphoid tumors were also reduced to a lesser degree by 1.0 mg. doses of hydrocortisone given to Group V mice on day 1, 21, or 50 (final incidence 67, 54, and 55 per cent, respectively). No significant inhibition of lymphoid tumor development was afforded by the 0.3 mg. dose level of hydrocortisone (Group IV). The mean latent periods and cumulative incidence curves for this group are similar to those observed in the control Group I, while Groups V and VI exhibit prolonged mean latent periods.
and their cumulative lymphoma incidence curves are flatter than those of Groups I and IV.

Some animals died within a few days or weeks after 3.0 mg. of hydrocortisone and exhibited no gross evidence of lymphoid tumor at autopsy. In some of these instances apparently viable tumor cells were still evident on histologic examination of the thymus and other tissues (fig. 3), whereas in others, careful microscopic examination failed to reveal viable tumor cells. Many of these tumor-negative thymus glands were greatly involuted; they weighed only 2.5 to 10 mg., as contrasted to normal age control thymic weights of 40 to 60 mg., and on microscopic study exhibited necrosis or extreme atrophy and depletion of lymphoid elements (fig. 4). Profound atrophy of the thymus and lymph nodes, and complete absence of viable tumor cells were also noted microscopically in the long-term survivors of all treated groups.

DISCUSSION

Lymphoid tissues respond dramatically, by cell death and acute involution, to the administration of adrenal cortical hormones. The administration of cortisone concurrently with, or as late as six weeks after, irradiation significantly depresses lymphoma incidence. Hydrocortisone, a powerful lympholytic steroid, is even more effective in preventing lymphoma development. In the present experiments, hydrocortisone was used therapeutically, rather than...
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Fig. 3.—Thymus 5 days after single massive dose of hydrocortisone, revealing pyknosis and extreme depletion of lymphoid elements, hemorrhage, edema, and a single focus of apparently viable tumor cells (arrow). X71.

Fig. 4.—Mediastinal tissues 23 days after 3 mg. hydrocortisone. Extreme involution of thymic lobe with no evidence of residual viable tumor cells. X 71.

prophylactically, since virtually all of the sacrificed controls were shown to have at least microscopic, and in some instances, frankly invasive or disseminated lymphoid tumors at the time of treatment, 50 or 100 days after the initial inducing dose of irradiation.

It is indeed remarkable that as early as 50 days after the beginning of
fractionated irradiation, 86 per cent of the animals already had thymic lymphomas in situ, and at 100 days the incidence was even higher (97 per cent). The validity of these microscopic diagnoses is supported by the fact that the corresponding placebo controls (Group I) developed an almost identical final lymphoma incidence. These control data also serve as a reference level against which the therapeutic efficacy of hydrocortisone or local X-rays can be measured with respect to prolongation of life and permanent cure. The neoplastic character of the early microscopic lesions has also been subjected to the test of isologous transplantability; these data will be reported more fully elsewhere. It is also of interest that some of the placebo-treated tumors present at day 50 or 100 did not kill their hosts until some 150 to 200 days later. The rate of evolution of autonomy clearly exhibits greater variation than the rate at which microscopic tumors are formed.

Single large doses of hydrocortisone (1.0 or 3.0 mg.) apparently destroyed incipient lymphoid tumors completely and permanently in a large proportion of animals thus treated, while a small dose (0.3 mg.), sufficient to exert a transient lymphocytolytic effect, was ineffective. There is need for comparable dose-response data for X-ray therapy, since the single dose of 1,000 r used in these experiments was chosen arbitrarily.

Paradoxically, the time of administration of hydrocortisone did not significantly influence its effectiveness in suppressing deaths due to lymphoid tumors. It might have been anticipated that animals treated with hydrocortisone at the beginning (day 1) or end (day 21) of the lymphoma-inducing course of X-radiation would have been more completely protected against ultimate lymphoid tumor death than animals whose treatment was withheld until day 50 or day 100, when either microscopic or macroscopic neoplasms were already present. Since some of the treated animals survived as long as 550 days after the start of the experiment and were then found to be completely free of gross or microscopic evidence of residual or recurrent lymphoid tumor, the use of the term cure seems justified.

Single doses of 1,000 r localized over the thymus area were somewhat less effective than the largest (3.0 mg.) dose of hydrocortisone at 100 days, but equally effective at 50 days. This difference in X-ray response at the two time intervals is attributed to the more frequent spread of lymphoid tumor cells beyond the thymus to other tissues of the animal by 100 days. In such instances, some of the tumor cells would have been outside the irradiated field, and their survival and growth could not have been affected. Thus, lymphoid tumor development could continue unabated in these animals. This interpretation is supported by the cumulative incidence curves; it can be seen that among mice given localized X-radiation over the thymic region at one hundred days, deaths due to lymphomas paralleled the control rate for approximately 165 days. The incidence recorded to that time (35 per cent) tallies well with the fact that one third of the microscopic lymphomas in the 100-day sacrifice group had already disseminated. In contrast, since the effect of hydrocortisone is systemic, lymphoid tumor cells residing in other tissues as well as in the thymus would be subjected to the cytolytic action...
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of this agent. It might therefore be expected that adequate doses of hydrocortisone would be curative even in disseminated cases. This expectation was confirmed by the equal response of the groups treated with hydrocortisone at 50 and 100 days.

Of great interest is the appearance of lymphoid tumors with prolonged latent periods (more than 300 days after the start of the experiment) in all of the experimentally treated groups. Only one of the 88 tumor deaths among the 94 animals of the irradiated control group (Group I) occurred after this time (469 days). In contrast, 42 (19 per cent) of the 225 lymphoid tumor deaths among a total of 408 mice in the experimental groups (Groups III to VI) occurred after 300 days (301–469 days). The appearance of lymphomas with prolonged latent periods may be due to: (1) failure of the therapeutic agent, in the dose employed, to destroy every last tumor cell, with ultimate recurrence of the original tumor; or (2) a second de novo tumor induction in once-cured animals in response to the original inducing stimulus of X-radiation. The former interpretation is supported by the distinctly dose-dependent character of the response to hydrocortisone, as well as by the observation of persistent viable tumor cells in some of the animals dying within a few days after injection of 3.0 mg. of hydrocortisone. It thus seems likely that the instances of lymphoma with long latent period are partial therapeutic failures, in which life span has been significantly prolonged, but in which death ultimately results from recrudescence of the original neoplasm.

Inasmuch as this animal investigation had its origins in clinical considerations, it seems appropriate to consider the import of these results for the clinical management of human malignant lymphomas. The fact that over half of a large group of animals with a hitherto fatal disease could be permanently cured by appropriate doses of lymphocytolytic agents administered early in the course of their disease should encourage renewed attempts at curative therapy in patients with apparently regionally localized malignant lymphomas. Since such early cases are relatively rare in any one medical center, there may be merit in cooperative studies whereby data from a number of centers can be pooled, and definitive results thus made available at a correspondingly earlier date.

Summary

Strain C57B1 mice in which thymic lymphosarcomas had been induced with total-body X-radiation were treated, at various times in the course of tumor development, with either hydrocortisone or local X-irradiation. Their survival was compared with that of placebo-treated controls. In other sacrificed control groups, the incidence and extent of lymphoma development at the time of treatment was established by microscopic examination.

Treatment with single massive doses of hydrocortisone or local thymic X-irradiation at 50 or 100 days after lymphoma induction resulted in permanent cure of half or more of the animals. For a given dose, hydrocortisone was equally effective when given at any time interval up to 100 days after the start of lymphoma induction. However, the therapeutic efficacy of hydro-
cortisone was a significant function of hormone dose, over the range 0.3 to 3.0 mg. In addition to the cure of many animals, significant prolongation of life was noted in animals dying of lymphomas in all of the treated groups.

**SUMMARIO IN INTERLINGUA**

Muses del racia C57B1, in que lymphosarcomas thymic habeva essite inducite per roentgeno-irradiation del corpore total, esseva tractate—æ varie tempores in le curso del disveloppamento del tumores—con hydrocortisona o con roentgeno-irradiation local. Le resultante supervivencia esseva comparate con illo de animales de controlo tractate con placebo. In altere sacrifice gruppos de controlo, le incidentia e le extension del disveloppamento de lymphoma al tempore del tractamento esseva establite per exames microscopic.

Le tractamento con massive doses solitari de hydrocortisona o con local roentgeno-irradiation del thymo a periodos de 50 o 100 dies post le induction del lymphoma resultava in curaciones permanente de un medietate del animales o plus. A base de doses equal, hydrocortisona esseva equalmente efficace quando administrate a non importa qual periodo usque a 100 dies post le comenciamento del induction del lymphoma. Tamen, le efficacia therapeutic de hydrocortisona esseva significativemente un function del dose in le region ab 0.3 ad 3.0 mg. A parte le curation de multes del animales, un significative augmento del longevitate esseva notate in omne Ic gruppos tractate inter le animales que ultimemente moriva ab le lymphomas.

**ACKNOWLEDGMENTS**

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**REFERENCES**

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Henry S. Kaplan, M.D., Professor and Executive, Department of Radiology, Stanford University School of Medicine, Palo Alto, Calif.

C. Susan Nagareda, Ph.D., Research Associate, Department of Radiology, Children's Hospital Society of Los Angeles, Los Angeles, Calif.
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HENRY S. KAPLAN and C. SUSAN NAGAREDA

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