The Effect of Cortisol on the Growth of a Transplantable Mouse Fibrosarcoma*

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Many studies have established the fact that cortisol and cortisone inhibit the growth of tumors of lymphoid origin (8, 19, 20, 23, 24). However, the effect of these steroids on other types of tumors is by no means certain. In most instances in which any effect on tumor growth is observed, these corticoids have been found to inhibit the tumors, but there have also been a few reports (5, 26) of enhancement of tumor growth. On the other hand, except for the lymphoid tumors, removal of the adrenal glands slows tumor growth in all cases where an effect is seen (4, 10, 14, 15, 17, 22, 26). This subject has been recently reviewed by Noble (18).

There are a number of possible explanations for the variability of the reported actions of these corticoids on tumor growth. In some cases, no effect of cortisol or cortisone can be seen in intact animals, whereas a measurable effect of the hormones may be observed after adrenalectomy (21, 26). Qualitative differences in the response of tumors to corticoids may represent differences in the tumor-host relationships. Studies on the effect of these hormones on heterologous tumor transplants have shown that the host response may profoundly affect the final growth of the tumor (1, 9, 12, 13, 28). Interpretation of the data in studies of tumor growth is frequently difficult. The density of tumor tissue varies with the degree of necrosis, for example, so that tumor weight may not be a true measure of the inhibition or enhancement of growth. In addition, no matter what index of growth is used, a high degree of variability is found among tumors transplanted into different animals, so that it is often difficult to obtain statistically significant data (25). A further complication is the fact that a wide range of hormone doses has been used, with many workers paying little attention to the relationship between tumor response and the dose of administered corticoi. There is reason to believe that quantitative, and perhaps even qualitative, differences might occur at different dose levels of exogenous hormone (5, 11, 18, 26, 27).

In the present study, various amounts of cortisol were administered to mice bearing a transplanted fibrosarcoma, in an attempt to construct a dose-response curve for the effect of cortisol on this particular tumor. The fibrosarcoma was selected as being of particular interest, since reported effects of adrenal steroids on this type of tumor are extremely variable. In view of the known action of these steroids on connective tissue in general, it was felt that the effects on such a tumor should be clarified. Since our preliminary experiments had shown that the hormone had no appreciable effect on tumors growing in intact animals, only data from adrenalectomized mice are presented herein.

MATERIALS AND METHODS

Male and female A/He CRGL X BALB/c CRGL F1 mice, between 2 and 3 months of age, were used in these experiments. The transplanted tumor was a fibrosarcoma which had arisen spontaneously in a hybrid mouse and which was in its eighteenth transplant generation at the start of this series of experiments. At the time of its selection for transplantation, this particular fibrosarcoma took about 10 days to reach palpable size and generally killed its host within 24 months. Initially, the tumor took in approximately 96 per cent of the animals. Because of the large number of mice involved, the experiments were staggered in time. To minimise the effect of any changes in tumor growth rate that might occur from one transplant generation to the next, a few animals for each group were used simultaneously. The results presented herein represent the data only from those animals which survived the entire experimental period of 50 days.

At the start of each experiment, all animals were gonadectomized, and all except the animals in Groups 1 and 9 were also adrenalectomized. Eight days after these operations, two pieces of tumor tissue were transplanted under the ventral abdominal skin by means of a trocar. On the 20th day of the experiment, the animals were weighed and the tumors measured by means of calipers. The largest diameter and the major diameter perpendicular to it were measured, the product of these being reported as "tumor area." The weighing and measuring procedures were repeated every 3 days for the remainder of the experiment.

On day 94, the animals in Group 9 were adrenalectomized,
and cortisol acetate treatment was begun in Groups 3–8. The cortisol was injected subcutaneously in 0.2 ml. of saline suspension. All animals were sacrificed 80 days after the beginning of the experiment, at which time the tumors were dissected out and weighed.

Throughout the experiment, the mice were maintained on a modified McCollum's Diet I (2) plus Purina chow. All cages were provided with plain tap water and a solution of 0.9 per cent NaCl.

Every 3d day, the tumor area for each animal was plotted on a graph such as those shown in Chart 1. The tumor area for each animal represents the mean of the two tumors measured. Similarly, the tumor weights reported (Table 1) were calculated from the mean tumor weight of each animal. The difference between the tumor area on day 32 and that on day 80 was also calculated (Table 1), providing an index of the relative effect of cortisol treatment on tumor growth in the various groups.

**RESULTS**

A significant decrease in the viability of the fibrosarcoma was noted during this series of experiments. The tumor was subsequently followed through its 36th transplant generation, at which time it took in only 10 per cent of the animals. A larger number of animals was used than has been reported here, because many of the implanted tumors regressed before treatment was initiated. In all cases where regression occurred, the animals were discarded. This was done only after it had been determined that the number of tumors that regressed was generally the same in treated as in control animals. For an undetermined reason, however, a larger number of such regressions occurred before day 34 in Group 4, so that an additional number of these animals was tested toward the end of the series of experiments to fill in the group. Probably for this reason, the average tumor growth appears somewhat retarded in this group (see Chart 1).

Some variability occurred between tumors transplanted into the same host. The difference between the final areas of the larger and smaller of the two tumors in the same host was significant in Groups 1, 2, 3, and 6. Because such variability did occur, it was considered more meaningful to report the mean area of the two tumors rather than the total tumor area per animal.

All intact control animals survived throughout the experiment. Approximately 20 per cent of the untreated adrenalectomized animals died. The mortality of adrenalectomized animals was considerably reduced when cortisol acetate was administered in doses of 0.35 mg/day or less, but was as great as or greater than that of the controls when higher hormone doses were used (33 per cent of the animals in Group 8 died).

The average tumor weights of the various groups are given in Table 1. The tumor weights in Groups 3 and 6 were higher than those of adrenalectomized controls (Groups 2 and 9), the differences being significant at the 5 per cent level of confidence. None of the other differences observed was statistically significant.

The increase in tumor areas is plotted in Chart 1, each line representing the mean tumor areas for all animals in a group. The segment of each curve to the left of the vertical line represents tumor growth before treatment was begun. Of the two groups (1 and 9) which were not adrenalectomized at this stage, only Group 1 showed a mean tumor area which was significantly larger than that seen in the adrenalectomized mice during the initial growth period. The inhibitory effect of adrenalectomy can be seen by comparing, at the right of the vertical line in Chart 1, the curves of Groups 1 (not adrenalectomized), 2 (adrenalectomized control), and 9 (adrenalectomized on day 34). The final tumor areas of animals in Groups 2 and 9 were significantly lower than those of Group 1. This confirms the work cited above (9, 14, 17, 22, 26).

The effects of various doses of cortisol acetate are shown by the remaining curves in Chart 1. The left-hand segments of these curves indicate that the growth of the tumors was similar in all groups (except Group 4, which was somewhat aberrant) before treatment was initiated. From the right-hand segments, it is evident that all doses of cortisol resulted in some enhancement of tumor growth. The amount of increase in tumor growth after cortisol treatment was dependent on the dose, however. The enhancing effect was greater with increasing doses up to 0.5 mg/day and became less evident at higher doses. The tumor areas of animals receiving 1.5 mg cortisol/day were not significantly greater than those of adrenalectomized control animals. The relation between hormone dose and tumor growth is more clearly shown in Table 1, which gives the difference between the tumor area on day 50 and the area on day 32 (2 days before treatment was initiated). The maximum increase in tumor area occurred after treatment with 0.5 mg cortisol/day.

To eliminate the possibility that these results were due to effects of the treatment on body weight, the mean body weight change was recorded for each group (Table 1). This was calculated by subtracting the total tumor weight from the final body weight and subtracting this corrected value from the body weight at the beginning of the experiment. Although all cortisol-treated animals lost weight, the magnitude of the weight loss can in no way be correlated with the effect on tumor growth.

Some of the tumors from each group were sectioned and stained with alum-hematoxylin and...
CHART 1.—Absolute growth of tumors in relation to the dose of cortisol acetate. The mean tumor area (sq. mm.) for each group was calculated from the averages of two tumors growing in each animal. See text for method of measurement. Segments of curves to the left of the vertical line represent pretreatment values; to the right, measurements obtained during the period of cortisol acetate treatment, which was initiated on day 34.
eosin. No histologic differences between tumors growing in the treated and untreated mice were noted, except that there appeared to be a slight increase in the degree of necrosis in tumors from the former groups.

**DISCUSSION**

The enhancement of fibrosarcoma growth by cortisol acetate was somewhat surprising to us, in view of the well known effects of this hormone on connective tissue in general. The explanation presumably lies in the connective tissue response of the host to cortisol treatment, as opposed to the response of the more autonomous tumor. That adrenocortical hormones can modify the host response to transplanted tissues is indicated by the fact that even some human tumors can be transplanted into laboratory animals which have been treated with cortisone (28). The relative effect of such hormones on the host as opposed to the tumor itself is variable and appears to depend on the type of tumor, its degree of autonomy, and its inherent growth potential (1, 9, 28). In this regard, it might be mentioned that no growth of the fibrosarcoma used in the present experiments was observed when this was transplanted into mice of another strain (A/Cal) which were treated with cortisol acetate (unpublished data). This may well be a result of the low growth potential of the tumor, making it unable to overcome the "acquired immunity" of the host (28) despite the hormonal treatment. Perhaps more pertinent to this discussion is the report by Green and Whitely (12) that tumors was observed, so that the increased necrosis after cortisol might be due only to the effect of this steroid on tumor size. No correlation between the degree of necrosis and the dose of administered cortisol could be detected from these data, however, but these observations were not quantitative. Variability in the degree of necrosis of the tumors probably accounts for the fact that no consistent or significant changes were observed in the tumor weights. As Talalay et al. (25, p. 834) have pointed out, "tumor weight may be regarded meaningful only when necrosis is minimal in a tumor sufficiently large so that errors intrinsic in the dissection are relatively small."

The results indicate clearly that the dose-response curve of this tumor is not linear. The differences in response to different doses of cortisol are,
in this study, only quantitative. A quantitative difference between different doses of whole adrenal extract has been reported by Brownell et al. (5), whereas Talalay et al. (26) found qualitative differences in the response of tumors to low and high doses of cortisone.

Although 1.5 mg cortisol/day did not reduce the body weight significantly more than did lower doses, such large quantities of steroid were decidedly toxic to the host. This may explain the poor response of the tumor to this dose. The animals treated with very large amounts of cortisol appeared to be in poor condition, and a number of them died before the end of the experiment. Hence, the treatment may have affected food intake. No quantitative measure of food intake was made during these experiments. The inhibitory effect on tumor growth of restricting the diet has been observed by Dobriner (8), by Ingle et al. (16), and also by Talalay et al. (26), who found that adrenalectomy inhibited tumor growth even in tube-fed rats, so that this effect cannot be entirely attributed to decreased food intake. Cortisol at high doses was also found to cause the deposition of a considerable amount of fat in the abdominal cavity, which might counter any weight loss caused by a decrease in food intake, although again no quantitative measurements were made of this phenomenon. Hence, it is possible that, in animals treated with large amounts of the hormone, an increase in fat production at the expense of body proteins, combined with a decreased intake of food, counteracts the direct enhancing effect of the hormone on these tumors. Whatever the cause of the differences in response to different quantities of cortisol, it is evident that the administration of such a hormone at only one or two dose levels is not sufficient to properly evaluate the action of the hormone on tumor growth.

**SUMMARY**

Various doses of cortisol acetate, from 0.1 mg. to 1.5 mg. daily, were administered to gonadectomized, adrenalectomized A/He CRGL × BALB/c CRGL F1 mice of both sexes bearing a transplanted fibrosarcoma. The changes in tumor areas which occurred before and during cortisol treatment have been reported. Final tumor weights were also reported but did not contribute meaningfully to the picture obtained.

It was observed that cortisol acetate, at all dose levels investigated, produced some enhancement of tumor growth. The maximum effect occurred at a dose of 0.5 mg/day, the degree of tumor enhancement being less at doses above or below 0.5 mg/day. These results cannot be explained on the basis of body weight changes of the cortisol-treated animals. It is evident that in evaluating the effects on tumor growth of a hormone such as cortisol, it is important to consider the dose-response relationships.

**ACKNOWLEDGMENTS**

We are indebted to Professors Morgan Harris and K. B. DeONE for their advice on this study. We also wish to thank Dr. John R. B. Beem of Merck, Sharp & Dohme, West Point, Pennsylvania, for generously supplying us with the cortisol (Hydrocortone) acetate used in this work.

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