The Effect of Corticosteroids and Altered Adrenal Function on Liver Regeneration following Chemical Necrosis and Partial Hepatectomy

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Summary

The inhibitory effect of cortisol on the mitotic rate of the proliferating liver of the mouse has been confirmed. When the initial liver damage had been caused by carbon tetrachloride, cortisol produced an inhibition with a linear log-dose response. Cortisol, corticosterone, and ACTH all produced inhibition of mitosis after partial hepatectomy. Various nonspecific procedures, such as starvation or slightly painful injections, also lowered the mitosis rate; this effect was abolished by adrenalectomy.

Introduction

It has been known since 1952 (25) that cortisone reduces the number of cells undergoing division in the liver following loss of parenchyma. This has subsequently been confirmed by histologic studies (7, 22) and by estimation of nucleic acid (9, 11) following partial hepatectomy, as well as by histology following carbon tetrachloride necrosis (15). Quantitative data on the mitotic response to different doses of steroids are not available, however, and the present work was undertaken to provide such information.

Materials and Methods

The experiments were conducted on adult albino male mice maintained on Oxoid breeding diet cubes and water ad lib. Partial hepatectomy was performed by the standard method (14). In the animals receiving carbon tetrachloride, 0.2 ml was given by s.c. injection. Cortisol (hydrocortisone) was injected s.c. in aqueous solution as the hemisuccinate. Corticosterone was administered s.c. as the free alcohol dissolved in propylene glycol, and ACTH as a solution in gelatin. The operations were carried out between 2:00 and 4:00 P.M., and the injections were given at 4:00 P.M. On the day of death 100 μg of colchicine was injected at 10:00 A.M., and the mice were killed at 4:00 P.M. In this way variations due to the diurnal rhythm of mitotic activity, which occurs in the normal (17) and regenerating (18) liver, were obviated.

The adrenalectomies were performed by the dorsal route. Immediately before operation 0.5 mg of cortisol hemisuccinate was injected s.c., and at the end of the operation a pellet of DOCA (1–2 mg) was implanted in a pocket between the skin and muscle. A week later the animals were very lively and were injected with carbon tetrachloride as described above.

The livers were fixed in Bouin's fluid and cut at 6 μm. To count the mitoses an Ehrlich eyepiece covering a field of 0.04 sq mm was used. All the resting and mitotic parenchymal nuclei in 50 fields from 2 blocks were counted. Regeneration was also expressed by the increase in weight of the remnant, according to formula: % regeneration = [(weight of liver at autopsy – calculated weight of remnant)/calculated weight of remnant] × 100. The weight of the remnant was calculated from the fact that in 25 control operations 65.8% of the liver was removed.

Results

Carbon Tetrachloride

In all the experiments of this group the mice were killed 72 hr after injection of 0.2 ml of carbon tetrachloride. With this dose, centrilobular hepatic necrosis is produced, the lesions being identical with those in the rat (6). A single dose of cortisol was injected 6 hr before death, i.e., at the same time as the injection of the colchicine. Groups of control mice were injected with carbon tetrachloride and colchicine at the same times as in the cortisol series. The doses of cortisol used were 0.1, 0.5, 2.5, and 12.5 mg; the results are summarized in Table 1. Increasing doses of cortisol caused a progressive fall in mitotic rate, the log dose-response being approximately linear. There was, however, considerable within-group variation, both in the control animals and in the ones receiving cortisol, as may be seen in the relatively large standard deviations. It must be emphasized that these counts were performed only on the liver parenchymal cells. Mitoses were also frequent in the Kupfer and sinus-lining cells, and although counts were not carried out on these, no obvious depression with cortisol was noted. Cortisol did not appear to cause any degeneration in the parenchymal cells such as has been reported in rats (13).

Partial Hepatectomy

The control series comprised hepatectomy alone and hepatectomy combined with injections of saline by the s.c. or i.p. route. The animals were killed 48 hr after the operation. Cortisol, corticosterone, or ACTH was given s.c. in 2 equal doses, one immediately after the operation, and the other 24 hr later. The total dose of cortisol was either 1 mg or 5 mg; the results are given in Table 2. It is apparent that both doses used caused a very significant fall in the mitotic rate. No dose-response effect was obtained, presumably because the smaller dose already caused a maximal response. As in the carbon tetrachloride series, considerable variation occurred within each of the groups.

Corticosterone, the principle corticosteroid secreted by the mouse adrenal, was injected into a 3rd series of mice (Table 2). Two injections of 0.5 mg dissolved in 0.25 ml of propylene glycol were given; as controls, 15 mice received 2 injections of 0.25 ml of propylene glycol only. The propylene glycol by itself caused...
a marked depression as compared with the uninjected or the saline controls. Some experiments to elucidate the cause of this will be described below.

ATCH in a total dose of 50 mU also produced a significant fall in the mitosis counts (Table 2).

The mitotic depression caused by the propylene glycol was rather unexpected. Two explanations may be advanced: (a) Propylene glycol may have a specific pharmacologic effect. As it is readily metabolized to pyruvic and acetic acids, this seemed unlikely, but the possibility could not be excluded. (b) The depression may represent a stress reaction to the pain of injection. In order to determine which explanation is correct, a series of 22 mice were subjected to partial hepatectomy and were injected with 0.3 ml of saline adjusted to pH 2.0 with hydrochloric acid immediately after operation and again 24 hr later; colchicine was given as before. It will be seen (Table 2) that a significant depression was produced.

Thus an obviously nonspecific, slightly painful injection produces a moderate fall in the mitosis count. It seemed probable that this reaction was mediated through the adrenals. To test this hypothesis, a series of mice were adrenalectomized and maintained on DOCA implants. Partial hepatectomy resulted in a very high mortality rate, and these attempts were therefore abandoned. Instead, 0.2 ml of carbon tetrachloride was injected s.c. 1 week after adrenalectomy. The mice were killed 72 hr later; the mitosis rate of 3.33% (Table 3) in the controls was lower than that of the normal mice receiving carbon tetrachloride (5.31%). On the other hand, 2 injections of acid saline 48 and 24 hr before death produced a value of 2.88%, which is not significantly different from the value for the adrenalectomized controls.

Thus adrenalectomy abolishes the depressant effect of the acid saline injections. Two mechanisms can be suggested whereby such injections might lower the mitotic rates in intact animals: (a) they cause the secretion of epinephrine, which has been shown to lower mitosis in the cornea (10) and skin (5); or (b) they cause the secretion of corticosterone. To investigate whether the 1st mechanism might be applicable, a further series of mice were injected with a total dose of 2 μg of epinephrine in 2 equal doses on the same time schedule as the steroids. A small fall was produced (Table 2), but this was not statistically significant.

As the various experimental procedures might involve a diminution of food intake, the effect of this factor was investigated. In an additional group of mice, food and water were allowed ad lib. up to the time of partial hepatectomy, but thereafter until the time of death 48 hr later, water only was allowed. The usual dose of colchicine was given 6 hr before death. As shown in Table 2, a highly significant fall in the mitosis count was found.

In all these experiments involving partial hepatectomy, the regeneration is also given on a wet weight basis in Table 2: these results will be discussed later.

**Discussion**

It is clear from the present results that cortisol does have a profound inhibitory effect on cell division in the liver. It seems almost certain that this is a specific effect. Injections of the same volume of saline produce only a small and nonsignificant fall in the mitosis count, so that nonspecific stress due to handling of the animals during the injection would not account for the low counts obtained in the cortisol group.

The principal corticosteroid secreted by the mouse adrenal is not cortisol or cortisone, but corticosterone (26). Strictly speak-

### TABLE 1

**Effect of Cortisol on Mitotic Rate in Mouse Liver following Injection of Carbon Tetrachloride**

<table>
<thead>
<tr>
<th>Dose of cortisol (mg)</th>
<th>No. of mice</th>
<th>% Mitosis (± S.E.)</th>
<th>Significance of difference from controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (controls)</td>
<td>100</td>
<td>5.31 ± 0.46</td>
<td>0.3 &lt; P &lt; 0.4</td>
</tr>
<tr>
<td>0.1</td>
<td>20</td>
<td>4.25 ± 1.00</td>
<td>0.02 &lt; P &lt; 0.05</td>
</tr>
<tr>
<td>0.5</td>
<td>20</td>
<td>3.96 ± 1.02</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>2.5</td>
<td>20</td>
<td>2.46 ± 0.64</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>12.5</td>
<td>20</td>
<td>1.34 ± 0.31</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

### TABLE 2

**Effect of Experimental Procedures on Regeneration of Mouse Liver following Partial Hepatectomy**

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>No. of mice</th>
<th>% Increase in wet weight (± S.E.)</th>
<th>% Mitosis (± S.E.)</th>
<th>Significance of difference of mitosis count from controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>36</td>
<td>72.5 ± 3.5</td>
<td>3.25 ± 0.50</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Saline</td>
<td>36</td>
<td>63.3 ± 3.8</td>
<td>3.06 ± 0.37</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Cortisol (1 mg)</td>
<td>20</td>
<td>64.9 ± 4.4</td>
<td>0.31 ± 0.11</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Cortisol (5 mg)</td>
<td>15</td>
<td>51.5 ± 5.2</td>
<td>0.72 ± 0.41</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>ACTH (0.05 IU)</td>
<td>16</td>
<td>63.3 ± 5.8</td>
<td>1.27 ± 0.27</td>
<td>P &lt; 0.005</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>15</td>
<td>66.5 ± 6.8</td>
<td>1.28 ± 0.47</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Corticosterone (1 mg)</td>
<td>15</td>
<td>79.7 ± 6.7</td>
<td>0.26 ± 0.13</td>
<td>0.025 &lt; P &lt; 0.05</td>
</tr>
<tr>
<td>in propylene glycol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid saline</td>
<td>22</td>
<td>56.6 ± 5.3</td>
<td>1.10 ± 0.25</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Epinephrine (2 μg)</td>
<td>16</td>
<td>79.2 ± 5.9</td>
<td>2.25 ± 0.74</td>
<td>P &gt; 0.20</td>
</tr>
<tr>
<td>Starvation</td>
<td>20</td>
<td>51.8 ± 6.3</td>
<td>0.72 ± 0.16</td>
<td>P &lt; 0.005</td>
</tr>
</tbody>
</table>

* Against saline controls.

* Against propylene glycol controls.
ing, therefore, cortisol is a pharmacologic rather than a physiologic substance in this species, and it was therefore decided to try the effects of corticosterone. The results were somewhat complicated by the fact that the vehicle employed, propylene glycol, itself causes significant depression of mitoses. Corticosterone did, however, have a significant depressant action by comparison with the propylene glycol controls. This conclusion is strengthened by the fact that ACTH also has a depressant effect.

Many of the previous workers have used very large doses of cortisone. In the present experiments the smallest dose used was 0.05 mg/day; on a simple weight basis this is roughly comparable to 100 ml of cortisol per day in man, so that even this dose is probably outside the physiologic range. As a dose of 0.1 mg of cortisol produced only a small depression of mitotic activity, it may perhaps be doubted whether control of mitotic rate in the liver is a physiologic function of endogenous corticosteroids, though such a role has been ascribed to these compounds (12).

On the other hand, under conditions of nonspecific stress, the adrenal does have an inhibitory effect. This is shown by the fact that rather painful injections of acid saline lower the mitosis rate in the intact but not in the adrenalectomized mouse. This effect is probably to be ascribed to the adrenal cortex rather than the medulla, as epinephrine caused only a nonsignificant fall in mitosis rate.

The finding that starvation for 48 hr lowers the mitotic rate confirms a previous finding (23), although Brues and Marble (3) had previously failed to observe this effect. Conversely, hyperphagia produced by hypothalamic lesions accelerates liver regeneration as assessed by the rate of DNA synthesis (19). Clearly, therefore, the possibility must be considered that injections or operative procedures may affect the rate of regeneration not by exercising a specific mechanism but simply by causing variations in food intake. The same considerations may also apply to other organs; for example, it has been shown that starvation lowers the mitosis rate in the skin (4, 21). In the present series, it seems unlikely that lowered food intake induced by the injections of cortisol was responsible for the fall in mitosis rate, as even larger doses of this compound do not affect feeding habits (2). It is possible, however, that the higher mitosis rate found in the mice injected with carbon tetrachloride as compared with the partially hepatectomized animals might be due to the greater anorexia produced by the operation than by the simple injection.

In the partially hepatectomized mice, although cortisol produced a marked lowering of the mitotic rate, the smaller dose was without significant effect on the increase in wet weight. The larger dose did retard the gain in weight of the liver remnant, and the results approached statistical significance (0.05 < P < 0.1). Previous workers (9, 22), employing even larger doses of cortisone, obtained a significant depression. The doses needed to produce marked inhibition of weight gain are well outside the maximum rate of endogenous secretion and the phenomenon thus seems to be pharmacologic rather than physiologic. This interpretation is supported by the fact that large doses of ACTH, as described above, failed to produce a significant depression in weight increase.

In one of the previous studies employing a moderate dose of cortisone (25), the proportion of glycogen and fat in the liver after partial hepatectomy was increased by cortisone, but the proportion of protein fell. Hence the net result was that the increase in wet weight was not affected by cortisone, but the protein gain was depressed. Thus measurement of wet weight is not a very satisfactory index of growth. In fact, no single index of regeneration is completely satisfactory, as the parameters may vary independently in the liver under different experimental conditions. For example, rats subjected to partial hepatectomy after treatment with lasiocarpine show normal DNA synthesis and normal wet weight increase but practically no mitoses (24); X-rays do not influence the wet weight increase but do inhibit the mitotic rate (20) and in this case the DNA synthesis is also depressed (1, 16).

In the case of the skin, it has been suggested that 4 hr is the maximum time for which a linear time-mitosis count can be obtained with the use of colchicine (O. H. Iversen, personal communication). For the liver, however, studies extending over 6 hr seem to be valid, as no instances of escape, as shown by normal anaphases or telophases, were seen in the present study. It has been claimed that in the skin (8) certain compounds produce a rise in the apparent mitosis count because they prolong the duration of mitosis rather than because they increase the rate of entry into mitosis. With colchicine, only the duration of prophase would matter from this point of view, and a relatively large variation in the duration would be needed to produce the large difference observed in the mitosis count. Under such circumstances, a marked increase in the number of nuclei in prophase would be found. In fact, however, such an increase was not found, and it may be concluded, therefore, that the variations recorded do reflect mainly the variations in the rate of entry into mitosis.

Acknowledgments

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References

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