Effect of Growth Hormone on Hepatic Carcinogenesis and Cirrhosis in AxC Rats Given N-2 Fluorenyldiacetamide

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SUMMARY

Inbred AxC strain male and female rats were fed 0.025% N-2-fluorenyldiacetamide in the diet. Growth hormone was administered to intact and castrated animals to determine its effect on carcinogenesis and cirrhosis of the liver. Growth hormone increased the incidence of hyperplastic and neoplastic lesions and cirrhosis in both castrated males and castrated females, but it did not exert any effect in the intact animals.

INTRODUCTION

Anabolic hormones are procarcinogenic and procirrhotic for the liver in male or female rats given N-2-fluorenyldiacetamide (3, 8, 9). Northandroline is the most potent, but testosterone is also very effective (3). Progesterone is less effective in promoting carcinogenesis but equally effective in promoting cirrhosis (9). The present experiment was carried out to see whether growth hormone exerted an anabolic or other effect on hepatic carcinogenesis and cirrhosis.

MATERIALS AND METHODS

Inbred AxC male and female rats 3 months old were used. Females weighed an average of 132 gm, males 185 gm. The experimental groups consisted of intact and castrated male and female animals, and intact and castrated male and female animals given growth hormone.1

In the castrated groups the gonads were removed 2 weeks before the experiment was started. The growth hormone was injected subcutaneously on the first day of the experiment. For the first 19 weeks it was given daily 5 days a week, and for the remainder of the experiment, 3 days a week. The dosage was 1 mg per 100 gm of body weight.

The carcinogen was added in the amount of 0.025% to Morris Diet #272 (3). The diet containing carcinogen was fed by modified pair feeding (based on cage consumption with 5 animals to a cage) for 4-week periods followed by 1 week on the basal diet until the carcinogen had been administered for 16 weeks. Following this period of feeding, the rats received Purina laboratory pellets. Animals were killed 38 weeks after the start of the experiment. Complete autopsies were done on animals dying during or at the end of the experiment. Tissues were fixed in 4% formaldehyde. Sections were stained routinely with hematoxylin and eosin. Pituitary glands were stained with periodic acid-Schiff orange G.

The changes in the livers were classified as (a) no hyperplasia, (b) hyperplasia, (c) hyperplastic nodules, (d) small hepatocellular carcinomas (5 mm or less in diameter), (e) well-developed hepatocellular carcinomas, (f) metastatic carcinoma, and (g) cirrhosis. These lesions have been described and illustrated in detail in previous publications (3, 10).

The results were analyzed statistically by a scoring method described in detail in an earlier paper (3). The method takes into account the hyperplastic lesions, the size and number of carcinomas, the presence of metastases, and the time required for the development of the lesions.

RESULTS

The numbers of animals with each type of lesion are given in the charts. The gross findings, the range of scores, and mean standard deviation are summarized in Table 1.

Animals not given exogenous hormones lost 26 to 30 gm during the first 4 weeks on the carcinogen-containing diet. Intact and castrated males and intact females given growth hormone lost 11 to 14 gm during the first 4 weeks. The animals regained the lost weight when they were returned to the basal diet during the fifth week and maintained or gained weight, except for minor fluctuations, until the end of the experiment. The castrated females given growth hormone gained weight throughout the experiment.

1Somatotrophic hormone, lyophilized (porcine), obtained from Calbiochem, Los Angeles, California, 90054. One mg is equal to 1 International Unit.

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One intact female and one castrated female given growth hormone had transitional cell carcinoma of the urinary bladder. There was focal squamous metaplasia over the surface of the carcinomas. In one intact male rat receiving growth hormone there was an astrocytoma of the brain.

**DISCUSSION**

Growth hormone was less effective than norethandrolone or testosterone in promoting hepatic carcinogenesis, but was equally effective in promoting cirrhosis. Its effects were more comparable to those observed previously with progesterone (9). Progesterone increased carcinomas and cirrhosis in intact male rats, but growth hormone did not. The increase was more remarkable in castrated males than in castrated females receiving progesterone.

The action of hormones in hepatic carcinogenesis and cirrhosis is related to their anabolic activity and more specifically to their effect on protein synthesis in the liver. The more effective hormones, norethandrolone and testosterone, are strongly anabolic (11). The hormones with less effect, progesterone and growth hormone, are weakly anabolic (2, 12). Testosterone and growth hormone increase the synthesis of protein in the liver (4, 6, 7). For growth hormone this is reflected by the incorporation of amino acid and stimulation of the synthesis of messenger ribonucleic acid (6, 7).

**Tumors of the Liver**

Growth hormone increased the incidence of hyperplastic and neoplastic lesions in castrated male rats ($P < 0.1$) and in castrated female rats ($P < 0.01$). There was no change in hepatic lesions in the intact males or females given growth hormone. All hepatic carcinomas, with one exception, were well differentiated histologically. One intact male had a poorly differentiated carcinoma that metastasized to the omentum.

**Cirrhosis of the Liver**

There was a marked increase in the incidence of cirrhosis in castrated males and castrated females receiving growth hormone, compared with castrated male and castrated female rats not given growth hormone.

**Other Organs**

Growth hormone given to castrated animals did not prevent the development of castration cells of the pituitary gland, atrophy of the prostate, seminal vesicles, or endometrium.
Growth Hormone and Hepatic Carcinogenesis

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of animals</th>
<th>Average body weight starting (gm)</th>
<th>Average body weight terminal (gm)</th>
<th>Average liver weight (gm)</th>
<th>Average no. of carcinomas per liver</th>
<th>Usual tumor diameter (cm)</th>
<th>Scores</th>
<th>Range</th>
<th>Mean</th>
<th>±</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact males</td>
<td>12</td>
<td>182</td>
<td>264</td>
<td>20.8</td>
<td>3-4</td>
<td>2.5</td>
<td>(19.3-350.1)</td>
<td>125.8</td>
<td>34.6</td>
<td></td>
<td></td>
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<tr>
<td>Intact males given growth hormone</td>
<td>11</td>
<td>185</td>
<td>262</td>
<td>12.5</td>
<td>3-4</td>
<td>1.0</td>
<td>(39.1-119.7)</td>
<td>64.5</td>
<td>7.5</td>
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<td></td>
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<tr>
<td>Castrated males</td>
<td>11</td>
<td>170</td>
<td>218</td>
<td>10.2</td>
<td>1</td>
<td>0.5</td>
<td>(9.6-31.8)</td>
<td>16.1</td>
<td>2.5</td>
<td></td>
<td></td>
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<tr>
<td>Castrated males given growth hormone</td>
<td>12</td>
<td>171</td>
<td>232</td>
<td>8.1</td>
<td>1</td>
<td>0.5</td>
<td>(10.7-36.7)</td>
<td>24.2</td>
<td>2.6</td>
<td></td>
<td></td>
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<tr>
<td>Intact females</td>
<td>11</td>
<td>140</td>
<td>185</td>
<td>6.9</td>
<td>1</td>
<td>0.5</td>
<td>(8.4-29.4)</td>
<td>11.7</td>
<td>1.9</td>
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<tr>
<td>Intact females given growth hormone</td>
<td>11</td>
<td>138</td>
<td>174</td>
<td>5.8</td>
<td>0</td>
<td>0</td>
<td>(9.1-15.7)</td>
<td>11.1</td>
<td>0.8</td>
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<tr>
<td>Castrated females</td>
<td>12</td>
<td>132</td>
<td>184</td>
<td>7.9</td>
<td>0</td>
<td>0</td>
<td>(3.2-15.3)</td>
<td>8.2</td>
<td>1.3</td>
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</tr>
<tr>
<td>Castrated females given growth hormone</td>
<td>11</td>
<td>133</td>
<td>194</td>
<td>6.8</td>
<td>1</td>
<td>0.5</td>
<td>(16.1-70.0)</td>
<td>25.9</td>
<td>4.7</td>
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<td></td>
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</table>

Summary of findings at autopsy and statistical data.

The ovary offered protection against growth hormone in this experiment. The ovary was also protective when norethandrolone, testosterone, or progesterone were given to females (3, 9). Progesterone and testosterone are known to be estrogen antagonists (1). It is concluded that growth hormone acts as an anabolic hormone in promoting hepatic carcinogenesis and cirrhosis in rats. Castrated animals are necessary to demonstrate this effect.

REFERENCES

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