Effect of 5-Fluorouracil on Noncancerous Tissue Growth*

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In the normal rat, exogenous preformed uracil is incorporated in vivo to an insignificant extent into liver nucleic acids. In contradistinction, a very much higher incorporation is observed in chemically (acetylaminofluorene) induced hepatoma, as well as in the "preneoplastic" liver of animals treated with this carcinogen (30). This utilization of exogenous uracil for the synthesis of nucleic acids was demonstrated in a variety of tumors (15), as well as in other rapidly growing tissues, the seminal vesicles, (d) growth hormone-induced growth of the epiphyseal cartilage.

MATERIALS AND METHODS

Fetal growth and survival.—Wistar rats (Barkbridge Farms, N.J.) maintained on Purina Laboratory Chow were used in all experiments. Females were mated in the evening when in heat, and vaginal smears were examined the next morning. The presence of spermatozoa was regarded as evidence of successful mating, and the day was recorded as day 1 of pregnancy. At different times (indicated in Table 1) in the course of pregnancy the animals were given injections intraperitoneally of fluorouracil on 3 successive days. Two dosage levels were employed: 25 mg/kg/day, and 12.5 mg/kg/day. In animals in which treatment was started prior to day 12 of pregnancy, the uterus was inspected by exploratory laparotomy both in the rats to be injected and in the pregnant controls. Treated animals and untreated pregnant controls were killed on the 20th day of pregnancy. At autopsy the following data were obtained: body weight of normal controls: 2.37 ± 0.05.

Based on these observations, a number of uracil analogs have been studied with regard to their inhibitory effect on tumor growth. The tumoristatic activity of 5-fluorouracil has been investigated extensively (14). The present paper deals with the influence of 5-fluorouracil on certain normal growth processes: (a) growth and survival of the fetus, (b) regeneration of the liver following partial hepatectomy, (c) testosterone-induced growth of such as intestinal mucosa (15), regenerating liver (6), and hormone-stimulated liver (5).

**Table 1**

<table>
<thead>
<tr>
<th>Days on FU</th>
<th>Dose, 25 mg/kg/day</th>
<th>Dose, 12.5 mg/kg/day</th>
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<tr>
<td></td>
<td>Surviving fet.</td>
<td>Mean fetal wt. (gm.)</td>
</tr>
<tr>
<td>6-8</td>
<td>0, 0</td>
<td>1.68, 1.92</td>
</tr>
<tr>
<td>8-10</td>
<td>0</td>
<td>2.01, 2.33</td>
</tr>
<tr>
<td>11-13</td>
<td>0/9, 7/10, 6/10</td>
<td>1.57, 1.68</td>
</tr>
<tr>
<td>12-14</td>
<td>10/10, 10/10, 0/11, 0/11</td>
<td>2.21, 2.03</td>
</tr>
<tr>
<td>13-15</td>
<td>9/9, 9/9</td>
<td>2.21, 2.03</td>
</tr>
<tr>
<td>17-19</td>
<td>9/10, 9/10</td>
<td>2.21, 2.03</td>
</tr>
</tbody>
</table>

FU: fluorouracil.
Mean fetal weight: each figure represents the mean weight of survivors of one litter (20th day of pregnancy).
Mean fetal weight of normal controls: 2.37 ± 0.05.

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1 Kindly supplied by Hoffmann-LaRoche, Inc., Nutley, N.J.
weight, weight of uterus containing the products of gestation, the weight of placentas, number and weight of live fetuses, number of dead fetuses and of resorption sites. The reasons for obtaining these data on the 20th day of pregnancy rather than at term were outlined in a previous publication (28). A number of litters were fixed in 10 per cent formalin, the skeleton was stained with alizarin and cleared by a modification of the technic of Dawson (9).

Liver regeneration.—Male Wistar rats (Barkbridge Farms, N.J.) were subjected to partial hepatectomy by the method of Higgins and Anderson, by which 67 per cent of the liver is removed (17). One-half of the animals in each experiment received fluorouracil by intraperitoneal injection daily for 4 days, the other half serving as controls. All were fed Purina Laboratory Chow, ad libitum, or by paired feeding. In some experiments, tube-feeding was employed; animals were fed twice daily, a medium-carbohydrate diet (18); in order to avoid feeding shock, tube-feeding was started about 2 weeks before operation, the daily volume of food being gradually increased. All animals were killed 96 hours after partial hepatectomy. Body weight and liver weight were recorded. In several experiments, aliquots of the liver were dried in an oven at 100°C. to constant weight, and the dry weight was obtained. Aliquots of the liver were used for N determination by the micro-Kjeldahl procedure. Liver samples were fixed in Bouin solution, imbedded in paraffin, and sectioned. After staining with hematoxylin-eosin, mitosis counts were made, as described above, and the mitotic index was calculated.

Growth hormone-induced growth of epiphyseal cartilage.—Female rats of the Sprague-Dawley strain, hypophysectomized on the 26th day of life, were purchased from Hormone Assay Laboratories, Chicago, Ill. Experiments were started 2 weeks following hypophysectomy. One group received 50 μg. of growth hormone2 daily by subcutaneous injection for 4 days (growth hormone controls). A second group received, in addition to the same growth hormone treatment, fluorouracil, 25 mg/kg/day by intraperitoneal injection for 5 days, beginning on the day preceding growth hormone administration. A third group received no treatment (controls). All animals were fed stock diet (Purina Laboratory Chow), with added milk, oranges, and carrots, ad libitum. Drinking fluid was 5 per cent glucose in 0.85 per cent NaCl.

In a second experiment, one group received growth hormone and fluorouracil, as described above. In this experiment the animals were paired on October 22, 1957, and were fed an 18 per cent protein diet (20). They also were given the glucose-saline drinking fluid as above.

On the day following the last injection of growth hormone, all animals were killed by exsanguination under ether anesthesia. The tibiae were prepared with silver nitrate for measurement of the width of the proximal epiphysis according to the procedure of Geschwind (12).

RESULTS AND DISCUSSION

Effect of fluorouracil on pregnancy.—Data are summarized in Table 1. When 5 mg. of fluorouracil (21–25 mg/kg) was administered on 3 successive days early in pregnancy (days 6–8, or 8–10), no fetuses survived. In one instance, resorption sites were still recognizable at autopsy on the 20th day of pregnancy; in the other two the uteri were involuted, and no trace of the products of gestation could be seen. With the same type of treatment later in pregnancy a varying number of fetuses survived; in some instances there was no fetal death. The mean weight of surviving fetuses was markedly impaired. Treatment on days 17–19 of pregnancy did not influence viability.

Male Sprague-Dawley rats (Barkbridge Farms, N.J.) were castrated through an abdominal incision. Three weeks later the animals were divided into three groups. One group received testosterone propionate2 in oil subcutaneously, 1 mg. daily for 3 days. The second group received the same treatment with testosterone, and in addition received four daily intraperitoneal injections of fluorouracil, 25 mg/kg, the first injection being given on the day preceding the first testosterone injection. The third group served as controls. On the day following the last treatment, all animals in the three groups received a subcutaneous injection of colchicine, 0.1 mg/100 gm; 8 hours later they were killed by exsanguination under ether anesthesia. The seminal vesicles were weighed, and an aliquot was dried to constant weight in an oven at 100°C. for dry weight measurement. A sample of the seminal vesicle was fixed in Bouin solution, imbedded in paraffin, and sectioned. After staining with hematoxylin-eosin, mitosis counts were made, as described above, and the mitotic index was calculated.

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2 Growth hormone (beef) was obtained from the Endocrinology Study Section of the National Institute of Health.
gestation had little if any influence on the fetuses; possibly larger doses at this stage of pregnancy might have proved damaging to the fetus. A dose of fluorouracil of 2.5 mg. (10–12 mg/kg) given on 3 successive days caused neither fetal death nor impaired growth of the fetuses. Examination of the skeleton in a number of surviving fetuses revealed frequent retardation of osseous development. Teratologic aspects were not within the purview of this investigation, but osseous malformations were seen in a few embryos; no search for visceral and nervous system abnormalities was made.

Similar observations on the effect of fluorouracil on the chick and rat fetus have been reported by Karnofsky et al. (22). The influence of a number of other antimetabolites on fetal survival and fetal development has been studied, including 6-mercaptopurine and other purine analogs, and azaserine (27). This subject has been reviewed recently (21). In addition, other agents used in cancer chemotherapy, such as roentgen rays (16) and nitrogen mustard (5–6), this may probably be explained in part by the fact that the growing embryo has metabolic characteristics different, quantitatively or qualitatively, from those of the adult organism. Certain of these characteristics the embryo shares with other rapidly growing tissues, as is demonstrated by the observations reported here. The antimetabolites studied in this respect (antifolic, 6-mercaptopurine, azaserine) interfere in some way with synthesis of purines or pyrimidines (27), and this would appear to be a possible common denominator for their actions on the fetus. In the case of fluorouracil, the mechanism of action has been investigated in detail by Heidelberger et al. (2, 7, 8), who have shown that fluorouracil is incorporated in the RNA, forming a “fraudulent” RNA, and also inhibits DNA synthesis. This effect of fluorouracil upon the growth of a tissue probably depends upon the capacity of that tissue for utilization of preformed uracil for nucleic acid synthesis. It is interesting in this connection that uracil-2-C14 is actively incorporated into the RNA of rat embryos following injection of the labeled uracil into the mother (unpublished observations).

The fact that in many pregnancies some fetuses died, whereas others survived, indicates differences either in sensitivity or in the amount of the antimetabolite taken up and incorporated. Similar observations have been made with the use of other compounds (27).

Liver regeneration: effect of 5-fluorouracil (Table 2).—In two experiments feeding was ad libitum. Fluorouracil was injected on 5 successive days, starting the day before hepatectomy; in one experiment the daily dose was 25 mg/kg, in the other 12.5 mg. Both groups lost much weight; the mean weight loss was 35.6 gm. and 29.2 gm., respectively, in sharp contrast to the partially hepatectomized controls (mean, −6.9 gm. and +1.6 gm.). Liver regeneration was markedly impaired in the fluorouracil-treated animals in both experiments. In view of the fact that liver regeneration, calculated on an actual weight basis, is diminished markedly in starvation (3, 29), these results are inconclusive.

Therefore, paired feeding was employed in eleven experiments. The following dosage levels of fluorouracil were used: 25 mg/kg in eight experiments, 12.5 mg/kg in one, and 6.25 mg/kg in three. The reduced food intake caused weight loss and diminished liver regeneration in the controls, with the exception of those pair-fed to animals receiving only 6.2 mg/kg. Of the eight groups treated with 25 mg/kg, two showed significant impairment of liver regeneration. No impairment was observed in

TABLE 2
Influence of Fluorouracil on Liver Regeneration

<table>
<thead>
<tr>
<th>Duration of regeneration</th>
<th>FU mg/kg/day×days of treatment</th>
<th>Feeding</th>
<th>Incidence of inhibition</th>
<th>Difference and &quot;P&quot; values†</th>
</tr>
</thead>
<tbody>
<tr>
<td>90°</td>
<td>12.5 ×4</td>
<td>ad lib.</td>
<td>1/1</td>
<td>15.6</td>
</tr>
<tr>
<td>&quot;</td>
<td>25 ×4</td>
<td>&quot;</td>
<td>1/1</td>
<td>16.0</td>
</tr>
<tr>
<td>&quot;</td>
<td>6.25 ×4</td>
<td>&quot;</td>
<td>0/1</td>
<td>9.5, &lt;0.001</td>
</tr>
<tr>
<td>&quot;</td>
<td>12.5 ×4</td>
<td>&quot;</td>
<td>0/1</td>
<td>16.5, &lt;0.01</td>
</tr>
<tr>
<td>&quot;</td>
<td>25 ×4</td>
<td>&quot;</td>
<td>2/6</td>
<td>22.1, &lt;0.05</td>
</tr>
<tr>
<td>&quot;</td>
<td>25 ×4</td>
<td>Force-fed</td>
<td>2/2</td>
<td>23.1, &lt;0.05</td>
</tr>
<tr>
<td>48°</td>
<td>25 ×2</td>
<td>Pair-fed</td>
<td>0/2</td>
<td></td>
</tr>
<tr>
<td>&quot;</td>
<td>6.25 ×2</td>
<td>&quot;</td>
<td>0/1</td>
<td></td>
</tr>
</tbody>
</table>

* The fraction indicates in how many out of the total experiments liver regeneration was significantly inhibited.

† The difference between per cent regeneration of controls and of fluorouracil-treated animals is given for those experiments showing inhibition. "P" is calculated by Fisher's "Student" method.

For the mother (see text under "Material and Methods") and the animals. The per cent liver regeneration was calculated for each means for controls and experimentals compared for each experiment.

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the three groups receiving the smaller amounts. These data also afford some indication of the toxicity of fluorouracil. The greater weight loss of fluorouracil-treated hepatectomized animals, as compared with hepatectomized controls, is associated with a greater reduction of voluntary food intake. Whereas in a group of partially hepatectomized animals (not charted) the mean daily food intake during 4 days after hepatectomy was 13.2 gm. (64 per cent of that preceding operation), fluorouracil-treated hepatectomized rats (25 mg/kg, for 4 days) consumed only 4.4 gm/day; intact rats treated with fluorouracil (25 mg/kg) for 4 further supported by an unpublished observation that the weight loss in a group of fluorouracil-treated, completely starved rats did not differ from that of similarly starved controls. Whether this is true only for the relatively brief period of treatment (4 days) cannot be stated at present.

Force-feeding was employed in two experiments, the controls and fluorouracil-treated animals receiving the same amount of food. The controls gained very little weight (mean weight gain in 5 days, 4.3 and 0.8 gm.) and the fluorouracil-treated animals lost very little (mean weight loss in 5 days, 5.9 and 5.0 gm.). In both experiments,

<table>
<thead>
<tr>
<th>TABLE 3</th>
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<tr>
<td><strong>INFLUENCE OF FLUOROURACIL ON WATER AND N CONTENT AND MITOTIC INDEX OF REGENERATING LIVER</strong></td>
</tr>
<tr>
<td>In each experiment there were ten control and ten treated rats. All figures are means for each group. Liver regeneration calculated as indicated in Table 3.</td>
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<tr>
<td></td>
</tr>
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<td>1</td>
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<td>2</td>
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<tr>
<td>3</td>
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</table>
* All differences between control and FU values are significant, P < 0.001.  
† C, hepatectomized controls.  
‡ FU, fluorouracil-treated, hepatectomized rats.

days consumed 8.4 gm/day (46 per cent of their pretreatment intake). Whereas the untreated hepatectomized rat reduces its food intake greatly on the day of operation and concomitantly loses weight, it regains its appetite within the next 24 hours, progressively increases its food intake, and regains weight. The fluorouracil-treated hepatectomized rat, on the contrary, shows progressive anorexia and weight loss, an exaggeration of the events induced in nonhepatectomized, fluorouracil-treated rats.

The fact that force-feeding almost completely abolishes the weight loss in fluorouracil-treated hepatectomized rats further supports the interpretation that the great weight loss induced by this antimetabolite in hepatectomized rats is due chiefly to anorexia, presumably of central nervous origin, rather than to specific toxic effects. This is liver regeneration was markedly and significantly impaired in the animals treated with fluorouracil (25 mg/kg).

Data on water content and N content of the regenerated livers were obtained in three experiments, two with paired-feeding and one with force-feeding (Table 3). In the two experiments in which liver regeneration was significantly impaired (Exp. 3, force-feeding, and Exp. 1, pair-feeding), the water content of the regenerated liver did not differ from that of the respective controls. The N content of the regenerated liver was identical with that of the controls in one (No. 3) and diminished in the other (No. 1). In one exp. (No. 2, pair-feeding) in which there was no significant inhibition of liver regeneration, the water content of the regenerated liver was higher in the fluorouracil-treated animals than in the controls, and there was no dif-
ference in N content (in per cent of wet weight). It would appear that in the well fed (force-fed) animals fluorouracil causes impairment of regenerative growth of liver without changing the composition of the regenerated tissue with respect to nitrogen and water content. It will be of interest to study the chemical composition of these regenerating livers more completely, especially since our data suggest that fluorouracil, in the presence of starvation, may alter the composition of the regenerated tissue.

Mitotic counts were obtained in the regenerated livers in two experiments, one in force-fed, the other in pair-fed animals (Table 3). In both experiments the mitosis in liver cells and Kupffer cells was markedly depressed in the fluorouracil-treated animals as compared with the respective controls. It is of interest that this fluorouracil-induced depression of mitosis was associated with inhibition of liver regeneration on a weight basis in the force-fed animals, whereas the pair-fed group showed no significant inhibition on this basis. The mitotic ratios were much lower in the pair-fed controls than in the force-fed controls; the former were partially starved, having lost 19 gm. in weight, whereas the force-fed controls maintained their weight.

Diminished mitotic activity in starvation has been reported for various tissues (4, 18) and may occur generally. We were concerned with this problem primarily because we wished to divorce the starvation effect from a specific effect of fluorouracil. This was achieved, as discussed above, by pair-feeding the controls down to the level of the fluorouracil-treated animals and by force-feeding the latter up to the normal (control) level. It was found that mitotic activity was much lower in the pair-fed (semi-starved) hepatectomized controls than in the force-fed hepatectomized controls. In view of the available information quoted above, reduction of mitotic activity in the regenerating liver of semi-starved rats would not be remarkable were it not for the observations of Brues et al. (3) and of Perez-Tamayo et al. (29) that the number of regenerated cells in the liver is identical in partially starved and in fully fed rats. If one accepts the indirect methods of determining total cell number as reliable, this discrepancy poses a nice problem. Purely hypothetically, it is conceivable that in starvation one type of liver cell would not go into mitosis, whereas another might have a briefer period of intermitotic "rest." However, in one study of chronic protein starvation, the "resting" stage of Walker tumor cells was considerably prolonged (34).

Several investigators have reported effects of carcinostatic agents on liver regeneration (10, 33), but their influence on food intake and body weight has not always been taken into consideration. A specific effect on liver regeneration may be simulated if the animals treated with a drug are partially starved. According to Brues et al. (3), regeneration is impaired in starvation, whereas Perez-Tamayo et al. (29) state that starvation has no influence on liver regeneration. However, in a comparison of liver regeneration in drug-treated and control animals, the possible error arising from drug-induced starvation would be present regardless of whether the starvation had a specific effect on regeneration or merely simulated such an effect. If the drug induces appreciable reduction of food intake and appreciable loss of body weight, it would appear important to study the effect of the drug in optimally fed (force-fed), animals, rather than in semi-starved, pair-fed controls, because in the latter case a complicating metabolic situation is introduced.

*Testosterone-induced growth of seminal vesicles (Table 4).*—Four weeks after castration the seminal vesicles were atrophic, and the microscopic sections showed absence of mitoses. Treatment with testosterone propionate, 1 mg/day for 3 days, induced intensive mitotic activity (mitotic index 19.9); simultaneous administration of fluorouracil, 25 mg/kg/day for 4 days, starting 1 day before testosterone treatment was begun, greatly reduced this effect of the hormone (mitotic index, 3.94). In the two following experiments, seminal vesicle weights were obtained in castrate rats treated in the same manner, except that no colchicine was administered. In both experiments the weight (both wet and dry) of the seminal vesicles was significantly increased by administration of testosterone propionate.

The weight increase was significantly smaller in the groups which received fluorouracil concomitantly with the hormone. The data refer to tissue weight, since the secreted contents of the seminal vesicles had been carefully blotted off. Whereas testosterone stimulation of both mitotic activity and over-all size (weight) of the seminal vesicle was significantly inhibited by fluorouracil, the inhibition of mitosis was much greater (80 per cent) than that of weight (45 and 41 per cent wet weight; 47 and 43 per cent dry weight). The gain in weight of the seminal vesicles, induced by testosterone, was associated with a higher water content of the tissue (decrease of dry weight in per cent of wet weight). Despite the fact that testosterone-induced growth of the seminal vesicles
was greatly reduced by administration of fluorouracil, the hormone-induced hydration of the tissue was not diminished.

**Influence on growth hormone effect (Table 5).**—The effect of growth hormone on the epiphyseal width in hypophysectomized rats was significantly impaired by administration of fluorouracil. In the first experiments (not tabulated) the antimetabolite was administered on 5 successive days, starting on the day preceding growth hormone treatment. Feeding was ad libitum. Fluorouracil (25 mg/kg/histoechnical techniques may permit better analysis of this phenomenon of growth inhibition. We may at present assume that it will involve at least inhibition of mitosis.

**GENERAL COMMENTS**

The data reported here indicate that 5-fluorouracil, a uracil analog capable of inhibiting various forms of malignant growth, also inhibits liver regeneration, fetal growth and survival, testosterone-stimulated growth of the seminal vesicle in the

### TABLE 4

**Influence of Fluorouracil on Testosterone-induced Growth of the Castrate's Seminal Vesicle**

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Treatment*</th>
<th>No. Animals</th>
<th>Body wt. change (gm.)</th>
<th>Seminal vesicle</th>
<th>Mitotic index ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Castrate controls</td>
<td>13</td>
<td>+6</td>
<td>30.6 6.7</td>
<td>78 ± 2.26</td>
</tr>
<tr>
<td></td>
<td>Testosterone propionate</td>
<td>13</td>
<td>+3</td>
<td>150.2 21.2</td>
<td>86 ± 0.29</td>
</tr>
<tr>
<td></td>
<td>Testosterone propionate</td>
<td>13</td>
<td>-12</td>
<td>69.0 10.0</td>
<td>85 ± 1.24 P &lt; 0.001†</td>
</tr>
<tr>
<td>2</td>
<td>Castrate controls</td>
<td>10</td>
<td>+6</td>
<td>74.1 17.2</td>
<td>76.9 ± 0.64</td>
</tr>
<tr>
<td></td>
<td>Testosterone propionate</td>
<td>11</td>
<td>+10</td>
<td>227.1 44.2</td>
<td>80.7 ± 0.3 P &lt; 0.001†</td>
</tr>
<tr>
<td></td>
<td>Testosterone propionate</td>
<td>11</td>
<td>-4</td>
<td>134.4 24.7</td>
<td>81.2 ± 0.3</td>
</tr>
<tr>
<td>3</td>
<td>Castrate controls</td>
<td>2</td>
<td>+9</td>
<td>0.00</td>
<td>19.9 ± 4.5</td>
</tr>
<tr>
<td></td>
<td>Testosterone propionate</td>
<td>5</td>
<td>+14</td>
<td>3.9 ± 1.1 P &lt; 0.02‡</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fluorouracil</td>
<td>4</td>
<td>-1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*See text for doses.
† "P" value (by Fisher's "Student" method) of difference against castrate controls.
‡ "P" value (by Fisher's "Student" method) of difference against testosterone propionate.

In the second experiment a semi-synthetic diet was fed, and the growth hormone-treated controls were pair-fed to the fluorouracil-treated animals. Fluorouracil (25 mg/kg) was given only on the 1st and 2d days of hormone therapy. There were five survivors (of ten) in the fluorouracil-treated group; the pair-fed partner of each was included in the control group. Despite the fact that the mean weight-loss was slightly greater in the control (2.4 gm.) than in fluorouracil-treated group (0.66 gm.), the latter showed a significantly lesser response to growth hormone. In these experiments, a technic (silver nitrate impregnation [12]) was employed which permits only rapid measurement of the width of the epiphyseal line. Standard histological and

### TABLE 5

**Influence of Fluorouracil (25 mg/kg) on Growth Hormone-induced Widening of Proximal Tibial Epiphysis of Female Hypophysectomized Rats**

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Treatment</th>
<th>No. Animals</th>
<th>Feeding</th>
<th>Epiph. width (µ)</th>
<th>p</th>
<th>Body wt. change (gm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control hypophysectomized</td>
<td>7</td>
<td>Ad libitum</td>
<td>145</td>
<td>‾</td>
<td>-2.4</td>
</tr>
<tr>
<td></td>
<td>Growth H</td>
<td>7</td>
<td>&quot; &quot;</td>
<td>272</td>
<td>&lt;0.02</td>
<td>+3.0</td>
</tr>
<tr>
<td></td>
<td>Growth H + FU</td>
<td>3</td>
<td>&quot; &quot;</td>
<td>147</td>
<td>-8.3</td>
<td>‾</td>
</tr>
<tr>
<td>II</td>
<td>Growth H</td>
<td>5</td>
<td>Pair-fed</td>
<td>207</td>
<td>&lt;0.05</td>
<td>-2.4</td>
</tr>
<tr>
<td></td>
<td>Growth H + FU</td>
<td>5</td>
<td>&quot; &quot;</td>
<td>156.7</td>
<td>-0.7</td>
<td>‾</td>
</tr>
</tbody>
</table>

Dosage schedule: see text. FU: fluorouracil. "P" of difference between growth hormone-treated and growth hormone plus fluorouracil-treated rats (Rank Test, Kruskall, and Wald).
castrate rat, and growth hormone-induced growth of the epiphyseal cartilage. In addition to increase in mitotic activity and dry weight, many rapidly growing tissues exhibit an increased water content. This is known to occur in response to growth hormone (24) and has been described as an early response of thyroid tissue upon incubation with thyrotropic hormone (1). We have observed an increase in water content of the adrenal glands and thyroid after treatment with ACTH and TSH, respectively.¹

Of particular interest are the observations which suggest that different phases of the growth process in the tissues studied, i.e., increase in mitotic activity, in dry weight, and in water content, may be influenced to different degrees by fluorouracil. In the case of testosterone stimulation of the castrate seminal vesicle, there was maximal inhibition of mitotic activity, less striking suppression of increase in dry weight, and no significant interference with the androgen-induced increment in water content. The cytologic changes induced by testosterone (25) appeared not to be influenced by fluorouracil. Similarly, the water content of regenerating liver in fluorouracil-treated rats was identical with that of controls, whereas mitotic activity was decreased by 90 per cent and increment in tissue mass by 32 per cent.

Dissociation of various components of the growth process has been observed under other experimental conditions. Brues et al. found a marked increase in the number of cells but not in tissue mass in regenerating liver in starved animals (3). Similar but statistically not significant observations were reported by Perez-Tamayo et al. (29). There is little available information concerning growth inhibition by antimetabolites. Gelfant et al. (11) have investigated the effect of aminopterin and nitrogen mustard on estrogen-induced growth of the uterus; mitotic activity was markedly reduced, whereas the increase in tissue mass was only moderately depressed. It would appear, therefore, in the light of our observations, that despite marked suppression of mitotic activity, probably through inhibition of DNA synthesis, cytoplasmic growth can still proceed, as evidenced by increase, although subnormal, in tissue mass and normal increase in hydration of the stimulated tissue.

There is now rather precise information as to the effects of 5-fluorouracil upon nucleic acid metabolism. It has been shown (a) to be itself incorporated into RNA, forming a "fraudulent" nucleotide, (b) to inhibit incorporation of orotic acid and of uracil into RNA, and (c) to interfere with DNA synthesis by inhibiting formation of thymidylic acid (2). Except for the relation of DNA to mitosis, it is impossible at the present time to do more than speculate as to the relationship, if any, between these metabolic effects of 5-fluorouracil and the observed differences in its effects on various components of the phenomenon of growth. However, the fact that these components can be dissociated under the influence of this anti-metabolite may provide an opportunity for further analysis of this complex process.

SUMMARY

The influence of 5-fluorouracil on nonmalignant tissue growth was studied, including fetal growth, liver regeneration, testosterone-induced growth of the seminal vesicle, and growth hormone-induced growth of the epiphyseal cartilage. All these processes were inhibited by 5-fluorouracil. Analysis of the growth inhibition in regenerating liver and in the seminal vesicle stimulated with testosterone revealed that tissue hydration, incident to growth, was not influenced by the antimetabolite. Furthermore, influence of 5-fluorouracil on mitotic rate was greater than that on increase of tissue mass. Such observations may contribute to a better understanding of certain mechanisms involved in the phenomena of growth.

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REFERENCES


¹ P. Smith, K. E. Paschakis, and A. Cantarow, unpublished observations.


20. ———. Effect of Diet in Rats on Adrenal Weights and on Survival Following Adrenalectomy. Ibid., 32: 410-14, 1943.


Effect of 5-Fluorouracil on Noncancerous Tissue Growth

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