EFFECT OF AMINOPTERIN ADMINISTRATION ON THE METABOLISM OF LIVER AND SMALL INTESTINE OF GUINEA PIGS AND RATS*

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Small amounts of Aminopterin, one of the antifolic metabolites, produce gastrointestinal effects such as nausea, vomiting, melena, and diarrhea in human beings (1, 2) and destruction of the epithelial elements in experimental animals (3, 4). A single injection of 240 γ of Aminopterin per kilo of body weight had no morphological effect when administered to young adult rats, but caused a marked reduction in oxygen uptake by gastrointestinal mucosa within 24 hours, followed by degeneration of all epithelial elements and subsequent massive hemorrhage into the gastrointestinal tract. Structural changes were always preceded by a decrease in oxygen uptake by intestinal mucosa (4). The guinea pig and the rabbit (5, 6) are more resistant to Aminopterin toxicity than is the rat. To investigate further the effect of Aminopterin on different animal species, the following comparative studies of the effect of Aminopterin on guinea pigs and rats were undertaken.

EXPERIMENTAL

Young female guinea pigs weighing approximately 250 to 300 gm. were fed ad libitum a commercial alfalfa diet to which was added 5 mg. per cent of ascorbic acid. They were injected subcutaneously with 240 γ of Aminopterin (lot No. 7-7717)† per kilo of body weight daily for 4 days. Young adult female rats weighing approximately 200 gm. were treated identically, but were maintained on Purina laboratory chow ad libitum. This dose of Aminopterin was chosen since it had been shown to produce severe

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diarrhea and melena in rats within a 4 day period (4). At the end of 4
days, six treated guinea pigs and six rats, as well as ten control untreated
animals of each species, were sacrificed. The liver sections and the first
6 to 8 inches of small intestine were removed, were washed clean with
saline, blotted, weighed, and prepared for folic and folinic acid analyses.
The tissues were homogenized in 0.04 M cysteine solution, pH 7. Liver
samples were diluted 1:10 and the gut samples 1:5. The homogenates
were autoclaved for 5 minutes at 15 pounds pressure, cooled, centrifuged,
and the centrifugates placed in the deep freeze until analyzed. The sam-
pies were analyzed microbiologically with Lactobacillus casei and Leuconos-
toc citrovorum as test organisms. The media used were those described by
Henderson and Snell (7) with slight modifications. NaCl was omitted
from the media, and in the folinic acid assay 10 mg. of CaCl₂·2H₂O were
added to each 100 ml. of media.

Another group of twelve guinea pigs of the same weight was given 240
γ of Aminopterin per kilo of body weight daily for 40 days. At the end of
this period, no gross deficiency signs appeared; i.e., loss of weight, ruffled
fur, or diarrhea. Four animals were selected at random and sacrificed for
metabolic studies. The first 6 to 8 inches of the small intestine were re-
moved, opened lengthwise, and washed with saline. The mucosal tips of
the villi were scraped off with a Stadie blade, and oxygen uptake was meas-
ured as previously described (4). Glucose was used as a substrate. The
remaining portion of the tract was also opened and examined for gross
lesions and promptly fixed in formalin. The formalin-fixed tissues were
imbedded in paraffin by the picric acid-dioxane method, cut in a routine
manner, and stained by Mallory's method with aqueous alum hematoxylin
and phloxine B (8). The oxygen uptake by mucosal tissue from six con-
trol untreated guinea pigs was also measured.

Guinea pigs and rats were also given a tracer dose of P³² on a weight
basis and sacrificed after 2, 4, and 8 hours. The various segments of the
gastrointestinal tract were laid open and the mucosa from the duodenum,
jejunum, and ileum scraped off and homogenized in water. The pro-
tein was precipitated with 10 per cent trichloroacetic acid, washed with
alcohol and ether, and dried. The phosphoprotein was then assayed for
activity, and the results are expressed as counts per minute per gm. of
protein.

Results

Table I presents the QO₂ (microliters of O₂ per hour per mg. of tissue,
dry weight) of intestinal mucosal tissue obtained from Aminopterin-treated
guinea pigs and from control untreated animals. The QO₂ of intestinal

* Hazard, J. B., unpublished data.
mucosa from treated animals was 14.2, while that from control guinea pigs was slightly less, approximately 13.

The \( QO_2 \) values for guinea pig intestinal mucosa were identical to those previously observed for normal rats (4).

**TABLE I**

*Respiration of Intestinal Mucosa from Aminopterin-Treated and Control Guinea Pigs*

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>No. of ( QO_2 ) values*</th>
<th>Average ( QO_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>35</td>
<td>12.6 ( \pm ) 0.8†</td>
</tr>
<tr>
<td>Treated†</td>
<td>4</td>
<td>28</td>
<td>14.2 ( \pm ) 0.9</td>
</tr>
</tbody>
</table>

* Total number of slices of intestinal mucosa taken from total number of animals.
† Mean \( \pm \) the standard error.
‡ Animals injected subcutaneously with daily doses of 240 \( \gamma \) of Aminopterin per kilo of body weight per day for 40 days.

**TABLE II**

*Folic and Folinic Acid Concentrations of Liver and Small Intestine in Control and Aminopterin-Treated Guinea Pigs and Rats*

The values are given in micromicrograms per gm. The numbers in parentheses represent the number of animals in each group.

<table>
<thead>
<tr>
<th></th>
<th>Folic acid</th>
<th>Folinic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Small intestine</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guinea pig (10)</td>
<td>1531 ( \pm ) 390*</td>
<td>48 ( \pm ) 9</td>
</tr>
<tr>
<td>Rat (10)</td>
<td>3051 ( \pm ) 220</td>
<td>384 ( \pm ) 59</td>
</tr>
</tbody>
</table>

Aminopterin-treated

|                  |            |               |            |                |
| Guinea pig (6)   | 190 \( \pm \) 64 | 891 \( \pm \) 156 | 6 \( \pm \) 1 | 38 \( \pm \) 11 |

* Mean \( \pm \) one standard error.

In Table II are the concentrations of folic and folinic acids in the first portion of the small intestine and in the liver of control and treated guinea pigs and rats. In the untreated animals, the rats had significantly higher concentrations of folic and folinic acid in their livers and small intestine than did the guinea pigs. However, in the case of both the guinea pig and rat, the concentration of these two metabolites is greater in the liver than in the first portion of intestine. Folic acid could not be measured in
the livers and intestine of animals after Aminopterin treatment by the method used. Residual Aminopterin in the tissues presumably inhibited the growth of the test organisms used for the determination of folate acid. Aminopterin administration did not change the concentration of folinic acid in liver. The concentrations of folinic acid were 181 and 890 μgm. per gm. of liver from untreated guinea pigs and rats, respectively, and 190 and 891 after Aminopterin treatment. However, Aminopterin caused a marked reduction in the concentration of folinic acid in the small intestine of the rat, but no measurable effect on folinic acid concentration of small intestine from guinea pigs. Before treatment the concentration of folinic acid in the small intestine of rats was approximately 160 μgm. per gm. This concentration fell to 38 μgm. per gm. of small intestine after Aminopterin administration. The folinic acid concentrations in guinea pig gut before and after treatment were 8 and 6 μgm. per gm.

In Table III are the rates of P\textsuperscript{32} incorporation in protein obtained from the gastrointestinal mucosa of rats and guinea pigs. At the end of 2 hours the specific activity of the phosphoprotein obtained from the mucosa of the duodenum, jejunum, and ileum of rats was 11,893, 6570, and 10,659, whereas for guinea pigs it was 1157, 989, and 1212, respectively. This difference in P\textsuperscript{32} uptake by mucosal tissue of guinea pigs and rats was also evident at the end of 4 and 8 hours, although less marked at the end of 8 hours.

**DISCUSSION**

Folinic acid, which may be an active form of folic acid, has been implicated in several enzyme systems (5). Although the metabolic and pathologic effects of Aminopterin on rat intestinal mucosa reported in a pre-
vious paper (4) were not related to a specific enzyme system, this was presumably the basis of its action. Since Aminopterin caused marked reduction in the concentration of folinic acid in the intestinal mucosa of rats, it is not surprising that the activity of gastrointestinal mucosa as measured by O₂ uptake is greatly reduced. No change in the activity of intestinal mucosa of guinea pigs was observed after Aminopterin treatment. This, too, was not surprising, since Aminopterin had no effect on the concentration of folinic acid of the guinea pig small intestine, which under normal conditions is very low. This would seem to indicate that those enzyme systems which require folinic acid as a cofactor are relatively inactive in the guinea pig intestine as compared with that of the rat. One can assume that folic acid or folinic acid plays an important rôle in cell division since the analogue, Aminopterin, is an antimitotic agent. By using the uptake of P³² as a measure of mitotic rate, the results expressed in Table III would indicate that the cells of rat gastrointestinal mucosa proliferate at a far greater rate than those of the guinea pig. No gross changes were seen in the small intestine of guinea pigs treated with Aminopterin comparable to the complete destruction of all epithelial elements seen in rats at the end of 4 days (4).

Aminopterin toxicity in the rat can be completely prevented by equivalent amounts of folinic acid or by very large amounts of folic acid (5). Since Aminopterin is not very stable, decomposing to folic acid, and since well nourished animals were used in these experiments, it is possible that the ratio of Aminopterin to folic acid available to the guinea pigs was not great enough to produce gastrointestinal changes.

SUMMARY

Rat liver and small intestine had considerably higher levels of both folic and folinic acids than were found in these organs of the guinea pig.

Aminopterin administration for a period of 4 days did not alter the concentration of folinic acid in the liver of rats and guinea pigs. Aminopterin caused a marked reduction in the folinic acid level of the small intestine of rats but did not affect the level in the small intestine of guinea pigs.

Aminopterin administration caused a marked reduction in oxygen uptake by rat intestinal mucosa, but had no effect on the oxygen uptake of intestinal mucosa of guinea pigs.

By using the uptake of P³² as a measure of cell proliferation, rat intestinal mucosal cells were found to proliferate more rapidly than those of guinea pigs. The possible relationship between the mitotic rate of intestinal mucosa and the relative sensitivity of guinea pigs and rats to Aminopterin is discussed.
BIBLIOGRAPHY

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