THE EFFECTS OF A FOLIC ACID ANTAGONIST (AMINOPTERIN) ON ALBINO RATS: A STUDY IN THE PATHOGENESIS OF SPRUE.*

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RECENT investigations of folic acid and its antagonists yielded many interesting but as yet not well-coordinated conclusions. Of particular relevance to this report are the observations on the effects of folic acid in macrocytic anaemias, notably sprue (Manson-Bahr, 1946; Speis, Lopez, Milanes and Aramburu, 1947), and of the folic acid antagonists in cases of leukaemia (Farber, 1949) and experimental tumours (Woll, 1948*a*).

In a study of chicks fed a synthetic folic acid-free diet, or an adequate diet supplemented with a folic acid antagonist (4-amino-pteroylaspartic acid), constant alterations were observed in the bone-marrow and gastro-intestinal tract (Woll, 1948*b*). These were identical in the diet-deficient and antagonist-treated animals. The marrow showed a transient reduction in the mature red and white blood cells and maturation arrest of both red and white elements, with an increase in the size of the stem cells. The process progressed rapidly to a total aplasia. In the gastro-intestinal tract there was, initially, marked hydropic swelling of gland cells of the small bowel and colon with cystic dilatation of the gland lumen. A comparable cytologic change was seen in the oesophageal mucosa. The process advanced to a marked atrophy and early scarring. The stomach escaped this. In order to verify the above observations on a different species of animal and with a more potent antagonist, essentially similar experiments, using albino rats and 4-amino-pteroylglutamic acid (aminopterin), were performed. The findings are here reported.

MATERIALS AND METHODS.

Seventy albino rats weighing 110 to 130 g. were divided into the following three groups:

1. Control: commercial food and water *ad lib.* (15 animals).
2. Diet as above, plus a daily dose of 25 μg. aminopterin administered orally in aqueous solution by catheter (35 animals).
3. Diet as above, plus a single oral dose of 100 μg. aminopterin given by catheter (20 animals).

In order to compare the effects of starvation and a water-free regime on the same colony of animals, an additional fourth group of 10 rats was kept without

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food and a fifth group of 10 rats without water. The cages for all groups were of a type which greatly reduced the chance of the rats eating their excreta.

The animals were killed with ether according to the following schedule: Group 1 (control): one to three animals at 3-day intervals from the 3rd to the 24th day of the experiment. Group 2 (daily dose of aminopterin): two to three animals were killed daily from the 2nd to the 24th day. Group 3 (single dose of aminopterin): two to three animals at 3- to 9-hour intervals from the 1st to the 3rd day of the experiment. The rats on the starvation and water-free regimes were killed in groups of 2 or 3 every 3 days for the first 10 days of the experiment. Autopsies were performed on all immediately after death. Representative blocks of tissue were fixed in formaldehyde or Zenker-formaldehyde solutions. Paraffin sections were prepared and stained with haematoxylin and eosin. In some instances special connective tissue, fat, and glycogen stains were made on suitably fixed material. In all cases direct smears of the femoral marrow were prepared, fixed in methyl alcohol and stained with haematoxylin and eosin or Wright's stain. Sections of intact femoral marrow were also prepared.

RESULTS.

Ante-mortem and autopsy gross findings.—Ante-mortem the following changes were of progressive severity for the duration of the experiment. The antagonist-treated animals were markedly malnourished and dehydrated. The fur was rough. There was no alopecia. Often the rats assumed a hunched position. There was no paralysis or evidence of muscle incoordination. The snout, paws, eyelids and tails were stained dark brown. This pigment gave a deep red glow under ultra-violet light. Diarrhoea, progressively more severe, became haemorrhagic. It was pronounced in animals which received the single large dose of the antagonist. In no case was there bleeding of other than gastro-intestinal origin.

At autopsy the chief findings were: Severe anaemia, dehydration of the muscles and subcutaneous tissues, greatly shrunken spleen, thymus, and lymph nodes. The bowel was either contracted, pale and opaque, with a swollen mucosal coat, or markedly distended with a foul-smelling pale grey or dark brown watery fluid. There were large ulcers of the small and large bowel. In all cases the stomach failed to show appreciable deviations from normal.

The femoral marrow was scant, deep red, and terminally pale brown, viscid. The adrenals were slightly larger than normal. They were otherwise not remarkable.

Microscopic examination.—The earliest changes were noted in the bone marrow. These were seen on the second day of the experiment. In the animals which received the large dose of antagonist, the alterations were observed in 24 hours. At first there was a sharp decrease in the number of mature granulocytes. This was followed by an absolute increase in the number of stem cells and decrease in normoblasts. The stem cells increased in diameter 40–60 per cent as measured by a micrometer under oil immersion. Their nuclear detail became indistinct rather than more delicate as in the megaloblasts of pernicious anaemia. The cytoplasm also increased in amount and stained more lightly than normal. Finally most of the normal cellular elements of the marrow disap-

peared, leaving an occasional erythroblast, a few lymphocytes and prominent reticulo-endothelial cells (Fig. 1, 2, 3).

The spleen, lymph nodes and thymus showed progressive depletion of the mature elements. There was no actual increase in the reticulo-endothelial cells. The contraction of these organs brought these cells into greater prominence. Early in the experiment both the thymus and mesenteric lymph nodes showed numerous irregularly shaped basophilic particles in the sinuses. Whether these represented fragments of chromatin is not certain. Phagocytosis was at no time prominent (Fig. 4, 5).

In the gastro-intestinal tract there was, initially, a marked swelling of the cytoplasm and nucleus of the cells of the glands, followed by severe disorganization of the normal cellular configuration, cystic dilatation of the gland lumen, and then severe atrophy of all epithelial elements. Cells in mitosis were seen only slightly less frequently than in the control animals. This was followed by severe ulceration, acute purulent exudate, haemorrhages, and early evidence of fibrosis (Fig. 6, 7, 8). Of interest was the finding that the Paneth cells escaped, until very late, the above-described degenerative changes.

The lesion in the gastro-intestinal tract followed quite consistently the following sequence: The first parts involved were the ileum and duodenum, followed by the colon and then the oesophagus (Fig. 9, 10). Later and rarely the tongue also was involved. The stomach always escaped this process entirely.

No appreciable departures from normal were seen in the other viscera. The adrenal, while heavier than usual, showed no appreciable morphologic alterations. In the brain the cortical neurons in the anterior half of the cerebral hemispheres appeared larger than normal and stained more lightly. However, at no time was there evidence of reactive changes. The skeletal system, peripheral nerves, cord and the pituitary glands were not examined with sufficient regularity to warrant valid conclusions. The animals on the control, starvation and water-free regimes failed to show any of the above-described lesions. The

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EXPLANATION OF PLATES.

FIG. 1.—Normal rat femoral marrow. Haematoxylin and eosin. × 1000 approx.

FIG. 2.—Femoral marrow of a rat which received aminopterin for 7 days. Note the swelling of the immature cells, loss of chromatin detail and reduction in the developed blood cells, especially granulocytes. Haematoxylin and eosin. × 650 approx.

FIG. 3.—Rat femoral marrow after 20 days on an aminopterin-supplemented diet. Note the severe pancytopenia, increased prominence of the reticulo-endothelial elements and the myxomatous character of the stroma. Haematoxylin and eosin. × 650 approx.

FIG. 4.—Normal rat thymus. The cortex occupies most of the field. Haematoxylin and eosin. × 300.

FIG. 5.—Rat thymus after 7 days of aminopterin-supplemented diet. Note the dark particles of varying size and shape in the cortex. Haematoxylin and eosin. × 300.

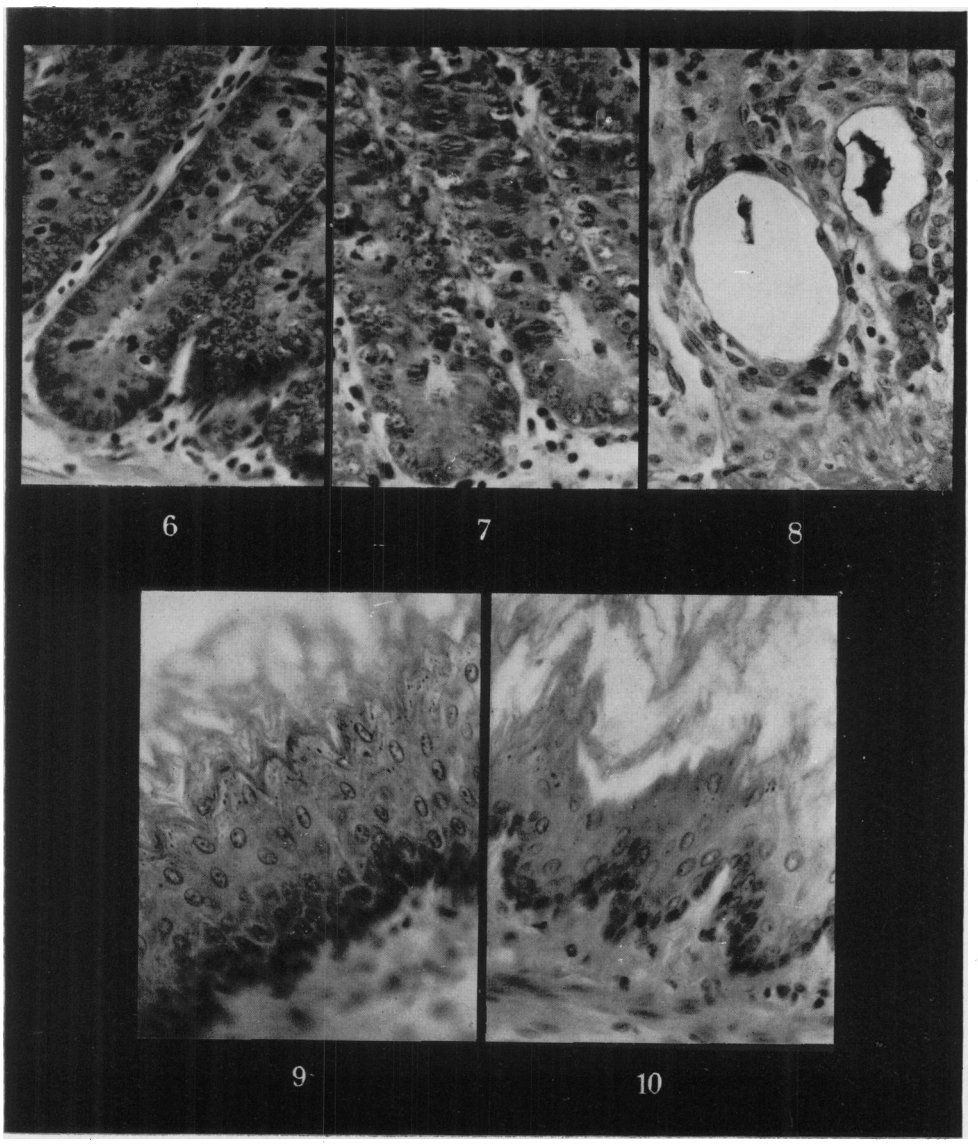
FIG. 6.—Normal rat duodenum. Haematoxylin and eosin. × 300.

FIG. 7.—Rat duodenum after 7 days of aminopterin-supplemented diet. There is marked nuclear swelling, a prominent nucleolus, disturbed cellular configuration and swelling of the cytoplasm. Haematoxylin and eosin. × 300.

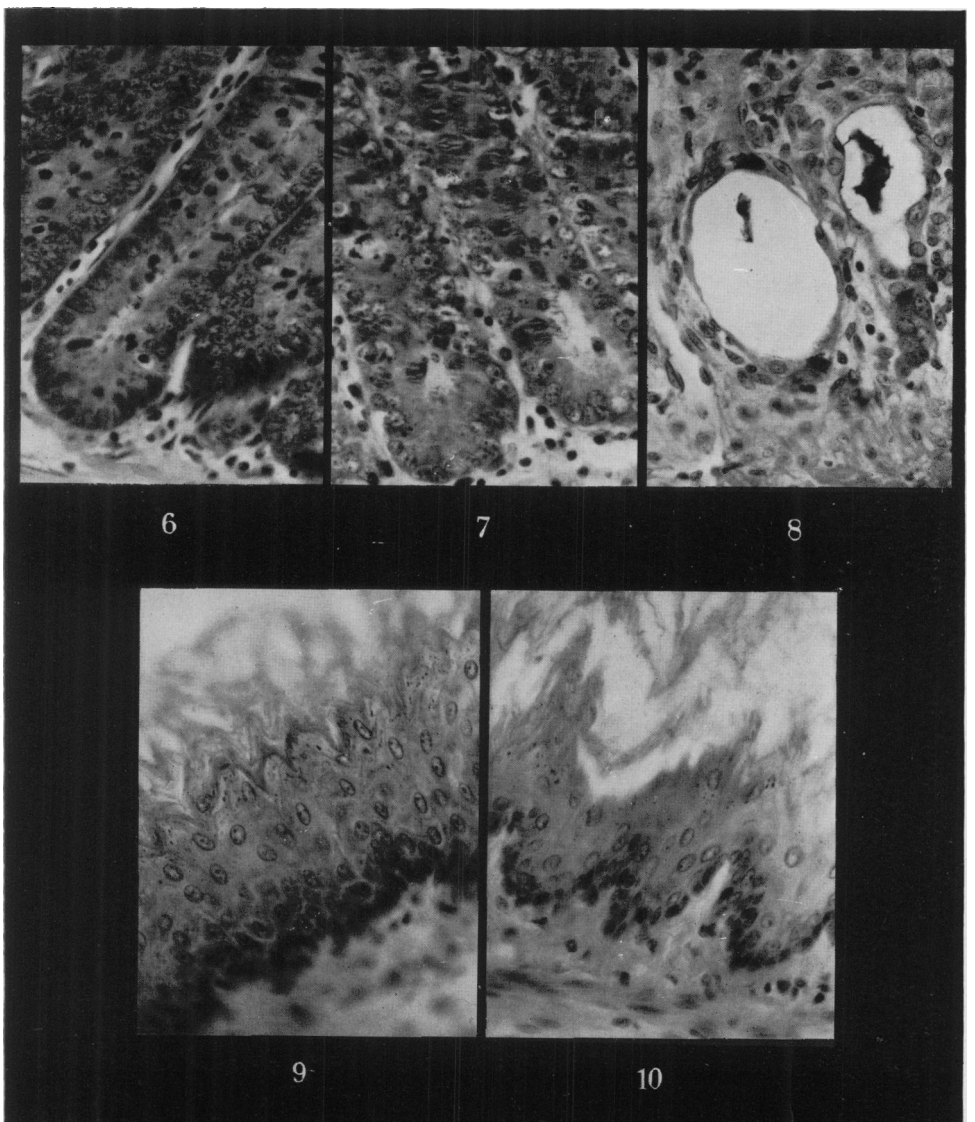
FIG. 8.—Rat duodenum after 15 days of an aminopterin-supplemented diet. Note the severe atrophy, cystic dilatation and fibrosis. Haematoxylin and eosin. × 300.

FIG. 9.—Normal rat oesophageal mucosa. Haematoxylin and eosin. × 300.

FIG. 10.—Rat oesophageal mucosa after 22 days of an aminopterin-supplemented diet. The sequences of maturation and keratinization are incomplete. Cellular outline and chromatin detail are lost. Activity in the basal and intermediate layers is apparently reduced. Haematoxylin and eosin. × 300.



Woll and Oleson.



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Woll and Oleson.

starved and dehydrated animals showed general changes of inanition characterized by generalized gross and microscopic reduction in the size of organs and parenchymal cells.

DISCUSSION.

The specific morphologic pathology of sprue, not related to pancreatic insufficiency, is fragmentary and conflicting. The lesions of the bowel described in this condition are quite similar to those seen in the above experiments. It is possible that the study of experimental folic acid deficiency, either diet- or antagonist-induced, may contribute to the knowledge of the pathogenesis of this disease.

SUMMARY.

Albino rats, fed a folic acid antagonist, were killed at frequent intervals, autopsied and the tissues examined microscopically. The findings were compared with those in control, food- and water-deprived animals.

The chief alterations were: (*a*) A pancytopenia and maturation arrest of the red and white blood cells at the level of the stem cell, with macrocytosis; this progressed to total aplasia; (*b*) atrophy of the gastro-intestinal tract, which had a specific pattern and did not involve the stomach.

The changes were essentially similar to those seen in chicks made folic acid-deficient by diet or antagonist.

The possible implications in relation to sprue are pointed out.

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