Megaloblastic Anemia Associated with Inhibition of Thymine Synthesis
(Observations During 5-fluorouracil Treatment)

By M. J. Brennan, V. K. Vaitkevicius and J. W. Rebuck

PROFOUND hematopoietic depression often occurs as a result of intensive treatment with 5-fluorouracil. Morphologic cellular studies of bone marrow before, during, and after administration of the drug have been undertaken in all but a few of 200 patients given this agent in the Oncology Division, Henry Ford Hospital, during the past 18 months. Clinical results of this chemotherapy are reported elsewhere.1

MATERIALS AND METHODS

All patients subjected to 5-fluorouracil therapy had far advanced cancer, no longer treatable with more conventional methods. Many of these patients had liver involvement, obstructive uropathies or extensive bone marrow replacement by tumor. For this study patients with such complications were excluded.

One hundred and forty-seven bone marrow specimens from 58 patients with metastatic cancer have been reviewed for this report. In these 58 patients, anemia, if present, was normocytic. No clinical evidence of hemorrhage or hemolysis was present. Care was taken to avoid previous biopsy sites in obtaining progress marrow samples though in all instances the same bone was used for all aspirations. From one to as many as ten observations were made during treatment in addition to the control study. The Turkel needle was used; aspirates were limited to 0.25 cc. volume and were smeared directly on glass slides for cytologic examination. A portion of clot containing marrow particles was embedded in paraffin and used for the estimate of cellularity. Leishman’s stain was employed for the smears and hematoxylin-eosin for sections. Five hundred-cell differential counts were made on direct smears.

5-Fluorouracil was administered intravenously in doses of 15 mg. Kg. (ideal body weight) per day for five days and 7.5 mg. Kg. every other day thereafter until clinical toxicity appeared. In certain instances, the dose pattern was altered to lower levels because of previous radiation or chemotherapy or debilitation caused by the neoplastic disease itself or its complications. Stomatitis and diarrhea of varying degree were regularly produced. Onset of these effects, or a fall in WBC to 2,000 or less, was taken to indicate cessation of therapy.

RESULTS

Twelve to 24 hours after the first dose, changes in metamyelocytes had begun to develop. Some cells increased markedly in size (fig. 1), nuclei became relatively pale and finely reticulated in appearance, while nuceli became

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Fig. 1.—(a) Multilobulated neutrophil. (b) Giant metamyelocyte with early cytoplasmic degeneration associated normal metamyelocytes—5-Fluorouracil. X1375.

Fig. 2.—Metamyelocyte with advanced cytoplasmic degeneration—5-Fluorouracil. X1375.
very prominent. Cytoplasmic changes, including a peculiar vacuolization were noted (fig. 2). Multilobulated granulocytes also appeared.

Subsequently, a marked rise in myeloid-erythroid ratio occurred from the usual pretreatment level of 3 or 4:1. This rise in the M-E ratio was not accompanied by any notable change in total cellularity until late in toxicity when a hypoplastic trend was noted. It was commonly well developed by 48 to 72 hours after the beginning of treatment.

At this time, also, qualitative changes in erythropoiesis had become apparent. Megaloblastic erythropoiesis became the dominant process at the time of onset of mucous membrane changes, usually on the fifth to the seventh day of treatment (fig. 3 and 4).

Within 48 hours of cessation of therapy, the values of the M-E ratio had passed their highest point and were in rapid decline towards normal or even slightly depressed levels. In some instances, giant plasma cells with many cytoplasmic vacuoles, reticulo-endothelial cells resembling those seen in Hand-Schuller-Christian disease (fig. 5), and megakaryocytes, multinucleated and deficient in platelet formation due to degenerative cytoplasmic changes (fig. 6) were found.

Peripheral neutropenia did not usually appear until one to four days after the onset of oral mucous membrane changes. The lowest point was not reached until 10 to 14 days thereafter. However, the recovery of marrow with a reversal to normoblastic hematopoiesis and more normal myeloid morphology was apparent within three to five days of discontinuance of drug administration.

A very marked increase in red cell mitotic rate was observed by the second to third day after cessation of 5-fluorouracil, but only modest increases in reticulocyte values in peripheral blood were met. After several courses of 5-fluorouracil therapy, a mild normocytic anemia usually developed. No significant macrocytosis was noted in any case.

Platelet counts, though diminished to less than 50,000/ml. at the time of maximal white cell depression, rose very quickly thereafter. Purpura was rarely noted in spite of distinctly subminimal platelet counts. A typical hematological course is represented in figure 7.

Concomitant changes in tumor cells are depicted in figures 8 and 9. Smears, fixed wet in ether-ethanol and stained by the method of Papanicolaou, or with Giemsa stain, revealed a disturbance of nucleocytoplasmic ratio, thinning of the nuclear chromatin net, cellular giantism, dyskeratosis, cytoplasmic vacuolization and frequent bi- and tri-nucleism in the cells of the oral, colonic, and vaginal mucosae. Comparable changes in the oral and vaginal mucosa in pernicious anemia and in megaloblastic anemia of pregnancy have previously been demonstrated.

When the myeloid-erythroid ratios obtained at the time of drug discontinuance were compared to the low point of the white blood cell count subsequently developing, a correlation of very high myeloid-erythroid ratios with severe leukopenia and normal ratios with freedom from that complication was noted (table 1).
Fig. 3.—(a) Control pretreatment polychromatophilic normoblast. (b) Orthochromatic megaloblast—5-Fluorouracil. (c) Polychromatophilic megaloblast—5-Fluorouracil. X1375.

Fig. 4.—(a) Normal basophilic normoblast. (b) Basophilic megaloblast—5-Fluorouracil. (c) Polychromatophilic megaloblast—5-Fluorouracil. X1375.
MEGALOBLASTIC ANEMIA WITH THYMINE SYNTHESIS INHIBITION

Fig. 5.—Cytoplasmic vacuolar degeneration marrow reticulum cell—5-Fluorouracil. X1375.

Fig. 6.—Cytoplasmic degeneration in promegakaryocyte—5-Fluorouracil. X1375.
Fig. 7.—Typical course of changes in bone marrow M:E ratio (continuous line), white blood count (dotted line), and platelet count (interrupted line) during and following 5-FU therapy in a patient with a disseminated adenocarcinoma of rectosigmoid.

DISCUSSION

Megaloblasts occur in the bone marrows of patients with widely varying conditions: pernicious anemia, the di Guglielmo’s Syndrome, megaloblastic anemia of pregnancy, sprue, intoxication with antifols, antimalarials, and anticonvulsant drugs. Vilter proposed a unifying concept, suggesting that all these conditions have one common denominator, namely deoxyribonucleic acid (DNA) deficiency. In the majority of the megaloblastic anemias there is reason to believe that deficient thymine synthesis is responsible for DNA shortage. Most megaloblastic anemias have been successfully treated with numerous compounds, all of which increase DNA synthesis. Thymidine, administered parenterally, produced complete remissions in two patients with pernicious anemia treated by Hausmann but Spray and Witts, and in one patient Rundles and Brewer were not able to produce the same effects. Therefore, Spray concluded that the “thymineless” explanation of megaloblastosis in pernicious anemia is no longer tenable. It was necessary for Vilter to use 15 Gm. of thymine to induce hematological remissions in his patients, while Spray used only 250 mg. of thymidine. Considering the molecular weight of both compounds this represented only slightly more than one per cent of the thymine dosage employed by Vilter. Although thymidine is a better precursor of bone marrow DNA than thymine, differences in the amounts administered may explain the different results obtained by those two investigators.
Fig. 8.—Group of adenocarcinoma cells (colon)—bone marrow aspiration. X800.

Fig. 9.—Same after 5-fluorouracil treatment. X800.
Table 1.—Changes in Myeloid-Erythroid Ratio Compared with the Degree of Leukopenia

<table>
<thead>
<tr>
<th>White Blood Count (minimum)</th>
<th>M-E Ratio (4th or 5th day of Rx)</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Below 500</td>
<td>215 : 1</td>
<td>6</td>
</tr>
<tr>
<td>500-2,000</td>
<td>85 : 1</td>
<td>15</td>
</tr>
<tr>
<td>2,000-4,000</td>
<td>26 : 1</td>
<td>33</td>
</tr>
<tr>
<td>Above 4,000</td>
<td>10.5 : 1</td>
<td>4</td>
</tr>
</tbody>
</table>

Thymine synthesis takes place through the action of an enzyme, thymidylate synthetase, acting on the deoxyribotide of uracil to attach a methyl group in the “5” position of the pyrimidine ring.12,13,14 The presence of N5, N10-methylene-tetrahydrofolic acid is required for this reaction. Vitamin B12 was shown to be required for utilization of formate in thymine synthesis in lactobacillus Leishmanii.15

The severity of the cytopathologic changes observed in thymine-deficient states may in part depend on the degree to which a pure thymineless state can be approximated. Thus, Cohen was able to modify the lethal effects of thymine deficiency in E. coli by restricting access of the cells to uracil, to purines, and to amino acids in a thymine-dependent strain deprived of thymine.13 Cohen also demonstrated that 5-fluorouracil produced the biochemical as well as the morphologic characteristics of thymineless death in E. coli.13

The outstanding biochemical characteristic of cells subjected to lethal thymine deficiency is the rise in ribonucleic acid versus deoxyribonucleic acid ratios in the lethally affected organisms.13 Cell death is proportional to this distortion. Therefore, it would appear that the less an agent interferes with protein and ribonucleic acid synthesis while simultaneously impeding deoxyribonucleic acid formation, the more potent it will be in terms of lethal cytotoxicity of this kind.

Lindner demonstrated in hypotetraploid mammalian tumor cells a rise in RNA and protein content per cell under 5-fluorouracil treatment while DNA fell to half the pretreatment weight per cell. Mitosis had continued during the period of treatment and the interesting conclusion was reached that perhaps cell division was taking place in spite of failure of DNA synthesis.16 No change in chromosome number accompanied the fall in DNA content per cell. No instance of such depletion mitosis in diploid cells has been noted. However, the delayed lethal effects of 5-fluorouracil in bacteriological and tissue culture systems are of interest in this connection.13 Furthermore, an implication is present that cytoplasmic growth, even in the absence of nuclear growth, may yet provide an effective signal for mitosis.

5-fluorouracil, having been converted in part to the deoxyribotide, becomes an irreversible inhibitor of thymidylate synthetase17 (fig. 10). A considerable portion of the remaining drug is incorporated into ribonucleic acid, especially that of the nucleus.18 It is possible that this “specious ribonucleic acid” accounts for some differences in the appearance of 5-fluorouracil intoxicated marrows from other megaloblastic conditions, mainly the difference in M-E ratio. It also might explain why the changes in bone marrow M-E ratio cor-
Fig. 10.—Suggested areas of 5-fluorouracil activity. Straight arrows indicate incorporation or transformation. Wavy line indicates metabolic block caused by 5-fluorouracil administration. Broken wavy line indicates variable effect in different systems tested.

relate better with the clinical toxicity than does the degree of megaloblastosis. Thymine treatment in 5-fluorouracil intoxicated rats led to many fatalities. We did not feel that at this time we were justified in treating 5-fluorouracil intoxicated patients with thymine. In studies now in progress in this laboratory on short-term human bone-marrow cultures, 5-fluorouracil, in dosages of 100 micrograms per 1 ml. of culture medium, produced megaloblasts. This change could be prevented by thymine but not by uracil (fig. 11). Similar changes were seen in HeLa cell culture. The details of these in vitro studies on human cells are to be reported at a later date.

Since 5-fluorouracil is known to inhibit thymine synthesis in bacterial and animal systems and since the megaloblastic changes in bone marrow culture as well as in cultured human tumor cells can be modified by thymine, it appears justifiable to speculate that the megaloblasts seen during 5-fluorouracil therapy in our patients were due to thymine deficiency.

SUMMARY

Megaloblastic changes in the erythrocytic series and cellular giantism in the myeloid line characterize the bone marrow response to 5-fluorouracil in man.

Cytoplasmic vacuolizations occur in reticulo-endothelial cells, megakaryocytes, and myeloid elements.

During development of these morphologic changes in marrow, associated
cellular giantism, polynucleism, and nuclear pattern changes appear in the oral, vaginal, colonic mucosa, and in some tumors resembling those encountered in the bone marrow.

The myeloid-erythroid ratio was found to be useful for the purpose of identifying dosage end-points.

The physiological implications of these changes as related to nucleic acid metabolism were discussed.

**SUMMARIO IN INTERLINGUA**

Alterationes megaloblastic in le serie erythrocytic e gigantismo cellular in le linea myeloide es caracteristicas del responsa del medulla ossee a 5-fluorouracil in le homine.

Vacuolisation cytoplasmic occure in cellulas reticulo-endothelial, in megacaryocytos, e in elementos myeloide.

Durante le disveloppamento de iste alterationes morphologic in le medulla, associate gigantismo cellular, polynucleismo, e alterationes configurational del nucleos se manifesta in le mucosa oral, vaginal, e colonic e in certe tumores que resimila illos incontrate in le medulla ossee.
Esseva trovate que le proportion myeloide-erythroidc es utile pro deter-
miliar punctos terminal de dosage.

Es discutite le signification physiologic que inhere in iste alterationes in
relation al metabolismo de acido nucleic.

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