Reports and Letters

Influence of the Concentration of Leukemic Inoculum on the Effectiveness of Treatment

Although the clincial treatment of acute leukemia with folic acid antagonists, first employed by Farber *et al.* (*1*), is often effective early in the course of treatment, it is generally considered that the ultimate failure of treatment is attributable to the emergence of resistant variants of the leukemic cells. This followed from the development by Burchenal *et al.* (*2*) and Law and Boyle (*3*) of mouse leukemias resistant to folic acid antagonists, such as amethopterin.

In this laboratory, leukemic mice were kept alive for an extended period of time with massive doses of amethopterin. The leukemic cells from such mice, however, showed on transplantation little, if any, evidence of resistance (*4*). It has also been observed that treatment of leukemic mice with an antagonist of folic acid late in the course of the disease was considerably less effective than treatment initiated early (*5*). The last-cited observations suggested that failure of treatment could result from an increased population of leukemic cells in the host (*4*). The current experiment (Fig. 1) demonstrates the importance of the number of leukemic cells in influencing the effectiveness of treatment.

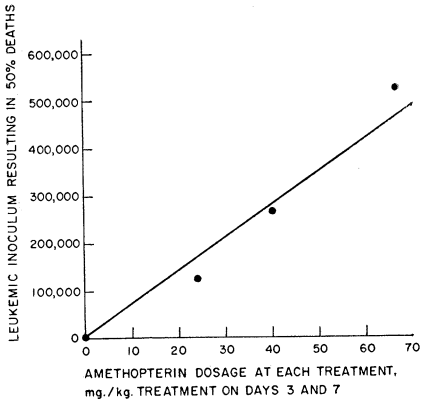

Fig. 1. Relationship between level of treatment with amethopterin and the size of the leukemic inoculum (cells per mouse) required to yield 50-percent mortality (EI_{50}). Early deaths from drug toxicity are excluded in computing the percentage mortality resulting from the leukemic inoculum. Without treatment, the EI_{50} was about 4000 cells.

The mice were inoculated intramuscularly with varying levels of leukemic (L1210) cells and were treated intraperitoneally with amethopterin on the third and again on the seventh day following inoculation with the leukemia. Three different dosages of amethopterin were employed, and, in addition, one set of leukemic mice was left untreated.

The mice were observed for mortality for 100 days following leukemic inoculation. Mice that survived 100 days were apparently "leukemia-free." Spleen implants from such mice did not result in leukemic growth. For each treatment level, and for the untreated group, the percentage of mice that died of leukemia increased with increasing inoculum. As the inoculum concentration was increased, it was apparently necessary to increase the dosage of drug to maintain the effect against the tumor.

Figure 1 shows the approximately linear relationship between the number of cells in the leukemic inoculum necessary to kill 50 percent of the mice (EI_{50}) and the dosage of amethopterin. From an EI_{50} of 4000 cells when no treatment was administered, there was an increase to more than 500,000 cells at the highest dosage of amethopterin employed, a relationship indicating that the level of treatment yielded better than 99-percent inactivation of the leukemic cells. The limitation to the continuation of this positive relationship is the toxicity of the drug to the host. At the highest dosage of the drug (67 mg/kg × 2), approximately 20 percent of the mice succumbed to drug toxicity. These animals were excluded from the calculations of the EI_{50}.

For a specific treatment, administered at a specified time after inoculation of the leukemia, this type of relationship describes the maximal potency of the drug. To improve treatment, it would be necessary to increase the effectiveness of the drug with respect to the leukemia without proportionately increasing the toxicity of the drug for the host. This may be accomplished, for example, by alteration of the schedule of treatment (*5, 6*) or by delayed administration of citrovorum factor (*6*).

The data indicate that the effectiveness of treatment is influenced by the number of leukemic cells in the host. Apparently, then, in addition to the possible emergence of resistant leukemic cells, and in addition to other factors, such as the possible diminution in the tolerance of the host to a particular treatment, progressive increase in the number of cells, despite treatment, may render a particular treatment progressively less effective. By the same token, the treatment of advanced leukemia may be hampered by the increased number of cells.

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Natural Selection Associated with the ABO Blood Group

Geneticists have suspected and suggested that human blood groups should be subject to the processes of natural selection (*1*). Except for hemolytic disease of the newborn, no conclusive evidence that this was so had been brought forward until the article by Aird, Bentall, and Fraser Roberts appeared in 1953 (*2*). An extensive, well-controlled, clinical study involving population groups throughout England and Scotland demonstrated convincingly the existence of an association between carcinoma of the stomach, peptic ulceration, and the ABO blood group. This report is concerned with the preliminary results of a similar study conducted in the United States. Buchanan and Higley in 1921 (*3*) and Mayo and Fergeson in 1953 (*4*), in the only similar American studies, concluded that there was no relationship between blood groups and malignancy and none between blood groups and any disease for which sufficient data were available to justify a conclusion.

The case material utilized in the study reported here was provided by

Table 1. Blood-type frequencies.

Typed	Total cases	Blood groups							
		O		A		B		AB	
		No.	%	No.	%	No.	%	No.	%
Gastric carcinoma patients	879	370	42.09	403	45.85	81	9.22	25	2.84
Peptic ulcer patients	1770	946	53.45	655	37.01	128	7.23	41	2.31
Controls	6313	2892	45.81	2625	41.58	570	9.03	226	3.58

patients seen and treated at the university and five other Iowa hospitals during the past 20 years (*5*). Unselected, consecutive cases of gastric carcinoma and peptic ulceration are the clinical material upon which this preliminary report is based. A histologic diagnosis, resulting from study of surgical or post-mortem tissue sections, was required for inclusion of carcinoma cases. Duodenal and gastric ulcer cases were included when (i) an iron-clad clinical diagnosis existed that was based on clinical, x-ray and/or gastroscopic findings, or (ii) a gross or microscopic diagnosis was established at the time of surgery or post-mortem examination. In order to insure that these criteria would be rigidly adhered to, all case records were reviewed by one or another of us. The extracted data were transferred to IBM punch-cards and sorted by machine methods. In order to test the significance of our results, they were subjected to statistical analysis by means of the chi-square and difference-between-percentages methods (*6*).

It was of the greatest importance to establish reliable values for blood-type frequencies among healthy individuals who were to be used as controls for comparison with the blood-type frequencies observed in the patient groups. The blood types of consecutive blood donors contributing to two hospital blood banks where most patient typings were done were recorded. Before giving blood, these individuals were screened to eliminate those with disorders contraindicating their use as donors. For the most part, they were relatives or acquaintances of the patients. Therefore, most of them came from the same population groups as the patients and had ethnological backgrounds similar to those of the patients.

The blood-type frequencies and the percentages these represent of the total control and patient groups are recorded in Table 1.

When blood-type frequencies in the control and patient groups are compared, it becomes clear that two significant associations exist. Members of blood type A are either more susceptible to, or have less resistance against, carcinoma of the stomach than members of the three other blood types. The significance of this relationship is at the 2-percent level. Members of blood type O, on the other hand, appear to have even greater vulnerability to, or less resistance against, peptic ulceration. The degree of significance here is at the significant level of 0.1 percent.

It is evident that the possession of blood type A is related to natural selection, in that such individuals have a greater probability of developing carcinoma of the stomach and consequently are less favored than members of the other blood types. Conversely, members with these other types are thus favored, except for the selection exercised on group O members by the increased likelihood that they will develop peptic ulceration.

The association of blood types, the genetics of which have been extensively studied and are well understood, to carcinoma of the stomach fits well with the heretofore suspected familial predisposition to this and other malignancies. A similar genetic factor, acting to cause peptic ulceration, is suggested by the demonstrated predisposition of type O individuals.

The hypotheses that may be advanced to explain these findings seem to fall into one of two general categories—that is, local factors acting in or on the stomach or general ones acting to control the individual's response to the carcinogen or ulcerogen. In the first instance, known mucopolysaccharide blood-group substances may act to increase tissue vulnerability to, or diminish resistance against, carcinogens and ulcerogens. In view of the large number of such substances that have already been isolated, resulting in the definition of the many blood groups, it seems likely that still other similar antigens remain to be identified that could well be responsible for these effects. Local tissue factors and enzyme activities that are unrelated to the red blood cell and its antigens remain less attractive possibilities. Perhaps more appealing, although little evidence thus far has been presented in its support, is the concept of fundamental, individual biochemical and/or physiological differences. Blood types, susceptibility to bacterial organisms, and predispositions to carcinogens and ulcerogens would reflect these fundamental differences in individuals, representing differences in response to similar environmental factors. Evidence that similar relationships do not exist for certain other malignancies and blood types as for carcinoma of the stomach does not rule out this explanation.

A relationship between natural selection and the ABO blood group is indicated by the results of this study through (i) a significant increase of type A among patients with carcinoma of the stomach, and (ii) a significant increase of type O among patients with peptic ulceration.

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5. Space does not permit acknowledgment of the individuals and hospitals participating in the collection of the data, which are to be included in more detailed reports that are now in preparation.
6. The statistical analysis was made by L. A. Knowler, department of mathematics and astronomy, State University of Iowa, Iowa City.

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Salivary Amylase in the Rat

In a review of the literature concerning amylase in the body fluids and tissues of the rat, no good quantitative data could be found on salivary amylase levels. Several sources (*1*, *2*) agreed that the rat does have amylase in its saliva but gave little other information. Quantitative data, of interest in this laboratory in connection with a larger study (*3*) on tissue amylases, have now been obtained and are reported here.

Fifteen Sprague-Dawley rats, fed on the usual laboratory diet, were used in this study. With the rats under ether anesthesia, flow of saliva was stimulated by intraperitoneal injection of approximately 1 mg/kg of pilocarpine (*4*). Saliva was collected by gentle suction on a micropipette leading to a small trap. By this means, 0.3 to 0.5 ml of saliva could be collected in 10 to 15 minutes. After saliva had been obtained, the rats were sacrificed, and blood was collected for serum amylase determinations.