and amount of absorption as the cause of the contradictory effects on body-temperature. Our experiments imply that the property of producing hyperthermia after subcutaneous injection and hypothermia after intraperitoneal injection is inherent in the nucleic acid molecule. The fact that the large nucleic acid molecule was active though the smaller hydrolysis products of nucleic acid were not suggests that molecular or particle size may be important in causing body-temperature changes. This raises the questions of phagocytosis of the material in the peritoneal cavity and its absorption by way of the lymph channels, and the possibility that hypothermia is associated with activity of the reticulo-endothelial system. Investigations of these questions are in progress and will be reported later.

Summary. Subcutaneous injection of 2 cc of a "15%" yeast autolysate in rats produced a significant rise in body-temperature. Intraperitoneal injection of the same material produced a significant fall in body-temperature. Both qualitatively and quantitatively similar results were produced by the injection of 2.5 cc of a 0.1% solution of the magnesium salt of yeast nucleic acid. Slight lowering of the body-temperature occurred in some animals after intraperitoneal injection of guanine. Subcutaneous injection of guanine had no effect on body-temperature. Adenine, uracil, xanthine, and allantoin had no effect on the body-temperature of rats by either route of administration.

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17061. Development of Macrocytic Erythrocytes in Leukemic Subjects Receiving Folic Acid Antagonist, 4-Aminopteroylglutamic Acid (Aminopterin).

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Temporary remissions in acute leukemia have been produced by the therapeutic administration of the folic acid antagonist 4-aminopteroylglutamic acid (aminopterin). Farber[1,2] has reported remissions in children. Dameshek[3,4] has produced equally good results in adults. The exact mechanism of the action of aminopterin is not known. It is a folic acid antagonist in that it possesses the property of inhibiting the growth of *Streptococcus faecalis R.* or *L. casei* in the presence of marginal levels of folic acid. Farber[2] noticed hypersegmentation of neutrophilic granulocytes in the peripheral blood and megaloblasts in the marrow in leukemic patients receiving folic acid antagonists. If aminopterin is a true folic acid antagonist one can theorize that morphologic changes might be produced in the erythrocytes. In a series of 25 patients (children and adults) with acute leukemia of all types treated with aminopterin, results were obtained in 7 of these individuals which support this theory.

Experimental. Daily doses of ½ to 1.0 mg of aminopterin were given intramuscularly. One cc of crude liver containing 2 U.S.P. units was given with each dose of aminopterin.

Frequent complete blood counts were done on all patients receiving therapy. Occasional bone marrow biopsies were also obtained. More complete studies were obtained when macrocytosis and anisocytosis was observed in the circulating red blood cells. These studies included red cell counts, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, morphologic observations on the erythrocytes and measurement of the mean erythrocytic diameter by the

1 Farber, S., Diamond, L. K., Mercer, R. D., Sylvester, R. F., and Wolff, J. A., *New England J. Med.*, 1948, **238**, 787.

2 Farber, S., *Blood, J. Hemat.*, 1949, **4**, 160.

3 Dameshek, W., *Blood, J. Hemat.*, 1948, **3**, 1057.

4 Dameshek, W., *Blood, J. Hemat.*, 1949, **4**, 168.

TABLE I.
Observations on Erythrocytes in Four Leukemic Patients Having the Most Marked Macrocytosis and Anisocytosis.

Age, yrs.	Sex	Days therapy	Total rbc.	Hgbn., g	MCH, γγ	Diameter, μ	Macrocytes	Anisocytes
3	M	0	2.57	8.0	30	7.0	0	0
		30	2.91	9.0	31	7.2	++	++
		65	4.10	11.8	29	7.8	++	++
3	F	0	*5.04	14.0	28	7.2	0	0
		90	4.79	13.0	27	7.5	++	++
13	M	0	3.91	10.4	27	7.0	0	0
		15	3.22	9.3	29	7.2	+	+
		30	3.21	9.8	30	7.5	++++	++++
4	F	0	2.20	4.9	22	7.0	0	0
		32	3.78	10.5	28	8.5	++++	++++

* Transfusions.

Haden-Hausser erythrocytometer.

Observations. An obvious macrocytosis and anisocytosis developed in 7 patients. A diagnosis of acute leukemia had been made in each instance by history, examination of the patient, peripheral blood studies and bone marrow biopsies. Although it is realized that cell types in leukemic diseases of children are extremely difficult to classify it is believed that 5 cases were acute lymphatic leukemia and 2 were of the acute monocytic type. Five of the patients were under 10 years of age and 2 were in their teens.

In 4 of the 7 individuals in whom morphologic changes were observed in the red cell series a very marked macrocytosis developed. These data are presented in Table I. In no instance did a severe anemia exist. All had responded well to aminopterin and the leukemic disease had at least been controlled to the extent that no transfusions were necessary. The macrocytosis and anisocytosis in the 7 patients usually became noticeable between the fifteenth and thirtieth days of therapy. Poikilocytosis was not noticed in any of the 7 patients. The mean diameter of the red cells as measured by the erythrocytometer became greater as will be noticed in Table I. Actually an accurate evaluation of the morphologic changes in the red cells could be made by direct microscopic examination of the red cells. Photomicrographs of the red cells in 2 subjects are shown in Fig. 1. The degree of macrocytosis and anisocytosis without poikilocytosis is indeed marked.

Bone marrow biopsies were done in 2 instances when the peripheral blood showed morphologic red cell changes. Even though the peripheral blood had returned to normal and the patients were markedly improved the marrow tissue was still markedly infiltrated and replaced with leukemic cells to the extent that only rare normal bone marrow cellular elements were observed. No true megaloblasts were observed in these 2 patients. In other individuals however who have had extensive therapy with aminopterin an exceedingly rare megaloblast has been observed.

Discussion. Farber[1] in his initial report emphasized the toxic nature of aminopterin. Many of the lesions produced are similar to those produced by a folic acid deficiency in the rat and monkey. These lesions are stomatitis, ulceration of the mucous membrane of the mouth, smooth tongue, pharyngitis, and atrophic changes in the intestinal epithelium. The morphologic changes in the erythrocytes consisting of macrocytosis and anisocytosis may be possibly due to an induced folic acid deficiency. It is of interest that poikilocytosis is not present in the 7 cases reported herein. Farber[2] and Dameshek[3,4] both emphasize that it is impossible to state at this time whether all the changes produced in acute leukemia by folic acid antagonists are primarily due to a folic acid deficiency. When untoward symptoms rapidly develop that are similar to those produced experimentally, the various types of

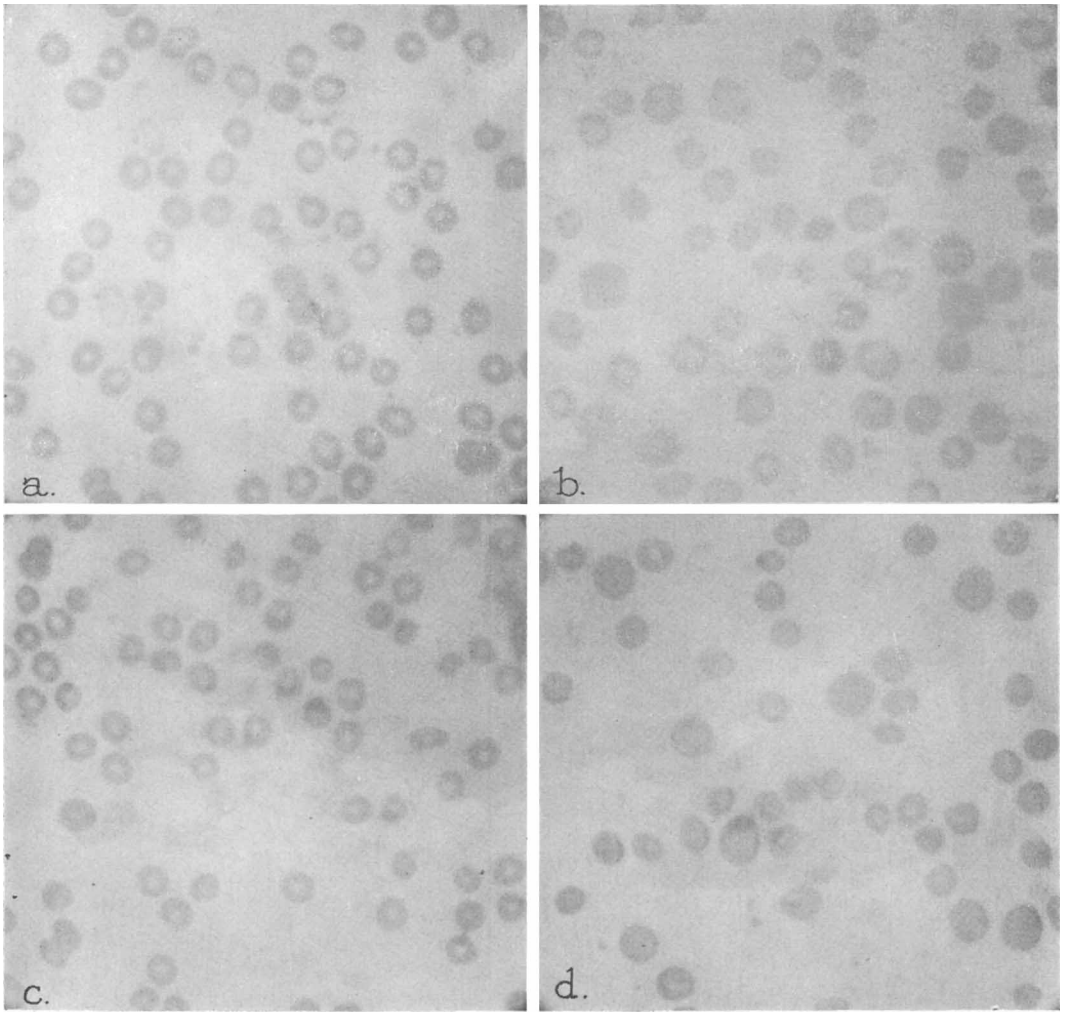

FIG. 1.

Morphologic changes in red blood cells during aminopterin therapy. A and b, patient 48-279, a, before therapy, and b, after therapy. C and d, patient 48-229, c is before therapy and d, after therapy. In each instance macrocytosis and anisocytosis are present after therapy.

therapy such as vitamin B preparations, liver extracts both crude and concentrated, and folic acid have not been more beneficial than the mere act of stopping the administration of the folic acid antagonist. Further observations on complicated biochemical systems must be made before definite conclusions can be reached.

Conclusions. In 7 of 25 human subjects with acute leukemia treated with the folic acid antagonist 4-aminopteroylglutamic acid (aminopterin) morphologic changes developed in the circulating red blood cells consisting of a macrocytosis and anisocytosis.

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