concluded that the removal of intravenously administered cholesterol was not significantly accelerated by either this mechanism or by any possible more direct action of these substances on cholesterol metabolism.

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Platelet Regeneration During Therapy of Acute Leukemia with Folic Acid Antagonists.* (17765)

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Temporary remissions in acute leukemia have been produced by the therapeutic administration of the various folic acid antagonists. Observations have varied as to the response of the blood platelets. Farber *et al.*(1,2) reported improvement in the platelet level in some of his cases. Dameshek(3) in a preliminary report stated that a definite increase in blood platelets must be considered as a part of the criteria in determining whether or not a remission had occurred but no other data are given. Meyer *et al.*(4) observed no significant effect on the platelet level of patients with acute and chronic leukemia treated with aminopterin (4-amino, pteroyl glutamic acid). Berman *et al.*(5) reported no regular response in platelets in nine cases of chronic leukemia treated with aminopterin. Stickney *et al.*(6) merely state that the platelets may increase in number during remissions. Sacks *et al.*(7) reported his findings in 14 cases of acute leukemia and in 4 cases with an initial thrombocytopenia a definite platelet response occurred.

In a series of 48 patients (children and adults) with acute leukemia of all types treated with folic acid antagonists, results were obtained which indicated that when a definite remission occurred the elevated platelet response was a part of the hematologic and clinical improvement. In the cases presented the regeneration of platelets and other formed elements of the peripheral blood followed a rather set pattern not mentioned in previous publications.

Experimental. The following folic acid antagonists were used: 4-amino, pteroyl glutamic acid (aminopterin); 4-amino, N^{10} methyl pteroyl glutamic acid (a-methopterin); 4-amino, pteroyl aspartic acid (amino-an-fol); nd 4-amino, 9 methyl pteroyl glutamic acid (a-ninopterin). These antagonists were administered intramuscularly or orally in approximately the same dosages as recommended by Farber(1,2) and Dameshek(3). Platelet levels were determined by the Rees-Ecker method, normal values in our laboratory being 225,000 to 325,000 per cu mm. The hemoglobin, red cell levels, leukocyte counts and differential counts were also frequently done in addition to bone marrow biopsies by the aspiration method. The leukocytes were studied qualitatively by the supravital method using Janus green and neutral red.

Observations. A definite response, either

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TABLE I.
Observations on Platelet Response in 9 Patients with Acute Lymphatic Leukemia Treated with Folic Acid Antagonists.

Age, yrs	Sex	Folic acid antagonist	Remission duration, days	Initial platelet count, per mm³	Platelets, days of therapy to 100,000 per mm³	Highest platelet level, per mm³
				× 1,000		× 1,000
3	F	aminopterin	110	28	8	310
10	M	aminopterin a-ninopterin	240	40	20	295
6	F	aminopterin amino-an-fol	200	26	16	264
5	F	aminopterin amino-an-fol	30	28	28	548
3	M	aminopterin	35	43	27	287
4	F	aminopterin	30	34	29	197
4	F	a-methopterin	150	42	24	394
5	F	aminopterin a-methopterin	30	70	31	360
2	M	aminopterin	55	74	21	310

hematologically and/or clinically, was observed in 25 of the 48 patients with acute leukemia and treated with folic acid antagonists. Eleven patients had an excellent remission both hematologically and clinically and in 14 subjects a definite improvement was noted but leukemic cells could be found in the peripheral blood, and some degree of enlargement of the lymph nodes and spleen could be detected. In the group of 25 patients who responded to some type of folic acid antagonist there were 9 patients who had an initial platelet count under 100,000 per cu mm and 8 of these had a definite rise to normal values and 1 to almost normal levels during their period of remission. All 9 patients were 10 years of age or under and had acute lymphatic leukemia. The accompanying table shows the data in the 9 patients observed. The increased platelet level in most instances was the first evidence of a good hematologic response and usually occurred in third and fourth weeks of therapy. The polymorphonuclear neutrophilic leukocytes began to respond at about the same time or shortly thereafter, this being followed later by a rise in the erythrocyte count and hemoglobin. Enlarged spleens, livers and lymph nodes receded in size. Bone marrow studies revealed a decrease in leukemic cells with a return of normal marrow elements including the megakaryocytes.

It is of interest that in those patients in a state of marked hemorrhagic diathesis the tendency to bleed disappeared before the platelets became elevated. These patients were given transfusions and hesperidin methyl chalcone, which may have had some effect on the bleeding phenomenon. However, the hemorrhagic tendency in patients not responding to therapy with folic acid antagonists was most difficult or impossible to control.

Discussion. The exact nature of the action of the folic acid antagonists is unknown. Theoretically one would expect a folic acid deficiency to possibly produce a thrombocytopenia, a marked decrease in platelets being observed in the macrocytic hyperchromic type of nutritional anemia. Farber(1) has emphasized the toxic nature of the antagonists. The lesions produced by the toxic effect of the compounds are similar to those produced by a folic acid deficiency in the rat and monkey. Stomatitis, ulceration of the mucous membranes of the mouth, smooth tongue, pharyngitis, and atrophic changes in the intestinal tract have been noted in these folic acid deficient animals. Farber(2) and Dameshek(3) both emphasize the difficulty in determining whether or not the lesions produced by the folic acid antagonists are primarily due to a folic acid deficiency. Our observations have confirmed these statements. Various types of therapy such as vitamin B preparations, including vitamin B_{12}, liver extracts, both crude and concentrated, and folic acid have not been more beneficial than merely

stopping the administration of the folic acid antagonist. It is indeed remarkable that a normal peripheral hematologic picture, including a platelet response, occurs under such therapy. Macrocytic erythrocytes and anisocytosis have been observed(8). A definite explanation of the response cannot be made at present.

Conclusions. In 48 human subjects with acute leukemia treated with folic acid antagonists, there were 9 of the 25 subjects who responded to therapy who had an initial thrombocytopenia. In 8 cases of acute leukemia there was a return of the blood platelets to normal levels and the megakaryocytes became more numerous in the bone marrow. In one other patient the platelets became elevated to slightly less than normal. The rise in platelets was usually followed by an increase in neutrophilic leukocytes. The regeneration of the red cells and hemoglobin then gradually responded. The periods of remission varied from 21 to 240 days.

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Comparative Tissue Explant Requirements of Columbia-SK, C(M) and M.M. Viruses, and of Theiler's Encephalomyelitis Virus.* (17766)

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Cultivation of the "murine strain of SK poliomyelitis virus"(1,2), here designated Columbia-SK virus(3), has been reported by Sanders and Jungeblut(2). This virus was originally reported as a rodent adapted human strain of poliomyelitis virus (Yale-SK strain), but has recently been established to be a member of the encephalomyocarditis (EMC) group of viruses and to be antigenically unrelated to the original or Yale-SK strain of poliomyelitis virus(4-6). Cultivation of the M.M. virus(7), also a member of the EMC group(4-6), in tissue explants, has been reported by Jungeblut(8). Enright and Schultz(9) first cultivated the Col. SK, M.M. and C (M) viruses in developing eggs. The last named virus stemmed from passage material of the Lansing strain of poliomyelitis virus and was later established by Schultz and White(6) to be antigenically similar to the Col. SK virus. It has been observed to induce typical flaccid paralysis in occasional rhesus monkeys(9) and to be more virulent for cynomolgus monkeys(6). Cultivation of the GD VII strain of Theiler's encephalomyelitis virus in tissue explants has been reported by Parker and Hollender(10), and its cultivation in eggs by Dunham and Parker(11), and Enright and Schultz(9). Cultivation of the FA strain in eggs has been reported by

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