Fig. 1 to 4 and 11 show agglutination of brucella, Fig. 5 to 10 that of typhoid H. The large rods in Fig. 12 are Cortisone rods. Fig. 1 and 2 were made from unstained preparations, all others from preparations stained with Janus Green. Fig. 9, 10, and 12 were magnified × 2000, all others × 1000.

plasma cell series, whereas typical small lymphocytes failed to show the phenomenon. These observations seem to show that it is the plasma cell rather than the lymphocyte which elaborates agglutinins.

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Prevention of Chemotherapeutic Effects of 4-Amino-N^{10}-Methyl-Pteroylglutamic Acid on Mouse Leukemia by Citrovorum Factor.* (18031)

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4-Amino-N^{10}-methyl-pteroylglutamic acid has previously been shown to be effective in prolonging the survival time of mice with transplanted leukemia AK4(1). This prolongation of survival time can be prevented by prior administration of massive doses of pteroylglutamic acid (PGA) or pteroyltriglutamic acid (PTGA)(2). Since the citrovorum factor (C. F.)(3) has been shown to be more effective than PGA in preventing the toxicity of 4-amino-PGA in the bacterium, *Leuconostoc citrovorum*(4), and in mice(5) attempts were made to prevent the antileukemic effect of 4-amino-N^{10}-methyl-PGA by C. F. (6). The detailed results of these studies are herewith reported.

Method. Experimental. The procedure for inoculating mice of the AKm stock with AK4 leukemia was similar to that reported previously(2). 0.1 cc of a saline suspension of leukemic spleen containing 1,000,000 cells was injected intraperitoneally. Forty-eight hours later, treatment with 4-amino-N^{10}-methyl-PGA was started at a dose of 2 mg/kg three times weekly for ten doses by the intraperitoneal route. Intraperitoneal injections of citrovorum factor were started twenty-four hours after the inoculation of the leukemia and then given fifteen minutes before each dose of the antimetabolite. Various dosage levels in units(3) of C. F. were studied against the standard dose of 4-amino-N^{10}-methyl-PGA. In one experiment various levels of C. F. and PGA were given one hour after each injection of 4-amino-N^{10}-methyl-PGA. In another experiment the C. F. was allowed to stand at pH 2.0 for 24 hours at room temperature before injection in order to destroy the C. F. activity(5). After this procedure all the folic acid activity but only 10% of the C. F. activity remained as shown by microbiological assay against *S. fecalis* and *L. citrovorum* respectively.

Discussion. The preparations of citrovorum

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1. Burchenal, J. H., Johnston, S. F., Burchenal, J. R., Kushida, M. N., Robinson, E., and Stock, C. C., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 381.

2. Burchenal, J. H., Kushida, M. N., Johnston, S. F., and Cremer, M. A., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 559.

3. Sauberlich, H. E., and Baumann, C. A., *J. Biol. Chem.*, 1948, v176, 165.

4. Sauberlich, H. E., *Arch. Biochem.*, 1949, v24, 224.

5. Broquist, H. P., Stokstad, E. L. R., and Jukes, T. H., *J. Biol. Chem.*, 1950, v185, 399.

6. Burchenal, J. H., Johnston, S. F., Broquist, H. P., and Jukes, T. H., *Cancer Research*, 1950, v10, 208.

TABLE I.
Prevention of Chemotherapeutic Effect of 4-Amino-N^{10}-methyl-PGA by C.F. and PGA.

Exp.	4-amino-N^{10}-methyl-PGA in mg/kg	C.F. in u/kg × 10^3	PGA in mg/kg	No. of mice	Range of survival time, days	Mean survival time, days
1	—	—	—	10	9-14	11.6
	2	—	—	10	21-35	27.6
	2	5000*	—	10	11-15	13.5
	2	2500*	—	10	11-15	13.1
	2	1250*	—	10	14-19	15.5
	2	625*	—	10	15-28	18.6
	2	—	7.5†	9	15-35	23
	2	—	3.7†	8	14-32	25
	2	—	1.8†	10	19-35	27.7
2	—	—	—	10	7-11	9.0
	2	—	—	6	22-28	25.3
	—	5000	—	10	7-12	8.1
	2	5000*	—	10	7-19	11.4
	2	2500*	—	10	11-17	14.2
	2	1250*	—	7	10-16	12.3
	2	625*	—	10	14-21	16.2
	2	312.5*	—	10	10-22	17.5
	2	78.1*	—	10	16-35	20.7
	2	39.05*	—	9	16-28	21.1
	2	—	15*	9	11-16	13.7
3	—	—	—	30	7-15	8.8
	2	—	—	23	21-28	24.3
	—	3000	—	11	7-10	7.7
	2	3000‡	—	10	14-22	16.1
	2	1000‡	—	9	13-28	19.4
	2	300‡	—	8	11-30	21.9
	2	100‡	—	10	14-30	22.8
	2	—	30‡	6	20-28	23.0
	2	—	10‡	9	20-29	25.2
	2	—	3‡	11	11-28	22.2
4	—	—	—	9	7-13	9.2
	2	—	—	10	15-27	22.8
	2	1250*	—	6	14-20	15.2
	2	2500* (acid hydrolyzed)	—	10	14-27	21.4
	2	1250* (acid hydrolyzed)	—	10	20-27	24.5
	2	625* (acid hydrolyzed)	—	8	20-27	23.1

* Injected ¼ hr before the injection of 4-amino-N^{10}-methyl PGA.
† Injected 1 hr before 4-amino-N^{10}-methyl PGA.
‡ Injected 1 hr after 4-amino-N^{10}-methyl PGA.

factor used in these experiments had an activity for *L. citrovorum* of 3,000,000 units(3) per cc and contained 3 mg/cc of organic material. Thus, this impure preparation of C. F. can be considered as having 1,000,000 units of activity per mg.

As can be seen from Table I, 625,000 units/kg to 1,250,000 units/kg (0.62 to 1.25 mg/kg) of C. F. are approximately as active as 15 mg/kg of PGA in preventing the antileukemic effect of 4-amino-N^{10}-methyl-PGA, and on a dry weight basis C. F. is thus 12 to 24 times as active as PGA. When PGA or C. F. were given one hour after the antagonist, 30 mg/kg of the former was without effect, but C. F. showed some effect at 3 mg/kg of dry material.

When the preparation of C. F. was allowed to stand at room temperature for 24 hours at pH 2.0, which did not diminish its folic acid activity, its effectiveness in preventing the effects of 4-amino-N^{10}-methyl-PGA was markedly decreased, further emphasizing that this effect of the preparation of C. F. was due

to its C. F. content and not to free folic acid possibly present as a contaminant or formed by the action of the hydrogen ion on the C. F. preparation.

Since 1 cc of the C. F. preparation containing 3.0 million C. F. units had a folic acid activity equivalent to approximately 1.0 mg of PGA as determined by microbiological assay with *S. fecalis*, 0.42 cc of the preparation, containing 1.25 million units, had a folic acid activity corresponding to 0.42 mg of PGA. Thus the preparation was 36 to 72 times as potent in preventing the antileukemic effects of 4-amino-N^{10}-methyl-PGA as could be expected from its total folic acid activity.

Summary. The effect of 4-amino-N^{10}-methyl-PGA in prolonging the survival time of mice with transplanted leukemia AK4 can be blocked almost completely by prior administration of 1/3 to 2/3 as much by dry weight of a preparation of citrovorum factor. Thus, citrovorum factor is at least 12 to 24 times as active as pteroylglutamic acid in preventing the antileukemic effect of 4-amino-N^{10}-methyl-PGA.

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Pressure Gradients in the Atria and Pulmonary Veins in Man. (18032)

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Careful analysis of the pressure records obtained from the pulmonary veins and chambers of the heart was carried out on 4 noncyanotic persons selected because of their varying anatomic and physiologic defects. In each instance, the pressure in the pulmonary vein was found to be higher than pressures measured in either or both atria. The cases form a unique group: in 2 the pulmonary vein, being anomalous, drained into the superior vena cava and the right atrium respectively; in 1 there was an atrial septal defect with a large left-to-right shunt; and in the fourth there was a patent foramen ovale, without demonstrable shunt, associated with moderate pulmonary stenosis. The observations on the pressure gradients are in accord with the early reports of Cournand and associates(1) and of Dexter and associates(2,3), who reported their findings in patients with atrial septal defect. One of the purposes of this study was to investigate, by detailed examination of pressure pulses, the concept that the gradient of pressure producing flow of blood from left to right atrium results from pressure transmitted through the pulmonary circulation by the right ventricle. This view was not expressed by Little and associates(4).

Methods. Records of the 4 patients adequate for analysis were obtained over a short sequence of time from various cardiac chambers. In each instance the tip of the cardiac catheter entered a pulmonary vein and was confirmed in a position several centimeters distal from the atrial opening by roentgenography. Recorded pressure and oxygen content of a sample of blood were also measured with the catheter tip in the same location.

Simultaneous records of blood pressure, of respiratory cycle and of electrocardiogram were made in each instance except in Case 1, in which respiration was not recorded. For measurement of pressure a strain gauge manometer was attached to the external end of the catheter. The reference point of zero pressure was located at the midpoint of the thorax at the level of the third rib at the

1. Cournand, A., Motley, H. L., Himmelstein, A., Dresdale, D., and Baldwin, J., *Am. J. Physiol.*, 1947, v150, 267.

2. Dexter, L., Haynes, F. W., Burwell, C. S., Eppinger, E. C., Sosman, M. C., and Evans, J. M., *J. Clin. Invest.*, 1947, v26, 561.

3. Hellems, H. K., Haynes, F. W., and Dexter, L., *J. Applied Physiol.*, 1949, v2, 24.

4. Little, R. C., Opdyke, D. F., and Hawley, J. G., *Am. J. Physiol.*, 1949, v158, 241.