left to future research.

Summary. The excised crayfish ventral nerve cord exposed to ultrasound (~35 watts/cm^2, frequency 1 mc) exhibited a reduction of spontaneous activity after several seconds exposure and recovered its original activity about one minute after the ultrasound was turned off. Frogs positioned so that ultrasound was incident on the dorsal surface over the lumbar enlargement of the spinal cord exhibited paralysis of the hind legs after 4.3 sec. exposure (at room temperature) and exhibited paralysis after 7.3 sec. exposure (at 1°-2°C). Histological examination of the sciatic nerves showed extensive degeneration of nerves and examination of the spinal cord indicated destruction of the lower motor neurones. It was concluded that the effect of ultrasound on the systems studied is produced by physical factors other than temperature.

We would like to extend our appreciation to Professor C. L. Prosser for his continual interest and helpful suggestions during the course of this research. We also wish to thank Professor Warren McCulloch for his interest in this work.

† Cavitation is a name applied to the phenomenon of formation of holes in liquid media.

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Effect of Citrovorum Factor in Pernicious Anemia.* (18491)

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(Introduced by Wm. Dock).

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Although Addisonian pernicious anemia is therapeutically well controlled by the parenteral administration of vit. B_{12}, the exact mechanism of response, and particularly the relationship of vit. B_{12} to pteroylglutamic acid (PGA), is still not well defined. Because of bacterial interrelationships of the citrovorum factor (CF) to vit. B_{12}, PGA, and thymidine, and because of the hematopoietic activity of vit. B_{12}, PGA, and thymine in pernicious anemia, it was decided to study the clinical and hematologic effect of CF in patients with pernicious anemia. CF(1) is a substance first obtained from liver extracts active against pernicious anemia, the unit of which has been defined as that amount of substance required for half maximal growth of *Leuconostoc citrovorum* 8081 in 72 hours on a basal medium. *L. citrovorum* shows some growth response to thymidine(2-5) or to large amounts of PGA, but none to vit. B_{12}(4,5). CF can replace PGA as a growth factor in all organisms that have been tested. Acid hydrolysis destroys the citrovorum activity, leaving the PGA activity unchanged(6,7). CF can be obtained from liver and yeast extracts(1,5) and has been isolated from the urine of rats and humans given PGA(8,9). Its biological synthesis by rat liver slices is

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TABLE I. Citrovorum Factor: Hematological Response in Patients with Addisonian Pernicious Anemia.

Case No.	1	2	3	4	5	6*
Citrovorum factor, mill. units I.M. daily	5	5	5	10	10	10
Initial RBC, mill./cu mm	1.46	2.55	2.19	1.30	2.65	1.75
RBC, mill./cu mm at 3 wk	3.09	3.56	3.27	2.51	3.75	
Expected RBC at 3 wk, mill./cu mm	3.15	3.69	3.54	3.04	3.76	
Day of reticulocyte peak	10	10	11	8	7	None
Max. reticulocytes, %	12.7	5.4	6.6	6.9	9.8	
Expected max. reticulocytes, %	24.5	10.0	16.0	27.0	9.0	20.5

* Subsequently received 120 γ B_{12} I.M. and responded with reticulocyte and RBC rise.

augmented by PGA and ascorbic acid(10). Folinic acid, a substance with CF activity, has been extracted from hog liver and synthesized chemically by hydrogenation of formylfolic acid(11-13). In experiments studying the inhibitory growth effect of the PGA antagonists, aminopterin and amethopterin, on *L. citrovorum*(7,14,15), *Strep. faecalis*(7), mice(7,16,17), chick embryos(18), and mouse leukemia(19), it was found that CF was very active in antagonizing the inhibition in a competitive manner. This was in contrast to PGA which gave little or no protection except when administered in large amounts, and to vit. B_{12} which showed no effect whatsoever. Thymidine, however, reversed aminopterin toxicity, but did so in a non-competitive fashion.

In an attempt to learn more about CF relationships, we undertook a study of the effect of CF in pernicious anemia patients, both with and without the addition of aminopterin.

Material and methods. Ten patients, ages 45 to 80 years, with Addisonian pernicious anemia hospitalized on the medical services of Kings County Hospital were studied. All received the usual hospital diet. In all cases the marrows were megaloblastic and histamine-fast achlorhydria was present. Red blood cell, white blood cell, hemoglobin, and hematocrit determinations were done 3 times a week on venous blood with Wintrobe oxalate mixture. Reticulocyte values were determined daily. The CF used† contained 20 million units per ml and was diluted with physiologic saline for doses of less than 10 million units. It was administered intramuscularly in all cases.

Results. The data on those patients who received CF alone are recorded in Table I. Where transfusions were given before therapy, the initial red blood cell counts noted are those after transfusion. Of the first 5 cases, only case 5 (Fig. 1) had a maximal reticulocyte response. This patient received 10 million units daily. Case 4, on the same dosage, had a submaximal reticulocyte peak and a submaximal rise of red blood cells at the end of 3 weeks. However, all 3 cases (Case No. 1, 2, 3) who received 5 million units daily had maximal red cell rises at 3 weeks despite submaximal reticulocyte peaks. All 5 patients had conversion of the marrow to a normal normoblastic one while on therapy.

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† The CF ("Leucovorin") was kindly supplied to us by Dr. J. M. Ruegsegger and Dr. T. H. Jukes, Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y. Twenty million units per ml is equivalent to 3 mg CF per ml.

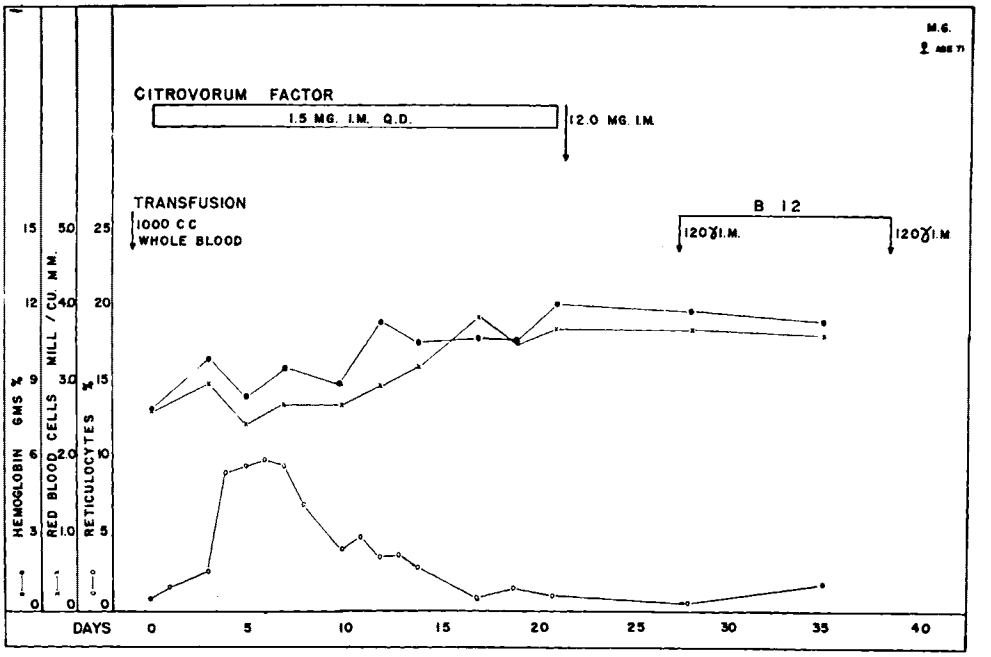

FIG. 1.

All had satisfactory clinical improvement, although it seemed somewhat less dramatic than that expected with vit. B_{12} or liver extract therapy. The glossitis in Case 3 was unaltered at the end of 3 weeks. There was no change in the neurological status in any of the patients during the 3 weeks of therapy.

However, one patient (Case 6) showed no response to CF in a dosage of 10 million units daily. On the eighth day of treatment she had a reticulocyte count of 0.1% and a falling red blood cell count, hemoglobin, and hematocrit, with a megaloblastic marrow. She subsequently responded to single intramuscular injection of 120 γ of vit. B_{12}.

In order to study maturation effect *in situ* in the bone marrow, one patient (Casel) received 10 million units of CF intrasternally. At the end of 48 hours there was partial conversion of the megaloblastic marrow to a normoblastic one at the same site, but a specimen of marrow aspirated simultaneously from the iliac crest showed a similar state of maturation.

Four additional patients were given aminopterin in addition to specific therapy with PGA and vit. B_{12} in an attempt to inhibit their hematopoietic response, so that subsequent administration of CF could be studied for its possible antagonistic effect. Case 7 (Fig. 2) received 0.125 mg aminopterin and 7 mg PGA intramuscularly on alternate days for a 10 day period. (This amount of PGA when given alone is expected to produce optimal hematopoietic response in a pernicious anemia patient). In this case there was a submaximal rise of 10.9%. Furthermore, there was a secondary reticulocyte peak of 6.1% in the second 10 day period during which time 20 million units of CF every other day was substituted for the PGA. Case 8 (Fig. 3) received twice the amount of aminopterin (0.25 mg) and the same amount of PGA (7 mg) every other day for a 10 day period with no reticulocyte response. Subsequent administration of CF in place of the PGA resulted in a submaximal reticulocyte peak of 6.9% on the 9th day.

Two additional patients were given vit. B_{12} simultaneously with aminopterin. One patient received 0.25 mg aminopterin, 30 γ vit. B_{12}, and 15 mg PGA intramuscularly every other day for a 10 day period. The other patient received 1 mg aminopterin and 10 γ vit. B_{12} on alternate days for a period of 10 days. Since both patients had a maximal reticulocyte response, CF was not given in a later period.

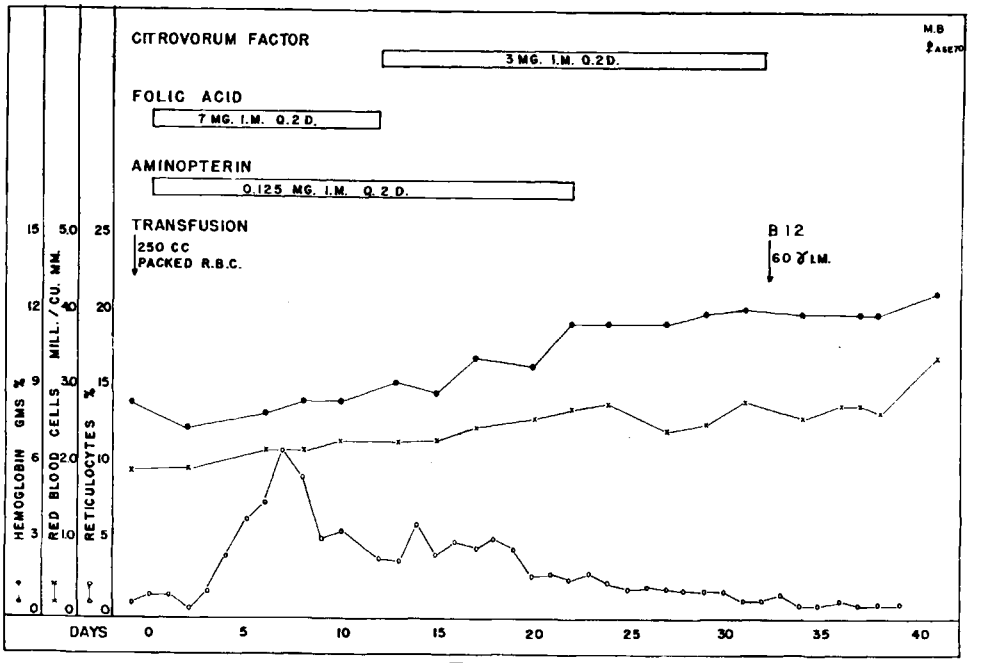

FIG. 2.

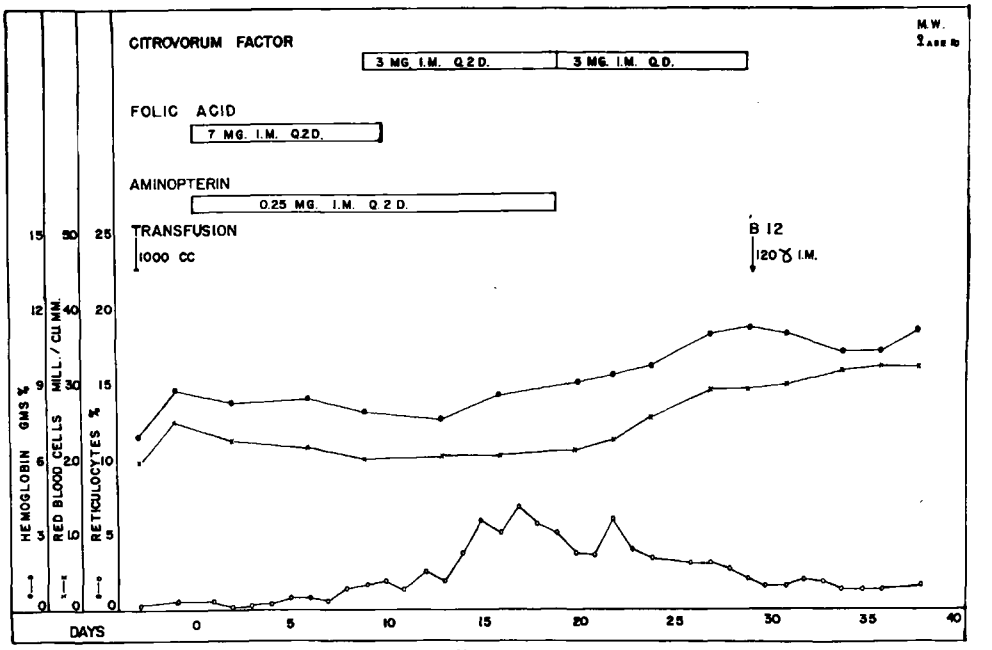

FIG. 3.

Discussion. Five of 6 patients with pernicious anemia gave satisfactory responses to the daily intramuscular administration of 5 to 10 million units (0.75 to 1.5 mg) of CF. In view of the fact that 4 cases gave maximal red cell rises at the end of 3 weeks, this amount might be considered to be equivalent to 1 U.S.P. unit of liver extract. However, the reticulocyte responses were only about half that expected, with the exception of one maximal response. Although a lack of correspondence between reticulocyte and red blood cell

response in pernicious anemia patients treated with liver extract has been reported by Clark (20), the reticulocyte responses seen here seem unusually poorly correlated with the erythrocyte responses, so that it may be possible that CF is relatively lacking in specific reticulogenic activity. Studies are in progress to determine whether daily doses of less than 5 million units of CF can produce a maximal erythrogenic response. The fact that one of the patients failed to give any response to 10 million units daily of CF is of considerable interest, but cannot be explained at the present time. Therapeutically and hematologically CF is not as dramatically effective as vit. B_{12} in the treatment of Addisonian pernicious anemia. This might be expected because of the closer chemical similarity of CF to PGA. For this reason, it will be of interest to observe the effects of CF in megaloblastic anemias associated with the presence of free HCl, particularly in the rare cases refractory to both PGA and to vit. B_{12}. CF has been ineffective in one patient with macrocytic anemia due to liver disease and in one patient with refractory macrocytic anemia, in both of whom the marrow was normoblastic. The lack of local maturation effect on megaloblasts in the marrow after intrasternal instillation of CF in one of our cases corresponds to the negative effect of PGA(21) under similar circumstances, and is in contrast to the local maturing action of vit. B_{12}.

Of considerable interest has been the effectiveness of CF in 2 of our patients in reversing the inhibitory effect of aminopterin. In one case PGA was without any effect, and in another case PGA was only partially effective, with amounts given on a comparable weight basis to the CF. However, we have not yet used amounts small enough to check the ratio determined by Burchenal *et al.* (19). These investigators found 625,000 to 1,250,000 units (0.18 to 0.33 mg) per kg of CF to be approximately as active as 15 mg per kg of PGA in preventing the antileukemic effects of amethopterin in mouse leukemia. This is a ratio of about 1:50 of CF to PGA on a weight basis.

Aminopterin had no inhibitory effect on the response of the two patients who received vit. B_{12}. This is in contrast to the finding of Meyer and associates(22), and may be due to a relatively insufficient amount of aminopterin. However, there is no reason to assume that the aminopterin-induced physiological changes are directly comparable to the defects present in pernicious anemia. Furthermore, it is quite possible that PGA and vit. B_{12}, although arriving at the same hematological endpoint in pernicious anemia, do so through distinct though related routes.

Summary. 1. Citrovorum factor, in amounts of 5 to 10 million units daily intramuscularly, has been shown to produce adequate hematologic and clinical response in 4 of 6 patients with Addisonian pernicious anemia, and a submaximal response in another. A 6th patient failed to respond altogether. 2. Local marrow instillation of citrovorum factor failed to produce enhanced maturation of megaloblasts *in situ*. 3. Citrovorum factor has been found to be more effective than pteroylglutamic acid in reversing aminopterin inhibition in patients with pernicious anemia.

We wish to express our gratitude to the members of the medical house staff, whose cooperation made this study possible.

Addendum. Although Bethell and coworkers (J. Lab. Clin. Med., 1948, v 33, 1477.) found that the simultaneous administration of 1 mg aminopterin and 1 γ vit. B_{12} daily for 16 days did not cause a hematologic response, this result may be due to the relatively small amount of vit. B_{12} administered.

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