tive cultures are detected and the reduction of bacteriological manipulation and material, plus the increased safety to the technician, compensate for the trouble taken in the preparation of the double medium. Furthermore, the technician in charge of identification of the organism receives only positive cultures and need not be warned about careless handling of the material. The possibilities of accidental infection are thereby reduced. The increase in the proportionate amounts of agar in the solid medium is necessary in order to prevent the deterioration of the agar layer. The rather high citrate content is intended not only to prevent clotting of the blood but also to reduce the phagocytic activity of polymorphonuclear leucocytes to a minimum. It is thought that the ease with which the Brucella colonies appear on the agar layer is due at least in part to the absence of plasma, thus reducing the bactericidal effect.

The 100 cc bottles have been found suitable for inoculation with 100 ml of blood. However, 200 or even 500 ml bottles may be used if one desires to culture larger amounts of blood.

Summary. A double medium for blood cultivation in brucellosis is described. The method saves time and material by avoiding transfers and affords increased safety against accidental infection to the personnel.

15718

Hematologic Effects of Pteroylglutamic Acid (Folic Acid) in Man.*

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Pteroylglutamic acid, commonly known as folic acid or *L. casei* factor, has been shown by several groups of investigators to be an effective hematopoietic substance in human macrocytic anemia.[1,2,3] Since 1945 when synthetic pteroylglutamic acid became available, 23 patients with macrocytic anemia have been treated with this material in our clinic. The findings obtained in 15 patients during the first few months of therapy have been reported.[4,5,6] Five patients have now been followed for approximately one year and 2 additional patients for over 6 months. In each instance, the clinical and hematological improvement which followed the administration of pteroylglutamic acid has been maintained.

Seventeen of the 23 patients studied were benefited by therapy. Hematologic findings at the initial and most recent visits to the clinic, and the amount and method of administration of pteroylglutamic acid are given in Table I. In all patients with pernicious anemia and nutritional macrocytic anemia there was a marked increase in the erythrocyte count, in hemoglobin and in the volume of packed red blood cells. In patients with sprue the blood picture was affected in only 3 of 5 cases. However, each patient improved in other respects including relief of glossitis and diarrhea, gain in weight, better absorption of nutrients from the intestinal tract as indicated by the glucose tolerance test, and diminution in steatorrhea. In one patient with cirrhosis of the liver there was a slight increase in the blood count after pteroylglutamic acid was administered while in 5 patients with macrocytic anemia of varied

* This research was supported by grants from the Nutrition Foundation, Inc., and the United States Public Health Service. Pteroylglutamic acid was furnished through the courtesy of Lederle Laboratories.

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TABLE I.
Effect of Pteroylglutamic Acid on the Blood Picture in Macrocytic Anemia.

Sex Race	Hematologic findings: Initial RBC	Initial Hb	Initial Het	Final RBC	Final Hb	Final Het	No. months observation	Pteroylglutamic acid therapy: mg/day	Time	Route
					Pernicious Anemia.					
WF	1.7	6.2	21	4.2	13.0	40	12	15‡	21D	IM
								15	7M	O
								5	3M	O
CM	1.7	4.9	14	4.0	13.0	44	12	15	21D	IM
								0	6M	—
								5	5M	O
WM	3.5	10.5	36	5.0	14.9	46	7	120	5D	O
								30	14D	O
								5	6M	O
CM	1.0	3.3	10	3.5	11.2	41	2	5	2M	O
WF	1.4	6.0	21	4.2	13.2	42	3.5	10	3M	O
WF	2.1	8.2	23	4.6	14.5	47	3.5	10	3.5M	O
CM	1.5	4.2	16	4.0	10.3	39	2	5	2M	O
					Nutritional Macrocytic Anemia.					
WF	1.8	6.0	20	3.9	12.0	39	12	15‡	21D	IM
								15	3M	O
								30	1M	O
								5	6M	O
WF	2.3	7.0	22	4.1	13.9	44	11	15‡	21D	IM
								30	2M	O
								5	6M	O
WF	2.3	9.5	25	4.0	12.3	39	13	5-15†	5M	O
								30	3M	O
								5	5M	O
WF	2.3	8.4	29	4.4	11.5	40	8	40§	2M	IM
								100‡	7D	O
								5	5M	O
					Sprue.					
WF	3.5	11.5	39	3.7	10.2	35	3.5	50	21D	O
								20-40§	2M	IM
WF	3.6	11.3	38	3.9	11.9	39	2.5	30	2M	O
WM	2.1	7.3	27	4.0	12.5	39	3	5	3M	O
WM	3.0	10.0	36	3.4	11.0	37	1	20¶	1M	IM
WF	3.4	10.9	37	3.8	11.5	38	1	10	1M	O
WM*	3.9	10.7	38	4.4	12.6	41	1	20	14D	IM

IM—Intramuscular administration; O—Oral administration; D—Days; M—Months; Het—Hematocrit-volume of packed erythrocytes %.

* Celiac disease.

† Therapy intermittent.

‡ Therapy omitted for 1-2 months after this period of treatment.

§ This dosage administered 3 times weekly.

¶ This dosage administered every 4 days.

etiology there was no appreciable change (2 had aplastic anemia, one each, anemia associated with regional ileitis and myxdema and one anemia of unknown origin).

The hematologic effects of pteroylglutamic acid closely resembled those obtained with liver extract. Within 2 to 6 days after therapy was instituted the percentage of reticulocytes in the peripheral blood increased, a maximum rise being attained in 5 to 14 days. When pteroylglutamic acid was given orally the increase in reticulocytes was similar to that which is usually observed when optimal amounts of liver extract are administered; when given parenterally the increase in reticulocytes was usually less than this (Fig. 1). During the first few days of treatment hemodilution occurred and edema was found on physical examination. The erythrocyte count, percentage of hemoglobin and the volume of packed red blood cells began to rise 7 to 10 days after therapy was instituted. The increase was rapid during the first month and then gradual until normal or slightly sub-

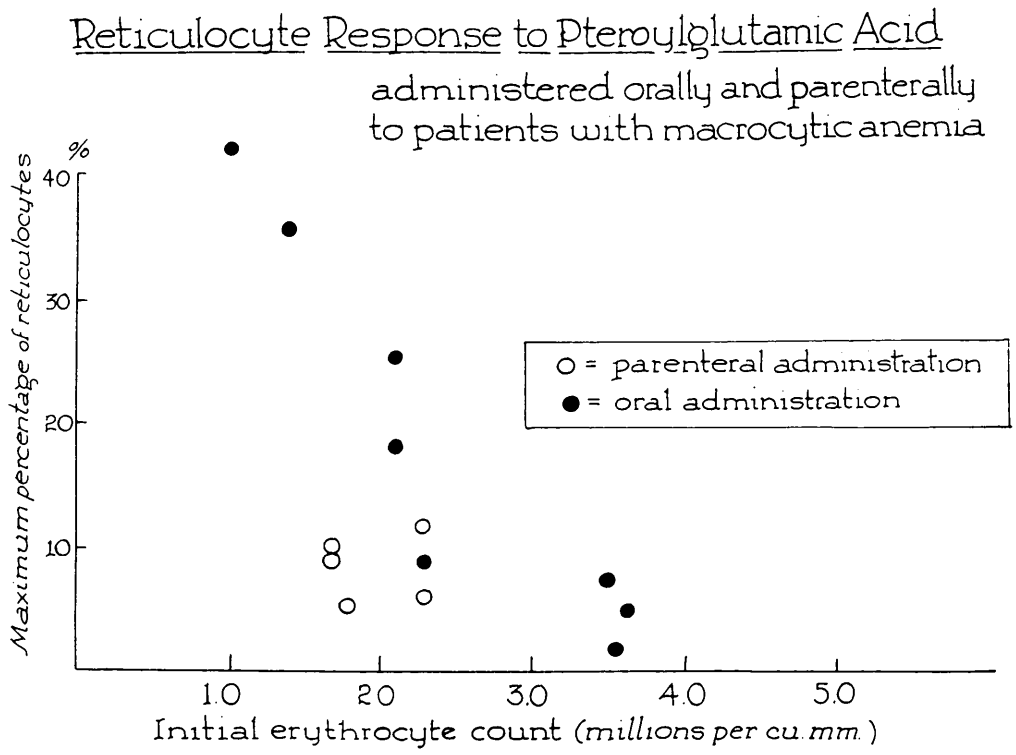

FIG. 1.

The amount of pteroylglutamic acid given parenterally was 15 mg or more daily; the oral dose in the four patients with a reticulocyte response above 15% was 5 to 10 mg daily.

normal levels were reached. The monthly erythrocyte counts for 7 patients who have been followed for over 6 months are given in Table II. The maximum rise occurred by the third month in most instances and this level has been maintained essentially unchanged. The count has remained below 4.5 million in a majority of the patients in spite of intensive treatment. This may indicate a difference between the effects of pteroylglutamic acid and of liver extract.

A dose of 5 mg of pteroylglutamic acid daily

TABLE II.

Erythrocyte Count in Patients with Macrocytic Anemia During Treatment with Pteroylglutamic Acid.

Sex Race	Initial count	Erythrocyte count (millions per mm³) monthly intervals												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Pernicious Anemia.														
W.F.	1.72	3.20	3.32	4.0	3.9	4.05	4.3	3.9	4.5	4.51	3.4	3.6	4.2	
C.M.	1.72	3.30	3.5	4.1	4.2	3.0	2.5	2.31	3.4	3.5	4.3	4.2	4.0	
W.M.	3.44	4.21	5.8	6.0	4.4	4.5	4.7	5.0						
Nutritional Macrocytic Anemia.														
W.F.	1.82	3.40	3.32	4.0	3.9	3.93	4.21,5	4.2	4.3	3.9	4.0		3.9	
W.F.	2.32	3.50	3.6	3.63	3.7	3.81	4.4	4.5	4.5	4.3	4.0	4.1		
W.F.*	2.31	2.92	3.00	3.12	3.40	3.93	4.0	4.1	4.21	4.0	4.0	3.8	4.0	4.0
W.F.	2.36	3.7	3.77	4.01	4.2		4.0	4.1	4.4					

* Therapy intermittent during first 5 months.

Amount of pteroylglutamic acid administered and changes in therapy are indicated by numerals as follows: (0) no therapy; (1) 5 mg daily; (2) 15 mg daily; (3) 30 mg daily; (4) 120 mg daily for 5 days then 30 mg daily for 2 weeks; (5) iron added to therapy; (6) 40 mg three times weekly; (7) 100 mg daily for 1 week then none for 1 month.

appeared to be approximately as effective as were larger amounts in producing reticulocytosis and an increase in erythrocyte count. The administration of 30 mg daily did not cause significant improvement over that seen with 15 mg a day (cases 4-5-6, Table II). However, the only patient (Case 3) whose red cell count reached 5 million was given very large amounts of pteroylglutamic acid initially. The 7 patients who have been followed for prolonged periods have received only 5 mg daily for the past 3 to 6 months. During this time the erythrocyte counts have remained essentially constant. This dosage seemed to be sufficient and may be more than the minimal amount needed for maintenance.

The initial leukocyte count was low (less than 3500 per mm^3) in 6 patients included in this study. In each instance there was a return to normal when pteroylglutamic acid was administered and the percentage of granulocytes increased. The rise in white cells began during the first week of therapy and reached a maximum in 1 to 3 weeks.

The bone marrow was studied in most of the patients treated with pteroylglutamic acid and showed a rapid return to normal. In pernicious anemia the marrow is hyperplastic, megaloblasts being the predominant cell. In one patient in whom serial studies were undertaken, megaloblasts had decreased in number at the end of 5 days and were rarely observed at the end of 2 weeks. In patients with nutritional macrocytic anemia the marrow was at times hyperplastic and at others hypoplastic. The predominant cell was the megaloblast in some instances, the normoblast in others. Regardless of the findings prior to therapy, the bone marrow became normal within 4 to 6 weeks.

Conclusion. The administration of pteroylglutamic acid was followed by definite improvement in 17 of 23 cases of human macrocytic anemia. The hematologic effects of pteroylglutamic acid closely resembled those of liver extract but differed in that oral administration was equally or more effective than parenteral and that restoration of the blood picture to normal was often incomplete.

15719 P

Antibiotic Activity of Certain Molds Against *Brucella.**

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Although a number of mold antibiotics have been described,[1] few reports indicate activity against *Brucella spp.*[2,3] The ineffectiveness of penicillin against bacteria of this group was early recognized,[4] and this observation has received repeated confirmation. Despite the demonstration of inhibition of Brucella by high concentrations of penicillin,[5,6] resistance of these organisms is so great that the drug has been successfully used in concentrations of 2-5 Oxford units per ml for selective isolation of Brucella from material containing other more sensitive bacteria.[6]

Experimental. In a preliminary study 202 mold cultures,‡ including strains of Aspergillus, Penicillium, Absidia, Rhizopus, Mucor, Chaetomium, Trichoderma, Sporotrichum,

* Aided by a grant from Swift and Company.

† The early part of this study was made at The Brucellosis Research Laboratory, Clayton Foundation, The University of Texas.

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‡ Cultures were kindly made available by Dr. M. B. Morrow, Department of Botany and Bacteriology, The University of Texas.