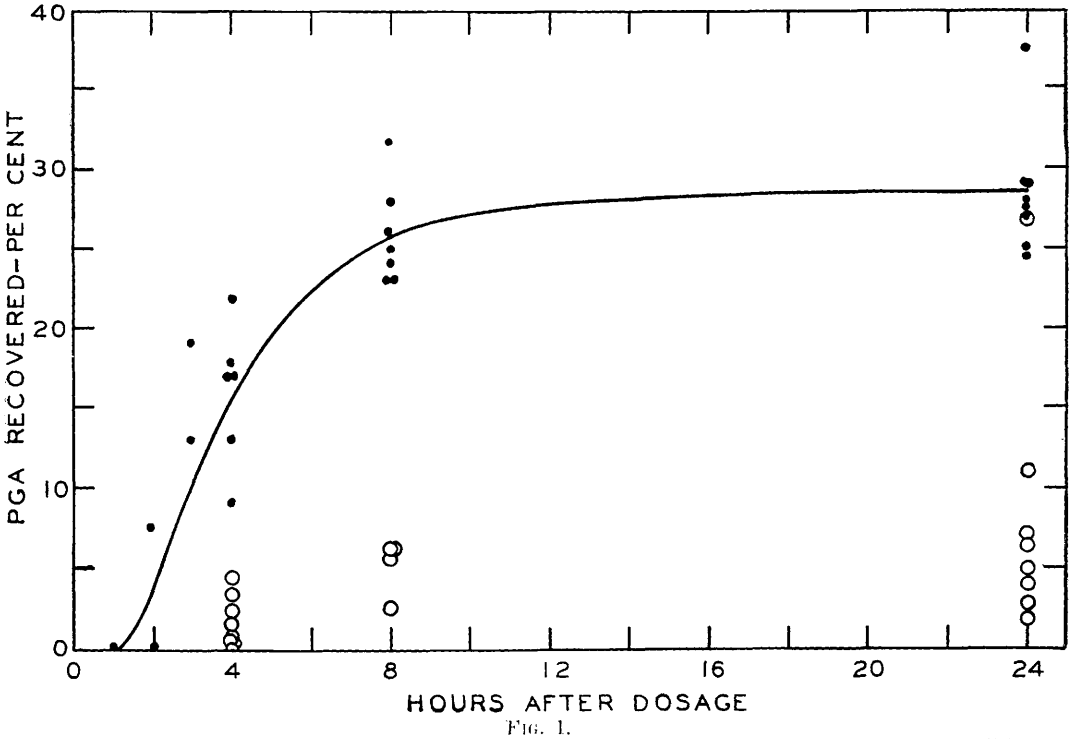

FIG. 1.

The percentage urinary recovery of pteroylglutamic acid after single oral doses of 5.0, 5.1, 10 or 16 mg. ●—Normal subjects; ○—hospital patients.

centage returns from single oral doses were much lower than in the normal subjects, a finding which is possibly indicative of a low degree of saturation.

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Effect of Folic Acid upon Primitive Erythrocytes *In vitro*.*

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Since the antianemic properties of pteroylglutamic acid (synthetic folic acid) were reported[1] there have been many studies to determine how this substance acts upon the bone marrow to stimulate the production of cells and to ascertain the relationship of pteroylglutamic acid to the antipernicious anemia principle. Efforts to demonstrate significant amounts of pteroylglutamic acid in highly potent antipernicious anemia liver fractions have failed.[2,3] Attempts made to liberate free pteroylglutamic acid from ground muscle treated with normal gastric juice according to the technic of Castle and Town-

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† With the technical assistance of Miss Betty Paulsen, Mrs. Francena Galbraith, and Miss Constance Brownell.

1 Spies, Tom D., Vilter, C. F., Koch, M. B., and Caldwell, M. H., *Southern Med. J.*, 1945, **38**, 707.

2 Clark, Guy W., *Am. J. Med. Sci.*, 1945, **209**, 520.

3 Stokstad, E. L. R., and Jukes, T. H., PROC. SOC EXP. BIOL. AND MED., 1946, **62**, 112.

TABLE I.

Substrate added to Tyrode solution*	Final conc., μg per ml	No. of reticulocytes per 1000 nucleated cells: Avg†	Range
None	—	7 (20)	3-8
Pteroylglutamic acid (folic acid)	4.9	8 (2)	8-8
	.05	8 (4)	6-9
	.0005	8 (2)	5-10
	.00005	7 (2)	7-7
Hexaglutamyl-pteroylglutamic acid (vitamin B_c conjugate)	1.0	8 (2)	7-9
	.1	7 (2)	7-7
	.01	6 (4)	5-8
Rat serum	—	13 (20)	9-34
Normal human serum	—	15 (4)	9-24
Human pernicious anemia serum‡	—	7 (1)	—
Human pernicious anemia serum‡ and liver extract	.005	13 (1)	—
Human pernicious anemia serum‡ and pteroylglutamic acid	.0025	8 (1)	—
Liver extract§	.1	11 (6)	6-18
	.01	11 (16)	9-18
	.001	13 (1)	—
	.0001	10 (2)	7-14
	.00001	6 (2)	6-6

* All substrates were dissolved in glucose-free Tyrode solution. In the cases where serum was added, 1 volume of serum was added to 3 volumes of glucose-free Tyrode solution.

† Numbers in parentheses indicate number of different experiments.

‡ Taken from case of pernicious anemia in relapse.

§ Liver extract powder provided by The Armour Laboratories. This extract (Lot No. 11285A) was used in the preparation of a 15 U.S.P. unit liver extract. 17 mg was equivalent to 1 U.S.P. unit.

send[4] in this and other laboratories likewise have failed. It would appear likely, therefore, that either the antipernicious anemia principle produced by the interaction of Castle's "extrinsic" and "intrinsic" factors, and that stored in the liver, are free of pteroylglutamic acid; or that pteroylglutamic acid is a component of the antipernicious anemia factor which ordinary methods of hydrolysis and assay fail to liberate and detect. The report of Welch *et al.*[5] that gastric juice did not liberate free pteroylglutamic acid from the heptaglutamyl (vitamin B_c Conjugate) form would support the latter view. From this report, there also appears to be some difference between the pernicious anemia patient and the normal individual with respect to the ability to break down the conjugated form to free pteroylglutamic acid. This finding agrees with the report of Bethel *et al.*[6] that patients having pernicious anemia fail to excrete increased amounts of pteroylglutamic acid in the urine when the heptaglutamyl conjugate form is fed. On the other hand, normal individuals are able to convert the conjugate form into pteroylglutamic acid and excrete it. In both instances pteroylglutamic acid when administered orally causes a marked increase in the urinary excretion of free pteroylglutamic acid.

In spite of the similarity between the clin-

4 Castle, W. B., and Townsend, W. C., *Am. J. Med. Sci.*, 1929, **178**, 764.

5 Welch, A. D., Heinle, R. W., Nelson, E. M., and Nelson, H. V., *J. Biol. Chem.*, 1946, **164**, 787.

6 Bethell, F. H., Swendseid, M. E., Bird, D. D., Meyers, M. C., Andrews, G. A., and Brown, R. A., *Univ. Hosp. Bull., Univ. of Michigan*, 1946, **12**, 42.

ical response obtained from the administration of antipernicious anemia liver fractions and pteroylglutamic acid in the treatment of the various blood dyscrasias it is becoming apparent that the actual stage at which these 2 substances stimulate erythropoiesis may not be the same. In addition, it is known that on a weight for weight basis, liver extracts are more potent than pteroylglutamic acid or its heptaglutamyl form.

The work reported here is the result of a series of experiments that has been conducted in this laboratory during the past year comprising a study of the effect of pteroylglutamic acid,‡ its heptaglutamyl conjugate form,§ and liver fractions highly potent with respect to their antipernicious anemia content upon red cell maturation *in vitro* in an attempt to secure information concerning the point at which these substances act upon red cell production. After removing the immature cells from other marrow constituents by means of centrifugation the resulting preparation permits a study of the maturation process independent of the modifying factors of the gastro-intestinal tract, the liver, and the bone marrow matrix which are found in the intact animal and hence it is possible to study this one phase of red cell production separately.

Table I summarizes some of the results which were obtained by using bone marrow cell survival technic based upon the method of Osgood and Brownlee.[7,‖] Rat bone marrow cells were incubated 3-5 hours at 37°C while suspended in a glucose-free Tyrode solution. At the end of the incubation period, reticulocyte counts were made using the wet preparation technic and staining with brilliant cresyl blue. Only "mature" reticulocytes were counted. Lang-Levy micropipettes[8] were used in adding the various constituents to the media. A standard liver extract of known concentration was included in each day's experiment.

It will be noted that neither pteroylglutamic acid nor its heptaglutamyl conjugate, appeared to bring about maturation of red cells from their primitive precursors to the mature reticulocyte stage. On the other hand, a liver preparation produced maturation quantitatively similar to that change observed when normal serum is added to the preparation. It should be noted that in a single instance serum from a patient with pernicious anemia in relapse did not show the presence of this maturation factor. It is possible that this experiment may indicate that the stimulant for erythropoiesis found in the blood is similar to that found in liver extract and not similar to pteroylglutamic acid.

The difference between the effect of liver extracts and of pteroylglutamic acid upon immature bone marrow cells has been noted in the presence of glucose, casein hydrolysate, or glucose and casein hydrolysate in the same medium so that it appears not to be a function of a metabolite or a combination of amino acids present. It has been observed that ferrous iron added to any of the above substrates appears to diminish maturation.

Summary. The above evidence indicates that pteroylglutamic acid, or its conjugated form, does not act directly in an unaltered state upon primitive red cells to mature them, whereas a highly potent antipernicious anemia liver preparation appears to cause a maturation comparable to that observed in the presence of serum. A single sample of serum from a patient having pernicious anemia in relapse appeared to lack the maturation factor found in normal human and rat serum. Pteroylglutamic acid failed to increase the maturation of bone marrow cells suspended in this serum.

‡ Courtesy of Lederle Laboratories, Pearl River, N.Y.

§ Courtesy of Dr. J. J. Pfiffner, Parke, Davis and Co., Detroit, Mich.

7 Osgood, E. E., and Brownlee, Inez E., *J. A. M. A.*, 1936, **107**, 123.

‖ Details of this method are to be published.

8 Levy, M., *C. R. trav. Lab. Carlsberg, Serie chim.*, 1936, **21**, 101.