cells it has been estimated that galactosemia in humans may occur as frequently as 1 in 18,000 births. Since galactosemic heterozygotes are generally asymptomatic the identification of a heterozygote of maternal origin among the mentally retarded suggests the possibility that the symptomology may have resulted from inadequate metabolism of galacose by the mother during maternity.

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Effect of Erythropoietin on Cultured Human Leukocytes.* (28969)

WILLIAM MCFARLAND AND FRANCES SOEHNLEIN (Introduced by D. H. Heilman)
Hematology Research Section, V. A. Hospital, and Georgetown University, Washington, D.C.

Phytohemagglutinin-stimulated cultures of mature human leukocytes produce a mitotically active cell which appears to be derived from lymphocytes(1,2). Since the lymphocyte has long been suspected of playing a role as stem cell(3,4), a primitive cell originating from lymphocyte precursors might be multipotential. This possibility and evidence that the hemoglobin type can be altered in irradiated mice by transplantation of leukemoid leukocytes(5) prompted us to study the effect of erythropoietin on cultured human leukocytes.

Method. Human peripheral blood leukocytes were cultured in the presence of phytohemagglutinin according to the technique of Moorhead *et al*(6). Cultures were set up in duplicate in each experiment so that one culture could serve as a control for the erythropoietin-treated culture. Erythropoietin was added in concentrations of .01, .05, 0.1, 0.5 and 2.5 units/ml culture medium at 0, 24 and 48 hours in separate experiments. At 66 hours colchicine was added to each culture and at 72 hours cells were harvested, exposed to hypotonic saline solution and fixed in methanol-acetic acid fixative. The cells were then resuspended and total cell counts determined. An aliquot of each culture was spread on a glass slide, air dried, stained with Orcein and studied for the per cent of cells in metaphase.

Results. Average total cell counts and per cent of cells in metaphase are shown in Table I. Erythropoietin appeared to have no significant effect on this cell system.

TABLE I.

Time of adding erythropoietin	Cell × 10^6/culture at 72 hr Mean (Range)		% cells in metaphase at 72 hr Mean (Range)	
	Control	Erythropoietin treated	Control	Erythropoietin treated
0	7.0 (3.3–9.6)	5.0 (2.1–7.0)	3.1 (1.9–5.0)	3.9 (2.0–4.8)
24	5.9 (3.5–8.8)	5.4 (2.0–9.4)	3.6 (1.5–5.8)	3.3 (2.2–4.6)
48	5.8 (4.0–6.9)	5.9 (5.2–6.5)	3.1 (2.0–6.1)	3.7 (1.6–7.4)

* The erythropoietin used in this experiment was sheep plasma erythropoietin concentrate, kindly supplied by the U.S.P.H.S.

Comment. Erythropoietin has not shown a consistent stimulatory effect in all *in vitro* experiments. However, in those experiments in which it has been effective, results suggest that its major site of action is at the stem cell level where it induces erythroid differentiation(7,8).

Failure to demonstrate erythropoietic effect in the present experiment might be interpreted as evidence against the stem cell as the site of action of erythropoietin. However, it seems more logical to conclude that the primitive cell that emerges as a result of phytohemagglutinin stimulation, even though derived from lymphocytes, may be completely lacking in potential for erythroid or any other differentiation. It may even represent a malignant transformation that is beyond control by normal humoral mechanisms(9).

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Determination of Evans' Blue in Plasma and Plasma-Dextran Mixtures By Protein Precipitation and Extraction.* (28970)

J. MAXWELL LITTLE AND CURTIS WILLIAMS†
Department of Pharmacology, Bowman Gray School of Medicine, Wake Forest College, Winston-Salem, N. C.

A rapid method for determination of Evans' blue dye (T-1824) in plasma has been described by Constable(1). This method involves the precipitation of a portion of the plasma proteins by addition of M/15 Na_2HPO_4 buffer, pH 7.5, and 3 N NaOH. The precipitate is acidified with HCl and extracted with n-butanol. The color in a mixture of the butanol extract and ethanol is determined spectrophotometrically. Besides the shortness of the procedure, other advantages claimed for the method are a lack of interference by variations in inherent plasma color, hemolysis and turbidity of the plasma.

The upper range of the linear relationship between extinction and dye concentration in plasma was quite limited, being no greater than 0.02 mg/ml plasma. Furthermore, we found this method to be unsuitable for determination of the dye in mixtures of dextran and plasma.

Since the method of Constable yields a precipitate representing only 1-2% by weight of the total plasma protein, it occurred to us that the narrow range of dye concentration precipitable by this method and the failure of proportional precipitation in a dextran-plasma mixture might be due to the incomplete protein precipitation under the conditions of the procedure.

This report is concerned with a modification of Constable's procedure which extends the range of linearity between opitcal density and dye concentration and which is equally suitable for normal plasma and for plasma-dextran mixtures.

Procedure. We have adopted the $CdSO_4$ precipitation procedure for plasma proteins of Little(2). The procedure for determination

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