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Glucocorticoids Suppress Formation of Erythroid Colonies Cultured from Human Fetal Liver and Umbilical Cord Blood (41308)

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Abstract. The effect of glucocorticoids on erythroid colony formation *in vitro* was studied in plasma clot and methylcellulose culture of human fetal liver and umbilical cord blood. At concentrations varying from 10^{-10} to 10^{-6} *M*, dexamethasone and hydrocortisone caused 25 to 90% reduction in the number of erythroid colonies present after 5 to 14 days of incubation. This suppression of erythroid colony production by glucocorticoids was not accompanied by an alteration in the ratio of γ and β globin chains synthesized by the erythroid cells.

The capacity of steroidal hormones to influence erythropoiesis has been under investigation for many years (reviewed in Ref. (1)). Androgens and androgen derivatives have been shown to stimulate erythropoiesis in clinical studies and in animal experiments *in vivo* (2). Certain 5β-H steroid metabolites enhance heme and hemoglobin synthesis in the cultured chick blastoderm (3), and in short-term suspension cultures of human bone marrow (4). In addition, the 5β derivatives have been reported to increase erythroid colony formation in clonal assays performed on rat bone marrow grown in semisolid media (5). However, no stimulatory effect on erythropoiesis by 5β-H steroids was demonstrable in a cooperative double-blind study of polycythemic mice (6).

The effects of adrenal corticosteroids on erythropoiesis are controversial. Studies *in vivo* on mice (7–9) and *in vitro* on fetal mouse liver (10), adult mouse bone marrow (10–12), adult rat bone marrow (5), human cord blood (13), human adult blood (14), human bone marrow (10, 15), and mouse (16) and human (17) erythroleukemia cells have suggested either stimulation (8, 10, 13–15) or inhibition of erythropoiesis (5, 7, 8, 11, 12, 16, 17) by glucocorticoids.

During the course of a series of investigations on *in vitro* erythoid colony formation, and fetal and adult hemoglobin synthesis by human fetal liver and umbilical cord blood (18, 19), we investigated the effect of several hormones on those cultures. Glucocorticoids consistently inhibited erythroid colony formation in a progressive manner with increasing dose of hormone.

Materials and Methods. Umbilical cord bloods were collected from human placentas at delivery under sterile conditions into heparinized vials. Fetal liver specimens were obtained with informed consent, at postmortem examination of prostaglandin-induced abortuses of 18–22 weeks of gestation, within 1 hr of delivery.

Erythroid colonies were grown from human fetal liver and umbilical cord blood in 0.1 ml plasma clots as previously described (18, 19). After layering over Ficoll–Paque (Pharmacia, Piscataway, N.J.) and centrifugation at 450*g* for 30 min, cells remaining at the plasma/Ficoll–Paque interface were collected for culture. One-tenth milliliter of a suspension containing 10^6 cells was added to 1 ml of culture medium containing 0.3 ml fetal calf serum, 0.1 ml of 10% bovine serum albumin in phosphate-buffered saline, 0.1 α-thioglycerol, 1:10,000 in NCTC-109, 0.1 ml asparagine, 2 mg/100 ml in NCTC-109, 0.1 ml NCTC-109, 0.1 ml citrated bovine plasma, and 0.1 ml of human urinary erythropoietin (final concentration 0.2–1.0 U/ml. One-tenth milliliter of bovine thrombin (1 U/ml) was added and 0.1-ml aliquots of the mixture were distributed into 0.2-ml culture wells and allowed to clot.

For culture of erythroid colonies in methylcellulose-containing medium, 1 ml of a suspension containing 10^7 cells from a

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Ficoll–Paque centrifugation were added to 11.5 ml of culture medium containing 2 ml of 2X Dulbecco's modified Eagle's medium, 5 ml of 2% methylcellulose, 3 ml fetal calf serum, 0.5 ml NCTC-109, 0.5 ml 10% bovine serum albumin, and 0.5 ml 1:10,000 α-thioglycerol. One-milliliter aliquots were then cultured in 35-mm petri dishes.

Human urinary erythropoietin was obtained from Dr. Peter Dukes, Children's Hospital of Los Angeles, through the offices of the Blood Diseases Branch, Division of Blood Diseases and Resources, National Heart, Lung and Blood Institute.

Dexamethasone, hydrocortisone, estradiol, etiocholanolone, testosterone, dihydrotestosterone, and progesterone were obtained from Sigma Chemical Company, St. Louis, Missouri, and dissolved in ethanol at 10^{-3} *M*. These stock solutions were diluted with NCTC-109 to provide the appropriate working concentrations. Diluted ethanol without hormone served as a control.

Cultures were incubated at 37° in an atmosphere of 96% air, 4% CO_2 in high humidity. Colonies in clot cultures were detected by benzidine–hematoxylin staining of glutaraldehyde-fixed clots. In methylcellulose cultures, erythropoietic colonies were detected by direct microscopic examination using a blue filter.

Globin chain synthesis in erythroid colonies was measured after incubation with [^{3}H]leucine followed by chain separation and liquid scintillation counting as previously described (19).

Results. When human fetal liver cells are placed in plasma clot culture in the presence of erythropoietin, erythroid colonies appear as early as Day 3 and reach a maximum number at approximately Days 9–10 (18). No colonies grow in the absence of erythropoietin and maximal response occurs in the range of 1.0–2.0 units of erythropoietin/ml (18). In the range of 0.2–0.6 U/ml there is a linear increase in erythroid colony number with increasing concentration of erythropoietin (Fig. 1, Table I). When dexamethasone was added to the medium and the cultures examined after varying periods of incubation there was a progressive decline in the number of erythroid colonies derived from human fetal liver samples with increasing concentration of steroid hormone. Several representative experiments are shown in Fig. 1 and Table I. The suppressive effect was observed over the whole range of concentrations of erythropoietin employed. At concentrations of 10^{-6} or 10^{-7} *M* dexamethasone, the number of erythroid colonies present was reduced to 10–30% of control cultures without steroid. Some suppression of erythroid colony formation occurred when concentrations of dexamethasone as low as 10^{-10} *M* were employed (Table I). This suppressive effect was not limited to dexamethasone but was also exhibited by the natural glucocorticoid, hydrocortisone (Table I). A control solution of the appropriately diluted ethanol vehicle had no effect on colony numbers (data not shown).

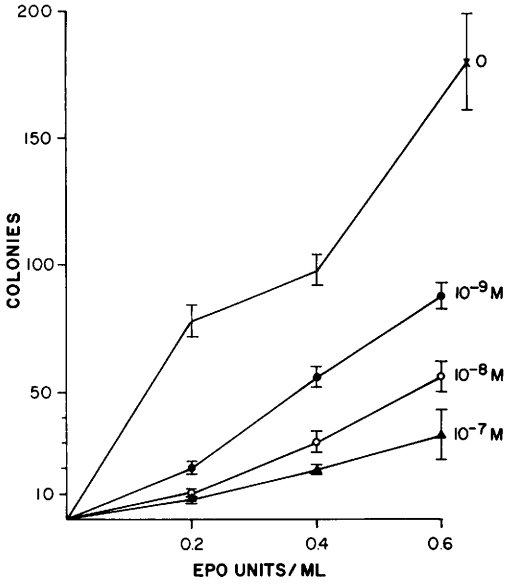

FIG. 1. Inhibition of erythroid colony formation by dexamethasone. Human fetal liver cells were incubated in 0.1 ml plasma clots for 10 days in the presence of either 0.2, 0.4, or 0.6 unit of erythropoietin/ml. The number of erythroid colonies ± SEM per 0.1 ml clot is shown for a representative experiment. The inclusion of increasing amounts of dexamethasone from 10^{-9} to 10^{-7} *M* resulted in progressive suppression of the number of erythroid colonies formed.

When cultures of umbilical cord blood were exposed to dexamethasone inhibition of colony formation also was noted. In these experiments 10^{-6} *M* dexamethasone reduced colony numbers from one-half to

TABLE I. EFFECT OF GLUCOCORTICOIDS ON ERYTHROID COLONY FORMATION[a]

Tissue	Culture Medium	Epo (U/ml)	Steroid	Steroid concentration (*M*) 0	10^{-10}	10^{-9}	10^{-8}	10^{-7}	10^{-6}	2×10^{-6}	Days of culture
Fetal liver	Plasma clot	0.2	Dexamethasone	78.5 ± 6.9	—	20 ± 2.4	10 ± 1.2	8.3 ± 1.4	—	—	10
		0.4	Dexamethasone	97.5 ± 5.6	—	55.8 ± 3.8	29.8 ± 4.2	19 ± 1.4	—	—	10
		0.6	Dexamethasone	180.3 ± 19	—	87.5 ± 5	56.3 ± 5.5	33 ± 10	—	—	10
Fetal liver	Plasma clot	0.5	Dexamethasone	195 ± 9.6	—	170.3 ± 10.4	142.3 ± 9.6	133.3 ± 2.5	—	—	5
		0.5	Dexamethasone	304 ± 18	255.5 ± 14.7	283.3 ± 13.2	254.5 ± 9.6	221 ± 7.7	98.3 ± 4.2	—	10
Fetal liver	Plasma clot	0.5	Dexamethasone	194.8 ± 10.5	—	140 ± 5.2	93.5 ± 15.8	63.5 ± 1.9	50.8 ± 3.1	—	5
		0.5	Dexamethasone	194.8 ± 11.4	—	—	63 ± 2.8	38.3 ± 1.3	14.8 ± 2.1	—	10
Fetal liver	Plasma clot	1.0	Hydrocortisone	143.8 ± 18.1	—	—	—	138.5 ± 6.2	94.8 ± 5.1	51 ± 8.1	5
		1.0	Hydrocortisone	283.8 ± 23.3	—	—	—	179.8 ± 7.6	168 ± 29.5	138.3 ± 14.1	7
Cord blood	Plasma clot	0.25	Dexamethasone	105 ± 9	—	130.9 ± 4.4	106.3 ± 8	93.8 ± 4.5	55.9 ± 6.1	—	7
Cord blood	Methyl-cellulose	0.5	Dexamethasone	242	217	—	207	97	70	—	10
Cord blood	Methyl-cellulose	0.5	Dexamethasone	78	64	78	74	68	22	—	7

[a] Results are no. of colonies formed; each determination is the mean of four or five clots ± SEM.

one-quarter of control values (Table I). Cells cultured in either plasma clots or methylcellulose were similarly affected (Table I).

Inhibition of colony formation by dexamethasone did not appear to affect the proportion of γ and β globin chains synthesized in culture. Although 10^{-6} *M* dexamethasone reduced the number of colonies at Day 10 produced by cord blood sample by approximately 25% the γ/β synthesis ratio was not altered (Table II).

Discussion. In our series of experiments adrenal corticosteroids were consistently suppressive of erythroid colony formation. This suppression was noted with both human fetal liver and cord blood cultures over a wide range of steroid hormone concentrations. Decreased erythroid colony growth was noted in the presence of erythropoietin concentrations ranging from suboptimal to maximally stimulating. These results were repeatedly reproduced in over 10 separate experiments.

Several previous authors have reported that glucocorticoids increased erythroid colony numbers in *in vitro* systems (10, 13–15). Differences in stage of development or in species responses may explain some of the discrepancy noted with those cultures (10, 15). In addition, in our human materials we have noted a rather great variability in colony yield among different tissue samples and occasionally within single experiments. As previously pointed out by others (11), variation in colony numbers, as reflected in considerable standard error in some prior reports, makes some interpretations of colony stimulation difficult. Extensive details of some previous reports of studies with normal human material are not available (12–14).

Interference with erythroid colony formation *in vitro* by glucocorticoids has been observed in experiments with normal rat (5) and mouse (11, 12) bone marrow. Similar suppression of colony growth occurred when murine (16) and human (17) erythroleukemia cell lines were examined *in vitro*. Recently several different glucocorticoids have been found to inhibit *in vitro* erythroid colony formation by murine fetal liver cells (20).

The mechanism for this glucocorticoid

TABLE II. GLOBIN CHAIN SYNTHESIS BY CORD BLOOD COLONIES

Cell sample	Dexamethasone	Erythroid colonies/ 0.1 ml ± SEM	Globin synthesis (γ/β)
Cord blood reticulocytes	0	—	1.09
12- to 14-day colonies	0	182.6 ± 13.2	1.04
12- to 14-day colonies	10^{-6} *M*	136 ± 4.7	0.99

induced decrease in colony formation is not at all understood. As pointed out by Gidari and Levere (12), the effect may be mediated by a direct action of the hormones on the erythroid colony progenitor or on other cell lines in the culture which normally interact with the erythroid line to promote erythroid differentiation. For example, there is conflicting evidence that interaction with T lymphocytes or macrophages may promote production of human erythroid colonies *in vitro* (21, 22). Dexamethasone has been shown to cause arrest in the G_1 stage with subsequent cell death in a human T-cell line *in vitro* (23). If T cells promote erythroid colony formation dexamethasone damage to T cells might be expected to impair erythroid colony growth. Recently Leung and Gidari (20) have presented evidence suggesting that glucocorticoids suppress erythroid colony growth by delaying entry of colony-forming cells into the S-phase of the cell cycle. Those authors have also postulated that this glucocorticoid effect may have a physiological counterpart modulating fetal erythropoiesis (20). Further experiments using more purified progenitor preparations and separated lymphoid subsets may help illuminate the mechanism of the glucocorticoid effect.

Finally our study could provide no evidence that glucocorticoids were capable of altering differential synthesis of γ and β globin chains *in vitro*. Hormonal influences may play a role in this differentiation process during human fetal development but experimental models have not yet clarified this problem.

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