THE INFLUENCE OF STEROID HORMONE METABOLITES
ON THE IN VITRO DEVELOPMENT OF ERYTHROID
COLONIES DERIVED FROM HUMAN BONE MARROW*

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Earlier studies from this and other laboratories demonstrated that certain naturally occurring steroid metabolites, formerly considered to be physiologically inactive end products of hormone metabolism, strongly stimulate porphyrin and δ-aminolevulinic acid synthase (ALA-S)[1] formation in cultured chick embryo liver cells (1, 2) as well as in vivo in the liver of the chick embryo (3). Steroid metabolites with a 5β-structure (A:B cis) were more potent in this respect than their 5α-epimers or their parent compounds. 5β-steroids which induce porphyrin synthesis in chick embryo liver cells have also been shown to induce the early synthesis of hemoglobin in the erythroblasts of the chick blastoderm (4) and a 5β-steroid receptor in this primitive avian tissue has been identified (5).

These steroid metabolites have been also reported to stimulate erythropoiesis in vivo in mice. For example, Gorshein and Gardner (6) described enhanced erythropoiesis in exhypoxic polycythemic mice after 5β-steroid administration as determined by ^{59}Fe incorporation into peripheral erythrocytes. Gordon et al. (7) reported similar results using both normal and polycythemic mice; in addition, their study showed that an antibody preparation against erythropoietin abolished the effects of testosterone, but not those of 5β-steroids. These data thus suggested that 5β-steroids can have effects on erythropoiesis that are independent of the action of erythropoietin. Besa et al. (8) later demonstrated that the 5β-compound, etiocholanolone (5β-androstan-3α-ol-17-one), increased erythrocyte mass in squirrel monkeys, confirming that such steroid metabolites may stimulate erythropoiesis not only in the erythroid tissues of birds, rodents, but also in vivo in primates. 5β-steroids have also been demonstrated to increase both heme and globin synthesis in suspension cultures of human bone marrow cells (9, 10).

The development of newer methodology for the study of hematopoietic stem cells in culture (11–13) made it possible to examine the mechanism and site of action of erythropoietin and steroids on the development of erythroid colonies in semisolid media (14). Using rat bone marrow cells, Singer et al. (15) reported that certain steroids enhanced erythroid colony growth in vitro and suggested that etiocholanolone, a prototype 5β-steroid, may act on early erythroid progenitor cells (16). The clinical

[1] Abbreviations used in this paper: ALA-S, δ-aminolevulinic acid synthase; BFU-E, burst-forming units-erythroid; CFU-E, colony-forming units-erythroid; DHT, dihydrotestosterone; URO-S, uroporphyrinogen-I synthase.

usefulness of this specific metabolite in the therapy of patients with severe bone marrow failure has been suggested by the studies of Besa et al. (17).

The present study was undertaken to examine the influence of steroid metabolite pairs which are epimeric at the C5 position on the development of erythroid colonies derived from human bone marrow using an in vitro semisolid culture method (12). This technique permits an evaluation of the effects of chemicals on erythroid colony growth; such growth is dependent on cellular proliferative activity. The data obtained show that steroid metabolites enhance the in vitro growth of both early primitive erythroid progenitors, i.e. burst-forming units-erythroid (BFU-E), and later erythroid progenitors, i.e. colony-forming units-erythroid (CFU-E) of normal human bone marrow; they indicate that 5β-compounds are generally more potent in this growth promoting effect than their 5α-epimers; and they demonstrate a steroid effect on a very early stage of erythroid differentiation.

Materials and Methods

Steroid Metabolites. Steroids were either purchased from Sigma Chemical Co. (St. Louis, Mo.) or prepared by standard synthetic methods from appropriate steroid precursors by Dr. H. L. Bradlow, Laboratory of Steroid Biochemistry, The Rockefeller University, N. Y. All compounds were purified by recrystallization from appropriate solvent mixtures. The purity of each compound was rigorously established by comparison with standards of known purity using two or more thin-layer chromatographic systems. No compound was utilized in these studies which was not shown to be homogeneous. Steroids were dissolved in propylene glycol and added to the bone marrow culture at a concentration of 10^{-7} M, which has been shown to be optimal for erythroid colony formation (15). C5 epimeric pairs of steroids were studied in all experiments.

Erythroid Colony Assay. Erythroid progenitors (BFU-E and CFU-E) were assayed using the in vitro culture technique which was originally described by Iscove et al. (12) with minor modifications. Briefly, aliquots of bone marrow specimens aspirated from iliac crests of healthy adults at the Memorial Sloan-Kettering Bone Marrow Donor Center were provided to us through the courtesy of Dr. M. J. Murphy, Jr. Diluted marrow cells (1:2 to 1:5) in α-medium (Flow Laboratories, Inc. Rockville, Md.) were applied to a 6.4% Ficoll 400 (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.), 9.8% sodium metrizoate (Nygaard Co., Accurate Chemical & Scientific Corp., Hicksville, N. Y.) solution as described previously (18), and interface fractions were collected. Mononucleated cells which had been harvested from the interface were treated with NH_4Cl:Tris HCl (pH 7.4): $KHCO_3$ solution (19) to lyse contaminating erythrocytes. 2×10^5 nucleated cells were resuspended in 1 ml of α-medium containing 0.8% methylcellulose (The Dow Chemical Co., Midland, Mich.), 30% fetal bovine serum (Flow Laboratories, Inc., Rockville, Md.), 1% bovine serum albumin (Calbiochem-Behring Corp., American Hoesct Corp., San Diego, Calif.), and 0.1, 0.25 or 0.5 IU of Step III sheep plasma erythropoietin (Connaught Laboratories, Ontario, Canada) and the cells were placed in a 35 × 10 mm plastic Petri dish (Lux Scientific Corp., Newbury Park, Calif.). The cell suspensions were incubated at 37°C in 5% CO_2 in air with saturated humidity. The number of red to reddish erythroid colonies containing >8 cells (CFU-E) was counted after 7 d of incubation, and the number of erythroid bursts having macroscopic dimensions of clusters of erythroblasts (BFU-E) was enumerated on day 14, using an inverted microscope without staining, as reported previously (20, 21).

Assay of BFU-E and CFU-E. Three sets of experiments were conducted. In the first, the influence of the continued presence of steroids on the development of BFU-E and CFU-E was examined in the presence of erythropoietin. Each steroid was added to cultures of normal human bone marrow cells at a 10^{-7}-M concentration and the cultures were incubated in the presence of 0.1 IU of erythropoietin. The numbers of CFU-E and BFU-E were counted on appropriate days as indicated above. Uroporphyrinogen-I synthase (URO-S) activity was determined as described earlier (22) in the cells which were harvested from duplicate plates after having enumerated the numbers of CFU-E on day 7.

Secondly, the effect of preincubation with steroids, followed by incubation with erythropoi-etin, on the development of BFU-E and CFU-E was examined. Cells were preincubated with 10^{-7} M of each steroid solution in α-medium containing 40% fetal calf serum and 10% bovine serum albumin for 48 h at 37°C in 5% CO_2-95% air. After a 48-h incubation, cells were washed with modified Ham's F12 medium (19) three times, and then resuspended in semi-solid medium containing methylcellulose in the presence of 0.5 IU of erythropoietin. BFU-E and CFU-E were counted on day 14 and day 7 of incubation, respectively.

The purpose of the third experiment was to evaluate the effect of delayed addition of steroids on the formation of erythroid colonies. Marrow cells were incubated in the semi-solid medium in the presence of 0.25 IU of erythropoietin, and either 5α- or 5β-androstane-3α,11β-diol-17-one was added to culture dishes on day 0, day 2, or day 4 at a 10^{-7}-M final concentration. These metabolites are derived from the adrenal hormone 11β-hydroxyandrostenedione. Neither the 5α- nor the 5β-metabolite of this adrenal hormone has androgenic properties. The numbers of BFU-E and CFU-E were counted as described above. For statistical analysis, the Student's t test was used.

Liver Cell Cultures. For studies of the effects of steroids on ALA-S synthesis and porphyrin formation in liver cells, serum-free cultures of chick embryo liver cells were prepared, and determinations of ALA-S activity and porphyrin concentrations were carried out as described previously (19).

Results

Benzidine staining, using acidic solutions for the detection of hemoglobinized cells, may also give rise to positive staining with granulocyte-macrophage colonies that contain peroxidases. To avoid false-positive results, red or reddish colonies without staining were counted as reported previously (20, 21). Morphological identification of erythroblasts was made after Wright-Giemsa staining of smeared cell preparations prepared by a Cytocentrifuge (Shandon Southern Instruments Inc., Sewickley, Pa.). Numbers of BFU-E and CFU-E increased in a dose-dependent manner as a function of erythropoietin concentrations as well as a function of the input cell number. No erythroid colonies (CFU-E) or erythroid bursts (BFU-E) were observed with steroid treatment alone in the absence of erythropoietin (data not shown).

Table 1 shows the effects of the continued presence of each steroid throughout the entire incubation period on erythroid colony formation. Out of six pairs of steroids tested, four of the pairs (A-D) were C19 compounds and two (E, F) were C21 steroids. The steroids alone were not stimulatory on erythroid colony formation in the absence of erythropoietin. However, when cultures contained 0.1 U of erythropoietin/ml, certain steroids enhanced the growth of both CFU-E and BFU-E. In this respect, 5β-steroids were nearly always more effective than their 5α-epimers. Specifically, all four androstanes (A-D) and one (F) out of two pregnanes (E, F) having the 5β-structure, were more effective in increasing CFU-E significantly as compared with control cultures which were treated with erythropoietin alone; both 5α-pregnane derivatives (E, F) also stimulated CFU-E formation and one of these compounds (E) was more active than the 5β-epimer. No C19 5α-steroid significantly increased BFU-E and CFU-E formation. All 5β-steroids increased BFU-E significantly. The extent of stimulation of erythroid colonies by the active steroids ranged from 200 to 260% for CFU-E and from 140 to 200% for BFU-E above controls.

We have previously reported that the activity of URO-S, a cytosolic enzyme in the heme biosynthetic pathway, is an early and sensitive marker for erythroid differentia-tion both in mouse Friend erythroleukemia cells (23) and in normal human bone marrow cultures (24). URO-S activity determined in cells harvested on day 7 after

TABLE I

Effect of Steroids on Erythroid-Colony Formation

Pair	Steroid	CFU-E*	BFU-E*
	Control (propylene glycol)	30.0 ± 3.0	23.0 ± 1.0
A	5α-Androstan-3α-ol-17-one	39.5 ± 5.5	26.5 ± 0.5
	5β-Androstan-3α-ol-17-one	73.5 ± 3.5‡	38.0 ± 3.0‡
B	5α-Androstan-3β-ol-17-one	38.0 ± 8.0	29.5 ± 2.5
	5β-Androstan-3β-ol-17-one	61.0 ± 6.0‡	31.5 ± 0.5‡
C	5α-Androstan-17-one	44.0 ± 3.0	25.5 ± 1.5
	5β-Androstan-17-one	79.0 ± 8.0‡	45.5 ± 0.5‡
D	5α-Androstan-3α,11β-diol-17-one	38.5 ± 7.5	23.5 ± 6.5
	5β-Androstan-3α,11β-diol-17-one	73.0 ± 6.0‡	37.5 ± 0.5‡
E	5α-Pregnane-3β,17α-diol-20-one	67.5 ± 0.5‡	26.5 ± 0.5
	5β-Pregnane-3β,17α-diol-20-one	50.0 ± 4.0	35.5 ± 0.5‡
F	5α-Pregnan-11-one	58.0 ± 4.0‡	29.0 ± 4.0
	5β-Pregnan-11-one	63.0 ± 6.0‡	37.5 ± 0.5‡

All cultures contained 0.1 U erythropoietin/ml. Steroids were added to a concentration of 10^{-7} M. No erythroid colonies were observed when erythropoietin was not added.

* CFU-E and BFU-E are expressed as mean numbers of erythroid colonies ± SEM per 2×10^5 input nucleated cells on day 7 (CFU-E) and on day 14 (BFU-E).

‡ $P < 0.05$, significantly different from the control value.

incubation with steroids and erythropoietin exhibited a general parallelism to the activity ratio of 5β- and 5α-compounds in inducing CFU-E formation (data not shown).

Preincubation of cells with 5α- and 5β-steroids (seven pairs) for 48 h, followed by incubation with erythropoietin, did not reveal any effect of either 5α- or 5β-steroids on the growth of CFU-E (Table II). On the other hand, in five of the seven pairs of compounds, the 5β-epimer significantly enhanced the growth of BFU-E (Table II).

Dihydroxycoprostane and trihydroxycoprostane, which are 5β-neutral sterols and normal intermediates in bile acid synthesis from cholesterol, also displayed significant stimulation of BFU-E, but not of CFU-E formation. These steroids have been shown to be porphyrinogenic in avian liver cells (25).

Table III depicts the effects of the administration of a pair of 5α- and 5β-steroids after initiation of cultures. The 5α-compound had no stimulatory effect on BFU-E and CFU-E formation irrespective of the time of addition to the cultures. The 5β-steroid enhanced the colony growth of CFU-E only when added simultaneously with the establishment of the cultures; this derivative also enhanced BFU-E formation when added within 2 d after the initiation of the cultures, but did not further affect erythroid colony development when added thereafter. These cultures contained 0.25 U/ml erythropoietin throughout the incubation periods.

The same steroid solutions in propylene glycol were tested for induction of porphyrins (13 or 16 μM) in serum-free cultures of chick embryo liver cells using our technique described previously (19) and induction of ALA-S (2.5 or 10 μmol/egg) in

TABLE II

Effect of Preincubation with Steroids on Erythroid-Colony Formation

Pair	Steroid	CFU-E*	BFU-E*
	Control (propylene glycol)	221 ± 2	37.5 ± 2.9
A	5α-Androstan-3α-ol-17-one	187 ± 1	44.5 ± 4.2
	5β-Androstan-3α-ol-17-one	200 ± 3	59.8 ± 2.1‡
B	5α-Androstan-3β-ol-17-one	223 ± 1	42.0 ± 3.0
	5β-Androstan-3β-ol-17-one	195 ± 16	61.5 ± 6.6‡
C	5α-Androstan-17-one	162 ± 13	41.8 ± 2.2
	5β-Androstan-17-one	231 ± 11	28.7 ± 5.2
D	5α-Androstan-3α,11β-diol-17-one	187 ± 9	35.0 ± 1.0
	5β-Androstan-3α,11β-diol-17-one	180 ± 1	54.3 ± 3.1‡
E	5α-Pregnane-3β,17α-diol-20-one	167 ± 2	53.3 ± 6.4
	5β-Pregnane-3β,17α-diol-20-one	185 ± 10	36.8 ± 6.1
F	5α-Pregnan-11-one	169 ± 13	37.5 ± 1.0
	5β-Pregnan-11-one	174 ± 11	61.8 ± 4.0‡
G	5α-Pregnan-3α,17α-diol-20-one	201 ± 2	46.8 ± 3.4
	5β-Pregnan-3α,17α-diol-20-one	185 ± 2	62.8 ± 2.6‡
	3α,7α-dihydroxycoprostane	204 ± 11	60.5 ± 6.0‡
	3α,7α,12α-trihydroxycoprostane	228 ± 14	50.8 ± 2.9‡

Normal human bone marrow cells were preincubated with steroids at a concentration of 10^{-7} M for 48 h. After washing three times, cells were incubated in semi-solid medium supplemented with methylcellulose in the presence of 0.5 U of erythropoietin/ml. CFU-E and BFU-E were enumerated as described in the text.

* CFU-E and BFU-E are expressed as mean numbers of erythroid colonies ± SEM per 2 × 10^5 input nucleated cells on day 7 (CFU-E) and on day 14 (BFU-E).

‡ $P < 0.05$, significantly greater than the control value.

TABLE III

Effect of Delayed Addition of Steroids on Erythroid-Colony Formation

Steroid	Day of administration	CFU-E*	BFU-E*
Control (propylene glycol)	0	85.0 ± 3.0	30.2 ± 2.0
5α-Androstan-3α-11β-diol-17-one	0	77.0 ± 1.0	34.5 ± 0.5
	2	67.5 ± 7.5	37.0 ± 3.0
	4	77.0 ± 11.0	31.5 ± 0.5
5β-Androstan-3α-11β-diol-17-one	0	125.0 ± 3.0‡	40.5 ± 0.5‡
	2	82.0 ± 1.0	42.0 ± 1.0‡
	4	66.0 ± 4.0	37.0 ± 5.0

Steroids were added at a concentration of 10^{-7} M on days as indicated in the table. All cultures contained 0.25 U of erythropoietin/ml.

* CFU-E and BFU-E are expressed as mean numbers of erythroid colonies ± SEM

‡ $P < 0.05$, significantly greater than the control value.

TABLE IV

Relative Activity of 5α- and 5β-Steroids on the Induction of ALA-Synthase and Porphyrins in Chick Embryo Liver

Pairs	Steroid structures	5β/5α inducing ratios				
		Human bone marrow			Chick embryo liver	
		CFU-E BFU-E (continuous incubation with steroid)		BFU-E (preincubation with steroid)	ALA-Synthase*	Protoporphyrin‡
A	(5β- and 5α)-Androstan-3α-ol-17-one (etiocholanolone and androsterone)	1.9	1.4	1.3	1.3	1.4
B	(5β- and 5α)-Androstan-3β-ol-17-one	1.6	1.1	1.5	1.8	2.1
C	(5β- and 5α)-Androstan-17-one	1.8	1.8	0.7	ND	4.7
D	(5β- and 5α)-Androstan-3α,11β-diol-17-one	1.9	1.6	1.6	1.7	0.7
E	(5β- and 5α)-Pregnane-3β,17α-diol-20-one	0.7	1.3	0.7	4.3	4.6
F	(5β- and 5α)-Pregnan-11-one	1.1	1.3	1.6	2.2	ND
G	(5β- and 5α)-Pregnan-3α-17α-diol-20-one	ND	ND	1.3	ND	ND

ND, not determined.

* ALA-synthase activity was determined in livers from chick embryo treated for 9 h with 2.5–10 µmol of steroid/egg as described previously (41).

‡ Porphyrin formation was determined in the serum-free cultures of chick embryo liver cells as described previously (19). Concentrations of steroids used were 13–16 µM.

§ Only the pair D showed an apparent lesser activity of 5β- than 5α-compounds (the ratio:0.7) on porphyrin formation. However, when 5 mM CaMgEDTA was supplemented together with the steroid to prevent the loss of protoporphyrin from the ferrochelatase step, the same pair of compounds displayed a 5β/5α-inducing ratio of 6.3.

the chick embryo liver *in ovo*.[2] These steroid concentrations were found to be optimal for induction in the respective model systems. As indicated in Table IV, 5β-steroids were generally more potent than 5α-steroids with respect to both the induction of porphyrinogenesis and the stimulation of ALA-S formation.

Discussion

The results of this study demonstrate that certain 5α- and 5β-steroid metabolites are effective in enhancing the growth, in cultured human bone marrow cells, of both BFU-E and CFU-E in the presence of a low dose of erythropoietin. In this respect, five of six steroids of the 5β-configuration tested, were effective in significantly increasing CFU-E (Table I) and all 5β-steroids increased BFU-E significantly (Table I). In contrast, only two 5α-steroids stimulated the growth of CFU-E, and no 5α-steroids stimulated the growth of BFU-E. No steroids demonstrated an inhibitory effect on erythroid colony formation.

These data are consistent with earlier observations which showed a greater potency

[2] Sassa, S., H. L. Bradlow, and A. Kappas. 1979. Steroid induction of δ-aminolevulinic acid synthase and porphyrins in liver. Structure-activity studies and the permissive effects of hormones on the induction process. Submitted for publication.

of 5β-steroids as compared with 5α-steroids in the induction of porphyrin-heme biosynthesis in avian embryonic liver (1–3), as well as the stimulation of erythropoiesis in developing avian blastoderm (4). This structure-activity relationship of steroid metabolites was subsequently shown to extend to heme and globin synthesis in human bone marrow cells in suspension cultures (9, 10). More potent actions of 5β-steroids, as compared with 5α-steroids, have also been shown by ^{59}Fe incorporation into erythrocytes in exhypoxic polycythemic mice (6, 7), ALA-S induction in the chick blastoderm (26), and by in vitro growth stimulation of CFU-E using rat bone marrow cultures (15).

Extensive studies on structure-inducibility relationships of these steroids on CFU-E formation by Singer et al. (15) clearly indicated that most 5β-metabolites are potent inducers of erythropoiesis in rodent bone marrow. The summary of 5β- and 5α-inducing ratios shown in Table IV confirms, in the tissue types indicated (human bone marrow and avian liver), the generally more potent inducing activity of the 5β-steroid structure. A common phenomenon occurring in these systems after treatment with 5β-steroids is the induction of ALA-S with a subsequent increase in porphyrins or heme (1–3, 27). Induction of this rate-limiting enzyme in heme synthesis has, in fact, been suggested as a key metabolic event in the mechanism by which steroid metabolites exert their stimulatory effects on heme synthesis (9, 27).

Because steroid metabolites may exhibit equal or greater biological potency in these systems than their precursor hormones, the role of hormone biotransformation to active derivatives in these systems remains a matter for further study; the phenomenon of metabolic conversion of parent chemical structures to biologically active derivatives by microsomal and other cellular enzymes is common and is known to extend to hormones. It should be noted that on a molar basis steroid metabolites are at least 100-fold more active in stimulating erythroid colony formation from human bone marrow cells than they are in inducing ALA-S or porphyrin formation in avian liver cells. This sensitivity of bone marrow cells to steroids including glucocorticoids extends to other species such as rats (15) rabbits (28), and man (29), suggesting an intrinsic biological characteristic of these cell types as compared with hepatic cells. Stimulation of BFU-E formation by the coprostane compounds is of particular interest because these neutral sterol metabolites of cholesterol are produced in large amounts in man.

The structure-inducibility relationship of these steroid metabolites to BFU-E, the most primitive erythroid progenitor cells, has not been extensively examined, except for the single pair of metabolites studied by Fisher et al. (28). These authors found, utilizing rabbit bone marrow cultures, that both the parent hormone, testosterone, and its metabolites, 5α- and 5β-dihydrotesterone (DHT) stimulated the in vitro growth of BFU-E in the presence of erythropoietin but that 5β-DHT was significantly more potent than the 5α derivative of the hormone (5α-DHT) in this action (28). When animals were pretreated with these steroids, both testosterone and 5β-DHT, but not 5α-DHT, gave rise to increased erythroid-colony formation in culture (28). These in vivo findings in rabbits contrast with earlier studies carried out by a cooperative group study utilizing polycythemic mice as a model test system (30). In any event, it appears from recent work that 5β-DHT is clearly more effective in stimulating erythroid differentiation in vivo in the rabbit than is 5α-DHT, and that this relative potency of 5β-DHT over 5α-DHT extends to cultured erythroid cells from this species (28).
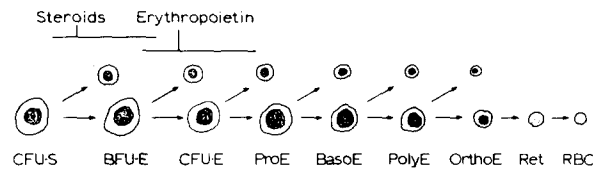
Fig. 1. Hypothetical scheme of sites of action of steroids and erythropoietin on erythroid progenitor cells. The site of erythropoietin (Ep) action has been considered to appear at the developmental stage between BFU-E and CFU-E. In contrast, the steroid action appears to occur at much earlier developmental stage of erythroid progenitors cells than Ep-sensitive precursors. It should be noted, however, that the steroid action on erythroid colony formation requires continuous presence or subsequent addition of erythropoietin. CFU-S, multipotential stem cell; Pro E, proerythroblast; Baso E, basophilic erythroblast; Poly E, polychromatophilic erythroblast; Ortho E, orthochromatophilic erythroblast; Ret, reticulocyte; RBC, erythrocytes.

Our results in this study represent the first demonstration in normal human marrow cell cultures that certain C19 and C21 metabolites stimulate erythroid colony growth and that 5β-metabolites are generally more potent in this action than their corresponding epimers. In our experiments, BFU-E formation was enhanced by short-term preincubation with or the continued presence of 5β-steroids in the cultures, whereas CFU-E formation was not enhanced by a preincubation with steroids; rather their continued presence throughout the incubation period was required. This suggests that the growth of erythroid colonies by day 7 may be, in part, a result of enhanced colony formation derived from steroid-responsive erythroid progenitor cells which may lie between BFU-E and CFU-E. In fact, human erythroid cells appear to represent a continuous and wide spectrum of erythropoietic precursors (21, 31). URO-S activity in cells which were harvested after 7 d of incubation, showed that 5β-steroids were, in general, more stimulatory of this enzyme activity than 5α-compounds (data not shown). This enhanced URO-S activity most likely reflects increased proliferation of erythroid cells in the cultures; however, in lymphocytes induction of the enzyme appears to take place after mitogen-stimulation (32).

The cellular response to erythropoietin is considered to appear in the developmental stage between BFU-E and CFU-E (33) (Fig. 1). In this respect, our results, after preincubation of human bone marrow cells with steroids, are interesting in that 5β-steroids increased only BFU-E formation but not CFU-E. This suggests that the effect of 5β-steroids on erythroid progenitors is directed towards an even earlier stage of erythroid differentiation than that of erythropoietin-responsive cells (Fig. 1). Singer and Adamson (16), using velocity sedimentation and cell-cycle kinetic techniques, reported that the etiocholanolone-responsive erythroid progenitors showed many similarities to those of BFU-E type and were different from erythropoietin-dependent CFU-E. Because the ^{3}H-thymidine suicide rate of etiocholanolone-responsive cells was substantially lower than that of erythropoietin-responsive cells, they concluded that this 5β-steroid probably acts on a separate, perhaps more primitive population of marrow cells (16). It is also known that an antibody preparation to erythropoietin abolishes the erythropoietic effect of testosterone in in vivo exhypoxic polycythemic mice (7, 34) but not that of 5β-steroids (7). Our results in Table III further substantiate the idea that 5β-steroids act principally on early erythroid progenitor cells; as noted, for example in Table III the 5β-derivative enhanced the growth of CFU-E colonies only when it was added on day 0, and increased the formation of BFU-E only when

it was added on day 0 or day 2, but not thereafter. These findings suggest that the functional responses of early erythroid progenitor cells to steroids may be rapidly lost in cultures after 48 h and that delayed addition of steroids to the cultured cells has little effect on the maturation of erythroid percursors.

Hormonal effects on erythropoiesis have been recognized for a long time (35). Differences of erythrocyte count, hemoglobin concentration, and hematocrit are known to exist between men and women and are considered to be a result of the effect of gonadal hormones (36). Androgen therapy for anemia in patients with a variety of blood disorders (37, 38), including aplastic anemia (39), was initiated almost two decades ago but still presents troublesome problems of masculinization and growth side-effects in women and children. Those steroid metabolites known to lack androgenicity (i.e. 5β-derivatives) may prove useful in treatment of certain intractable anemias of man, but extensive clinical studies of this possibility have not been undertaken to our knowledge. Recently, Besa et al. (17) reported that one 5β-steroid, etiocholanolone, could stimulate erythropoiesis in some patients with aplastic anemia. It should be noted, however, that certain 5β-steroids (including etiocholanolone) which are known to induce heme synthesis also have potent inflammatory and fever-producing activity in man (40); certain other 5β-derivatives which can activate the heme pathway do not have these properties. Thus, a careful selection of 5β-steroids with appropriate consideration of their structural and biological properties is necessary for human studies.

It should be emphasized that the steroids utilized in this study are derived from the in vivo biotransformation of endogenous hormones. The localization of the developmental stage at which these steroids act on erythroid progenitor cells is of considerable fundamental interest and may provide useful information regarding the mechanisms and sites of steroid actions on cell growth. It may also facilitate the use of these physiologically derived compounds for the stimulation of erythropoiesis in certain forms of bone marrow failure in man as we have suggested earlier (1, 4).

Summary

Certain C19 and C21 steroid metabolites, when incubated with normal human bone marrow cells in culture, increased the number of erythroid colonies in the presence of erythropoietin. Among a number of pairs of C5 epimeric steroids tested, most 5β (A:B cis) steroids stimulated the growth of both early erythroid progenitor cells (BFU-E) and late erythroid progenitor cells (CFU-E), whereas only a few 5α-(A: B trans) steroids stimulated the growth of CFU-E. No 5α-compounds of six pairs of steroids studied were found to stimulate BFU-E formation. This structure-activity relationship conforms with that previously observed in studies of steroid induction of ALA-synthase in avian embryo liver cells and hemoglobin synthesis in the cultured avian blastoderm.

When human bone marrow cells were preincubated with the steroids for 2 d, followed by incubation with erythropoietin, only the 5β-compounds stimulated the growth of BFU-E. Similarly, when addition of steroids was delayed in relation to erythropoietin in the culture, only the 5β-derivative of a pair of C5 epimeric compounds displayed an enhancing effect on the growth of BFU-E. This effect required that the steroid addition be made no later than 48 h after initiation of the culture. These data demonstrate that certain natural steroid metabolites significantly

stimulate erythropoiesis in normal human bone marrow cells in culture. They also indicate that 5β-compounds are more stimulatory than their 5α-epimers, and they suggest that these 5β-steroids act preferentially on very primitive erythroid progenitor cells, probably on BFU-E.

References

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