Hormonal Stimulation of Erythropoietin Production and Erythropoiesis in Anephric Sheep Fetuses

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ABSTRACT The effect of testosterone (DT) and thyroxin (L-T4) on erythropoiesis and erythropoietin (Ep) production was studied in control and nephrectomized sheep fetuses beginning at about 100 d of gestation. Fetuses were given injections of either 1.2 mg/d × 13 of L-T4, 12 mg, once every 5 d × 3 of DT or the vehicle alone. Fetal plasma samples for Ep determinations were obtained before and at intervals after the start of the treatment. Reticulocyte and hematocrit levels, and the percent erythrocyte-^{59}Fe uptake values were used to assess erythropoiesis in each fetus. No Ep was detected in plasmas of control fetuses, while significant amounts of Ep were present in plasmas obtained from DT- and L-T4-treated intact fetuses. Bilateral nephrectomy did not diminish the Ep response to DT and L-T4. In both intact and nephrectomized fetuses, treatment with DT resulted in the production of significantly greater amounts of Ep than L-T4. The rise in Ep in all groups was accompanied by increases in reticulocytes (2.2±0.2% vs. L-T4:8.1±0.4% and DT:7.6±0.7%), percent erythrocyte-^{59}Fe uptake (20.5 ±2.9% vs. L-T4:36.7±3.8% and DT:39.1±4.0%) and hematocrit (31.2±2% vs. L-T4:41.8±3% and DT:48.6 ±4.2%). The enhanced erythropoiesis in all groups of nephrectomized fetuses was dependent upon the presence of Ep, because the administration of anti-Ep to these fetuses resulted in the suppression of erythropoiesis in all three groups.

These data demonstrate that (*a*) DT and L-T4 are effective promoters of extrarenal Ep production, thereby enhancing erythropoiesis in intact and nephrectomized fetuses; (*b*) DT is a stronger stimulus of extrarenal Ep formation than L-T4; and (*c*) Ep is required for the expression of the erythropoietic effects of L-T4 and DT.

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INTRODUCTION

Erythropoiesis in the mammalian fetus and the adult is regulated by the hormone erythropoietin (Ep)[1] (1, 2). Although Ep is produced mainly by the kidneys in the normal adult (3), a number of studies have demonstrated the existence of extrarenal sources that, when appropriately stimulated, can produce about 5–10% of the Ep normally evoked in intact adults (4). The most important extrarenal source of Ep in the adult is the liver (5). By contrast, the liver represents the primary source of Ep during fetal and early neonatal periods (6, 7). The production of Ep by the liver in the fetus-newborn is regulated by mechanisms similar to those that control renal Ep production in the adult (6, 7). Thus hypoxia represents the fundamental erythropoietic stimulus for both organs (7). The switch from hepatic to renal production of Ep, which occurs soon after birth, is accompanied by the effective loss of hepatic Ep formation. It is likely that the decline in hepatic Ep production is associated with increased maturity of the liver. However, the possibility that renal Ep exerts a suppressive influence on this aspect of liver function can not be ruled out. The decreased hepatic Ep production is apparent from the observations that an adequate rate of erythropoiesis is not maintained in renoprival adults (8, 9). Thus anemia is a fairly constant feature of renal dysfunction in man (10, 11). Although erythrocyte abnormalities, chronic blood loss, deficiencies in certain essential elements required for heme synthesis, and inhibitors of erythropoiesis may be contributing factors, it is generally suspected that the underlying cause of the anemia in this disorder is a decrease in Ep production (11, 12). A recent study employing plasma concentrates (13) has shown the presence of detectable levels of Ep in anephric patients (14). However, the levels were significantly below those found in uremic-nephric patients with comparable degree of anemia.

[1] *Abbreviations used in this paper*: DT, testosterone cyclopentyl propionate (depo-testosterone); Ep, erythropoietin; L-T4, L-thyroxin.

Reactivation of hepatic Ep formation can be achieved by exposure of the nephrectomized adult to hypoxia-producing stimuli (4, 15). However, studies with laboratory animals have shown that the intensity of hypoxia needed to activate the extrarenal site is significantly greater than that required by the kidneys (15). Stimulation of hepatic Ep formation in renoprival adults is of considerable clinical interest because it may provide a more physiological approach to the treatment of the anemia in these individuals; normalization of erythropoiesis in uremic-anemic rats with chronic administration of Ep has been demonstrated (16).

Androgens have been employed in the treatment of the anemia in some patients with chronic renal failure (17–20). The therapy has been generally more effective in patients with residual renal tissue (19), but certain of the androgenic compounds have also been effective in some anephric patients receiving adequate hemodialysis (18). In some patients with chronic renal failure (with or without residual renal tissue) the androgen-induced erythropoietic stimulation was accompanied by an increase in circulating Ep levels, whereas increased Ep levels were not demonstrated in others (17–19). It is probable that in the latter group of patients, enhanced erythropoiesis was also associated with increased Ep production, which the relative insensitivity of the bioassay did not detect.

It is now firmly established that the erythropoietic effect(s) of androgens is mediated, at least in part, through increased production of Ep (21–23). Studies employing nephrectomized adult animals have shown that this effect is dependent on the presence of kidneys (24). However, the efficacy of androgens in promoting extrarenal Ep formation was not determined in these studies partly because the experimental models used (usually rats) did not allow for adequate evaluation. In the present study, we have employed the sheep fetus as an animal model for the study of factors influencing the production of extrarenal Ep. Chronic fetal sheep preparations have been employed extensively in the study of fetal growth and developmental physiology and have proven particularly suitable for the study of mechanisms regulating fetal erythropoiesis. Specifically, we have examined the effects of two well-established erythropoietic agents (testosterone and thyroxin) on hepatic Ep formation in the presence and absence of the kidneys. Because of the unique position of the fetus, which permits relatively long-term observations, we have been able to assess the efficacy of these two agents in promoting extrarenal Ep formation in anephric fetuses. The results demonstrate that testosterone and thyroxin stimulate hepatic Ep production, thereby enhancing erythropoiesis in the anephric fetus.

METHODS

These studies were conducted in fetal sheep at about 100–110 d of gestation (normal gestation, 145 d). Pregnant ewes with confirmed dates of conception were obtained from local suppliers, observed, acclimated for at least 5 d, and then fasted for 24 h before surgery. Progesterone (Lipo-Lutin, 250 mg, Parke, Davis & Co., Detroit, Mich.) was administered intramuscularly on the day before surgery and daily thereafter. The studies were performed over a 2-yr period.

12 groups of five to eight fetuses each were established (Table I). The fetuses in all groups were prepared for study by inserting a catheter into each of two fetal femoral arteries. The procedure for the placement of these intravenous catheters has been reported elsewhere (25). In addition, each fetus in groups 4–12 was fitted with three separately placed subcutaneous catheters by creating pockets under the fetal skin and securing the catheters in these spaces. The following additional surgical procedures were performed at the time of catheter emplacement. Fetuses in groups 2 and 7–12 were bilaterally nephrectomized, while the fetuses in group 3 were subjected to partial hepatectomy (80–90% of liver removed). These operations were performed through a single ventral incision as described earlier (26). The various catheters were clearly labeled and brought out through the uterus and maternal abdominal walls and secured to maternal skin. The amniotic fluid lost during surgery was replaced by warm isotonic saline containing antibiotics (25). The uterus and abdominal walls were closed around these tubes in layers. Heparinized prewarmed sterile saline was used to maintain intravenous catheter flow. Ampicillin (250 mg) was injected daily into each fetus via the intravenous catheter to minimize the chance of infection (25). The intravenous catheters were employed to obtain blood samples from the fetus and to administer radioiron and anti-Ep to the fetus. Subcutaneous catheters were used to administer thyroxin or testosterone, as well as the vehicle in which these hormones were dissolved. All animals except those in groups 1–3 were allowed to recover from surgery for 24 h before further treatment; groups 1–3 were allowed 4 h for recovery. The fetuses in groups 1–3 were rendered anemic by removing a 50–55 ml sample of blood (representing 42% of blood volume) from each fetus at the end of the recovery period. Plasmas from this initial bleeding served as control (0 h) samples.

The production of Ep in response to anemia in these groups was monitored by obtaining plasma from each fetus 12 h after the induction of anemia. The Ep activity of these and all other plasma samples were assayed in exhypoxic polycythemic mice. The plasma from each fetus was assayed separately

TABLE I

*Experimental Groups and Summary of Treatments**

Group No.	Type of fetus	Treatment
1	Intact	Phlebotomy
2	Nephrectomized	Phlebotomy
3	Hepatectomized	Phlebotomy
4	Intact	Vehicle
5	Intact	L-T4
6	Intact	DT
7	Nephrectomized	Vehicle + normal rabbit serum
8	Nephrectomized	L-T4 + normal rabbit serum
9	Nephrectomized	DT + normal rabbit serum
10	Nephrectomized	Vehicle + anti-Ep
11	Nephrectomized	L-T4 + anti-Ep
12	Nephrectomized	DT + anti-Ep

* See text for detailed description of the groups and schedules of treatment.

using five mice per group. Each test sample was administered in divided doses of 0.5 ml/d per mouse × 2 beginning on day 5 after hypoxia. Radioiron (0.5 μCi) was given on day 7 and the percent erythrocyte-^{59}Fe incorporation determined 72 h later (27).

15 ml of heparinized blood was obtained from each fetus in groups 4–12 immediately after the recovery period and was used to obtain pre-treatment (day 0) percent reticulocytes, percent hematocrit, and plasma Ep levels. Each fetus in groups 5, 8, and 11 was given subcutaneous injections of 1.2 mg thyroxin (L-T4) dissolved in 0.5 ml of the vehicle (0.0025 M NaOH in saline)/d, 5 d/wk for a total of 13 injections. Each fetus in groups 6, 9, and 12 received subcutaneous injections of 12 mg of a long-acting preparation of testosterone (testosterone cyclopentylpropionate; depo-testosterone [DT]) once every week for 3 wk. Each dose of DT was dissolved in 1 ml of peanut oil. The animals in groups 4, 7, and 10 served as controls and were given subcutaneous injections of a mixture of 0.0025 M NaOH in saline and peanut oil once every 3 d. The site of injections in groups 4–12 was changed with every dose by employing different subcutaneous catheters. 15-ml samples of heparinized blood were obtained from each fetus at intervals during the treatment period for reticulocyte, hematocrit, and Ep determinations. These intervals are indicated in the tables.

The effect of L-T4 and DT on erythropoiesis in these fetuses was also monitored by administering 50 μCi[^{59}Fe]Cl_3 to each fetus via the intravenous catheter 24 h after the last injection of the hormone. The radioiron was preincubated with 2 ml of the fetus's plasma for 30 min at 37°C before injection. Plasma used for this purpose was separated from 5 ml of blood obtained from each fetus immediately before use. Percent incorporation of radioiron into fetal erhtyrocytes was determined 36 h later (2). Blood volume of the fetus was assumed to be 5% of body weight, which was determined at the termination of the study.

The effect of neutralizing the circulating Ep in control and hormone-treated nephrectomized fetuses on erythropoiesis was determined as follows. Each fetus in groups 10–12 was given daily injections of 1 ml of a preparation of anti-Ep capable of neutralizing ≅22 IU of sheep plasma Ep (Step III, Connaught Laboratories, Toronto, Canada) for 3 d. The procedures for anti-Ep preparations, assay, and handling have been described (2). The first dose of anti-Ep was given 48 h before the last injection of the hormones. 1 d after the last anti-Ep injection, each fetus in groups 10–12 received 50 μCi of radioiron as described above; anti-Ep and [^{59}Fe]Cl_3 were given via the intravenous catheters. Reticulocyte numbers and percent erythrocyte-^{59}Fe uptake were determined 36 h after the administration of radioiron.

RESULTS

Although five to eight fetuses were employed in each group, not all survived the various surgical and experimental procedures. The number of surviving animals in each group is indicated in legends to tables and the figure. It should be pointed out, however, that all but one of these fetuses died within the first 72 h of the study; one fetus died 8 d after surgery. None of these losses can be attributed to the effect of nephrectomy. In general, it has been shown that the kidney is not necessarily essential to the survival of the fetus, although the overall growth may be somewhat retarded (28). This unique fetal characteristic has permitted these studies. The nephrectomized fetuses in these studies exhibited moderate increases in creatinine and blood urea nitrogen levels (creatinine: 1.4±0.5 vs. 4.3±0.9 mg%; blood urea nitrogen 9.8±2.8 vs. 62±8 mg%). No significant change in fetal body weight was observed in the hormone-treated groups. However, because of significant variations in the volume of blood removed from each fetus at the termination of the study, before weighing, it is possible that such differences were not recognized.

Initial studies (groups 1–3) demonstrated once again that the liver is the primary site of Ep formation in the fetus. Plasma samples obtained from fetuses soon after the completion of surgery did not elicit a significant erythropoietic response in exhypoxic polycythemic mice. This is in accord with our previous finding that the various surgical procedures employed in these studies are not sufficient by themselves to induce increased production of Ep in these fetuses (2, 6, 25). Induction of anemia by the removal of a large volume of blood (corresponding to 1.5–2% of fetal body weight), however resulted in the appearance of significant amounts of Ep in fetal circulation (1.3 IU Ep/ml of plasma). Removal of both kidneys before the induction of anemia did not inhibit the Ep response of these fetuses (1.1 IU Ep/ml plasma), while the removal of ≅90% of the liver caused the suppression of Ep formation in response to bleeding (0.14 IU Ep/ml plasma).

As can be seen in Table II, plasmas from L-T4 and DT treated intact fetuses, but not vehicle-treated animals, exhibited significant erythropoiesis-stimulatory activity in exhypoxic polycythemic mice. This increased erythropoietic activity was detected soon after treatment with L-T4 and DT had been initiated and persisted for the duration of the treatment period (Table II); DT caused the production of greater amounts of Ep than L-T4.

Table II also shows the effects of L-T4 and DT on Ep production in bilaterally nephrectomized fetal sheep. As in intact fetuses, the administration of these hormones resulted in increased plasma erythropoietic activity. Once again, plasmas from DT-treated fetuses exhibited greater erythropoiesis-stimulatory activity. That this activity was caused by the presence of Ep in these plasma samples was shown by the fact that treatment of these plasmas with anti-Ep (27) before injection into polycythemic assay mice resulted in the complete inhibition of their erythropoietic activity.

Results presented in Table III demonstrate that this increase in Ep production was accompanied by enhanced erythropoiesis in treated fetuses. Generally, elevated reticulocyte and hematocrit values were noted in DT and L-T4 treated normal and nephrectomized fetuses. Increased reticulocyte numbers were first detected on day 7 of treatment and remained elevated throughout the study period. Significantly increased erythropoiesis was also evident from the increased incorporation of radioiron into erythrocytes of the hormone-treated fetuses (Table III).

TABLE II

*Effect of DT and L-T4 on Ep Production by Normal and Bilaterally Nephrectomized Sheep Fetuses**

Fetus	Treatment	Days	Erythrocyte-^{59}Fe incorporation (mean±SEM)‡									
			0	1	3	5	8	10	12	15	20	22
			%									
Normal (5)§	Vehicle		0.7±0.2	1.2±0.3	—¶	—	0.9±0.3	0.8±0.1	—	0.9±0.3	1.3±0.3	—
Normal (4)	L-T4		0.8±0.2	—	—	2.5±0.5	—	—	5.0±0.7	7.6±1.2	—	6.9±0.8
Normal (4)	DT		1.0±0.2	0.8±0.1	—	4.4±0.8	—	—	8.7±1.1	13.2±1.6	—	14.8±2.3
Nephrectomized (3)	Vehicle		0.5±0.1	0.6±0.2	0.9±0.1	0.7±0.2	0.4±0.1	0.8±0.2	0.3±0.1	0.9±0.2	—	—
Nephrectomized (4)	L-T4		0.9±0.1	—	2.0±0.3	4.2±1.1	8.1±1.5	9.7±1.8	7.3±1.2	8.9±1.0	10.7±1.7	9.9±0.9
Nephrectomized (4)	DT		0.2±0.0	0.4±0.1	5.1±0.6	11.3±1.8	15.3±2.1	14.6±1.9	15.0±2.1	14.1±2.0	13.9±1.3	16.1±2.7

* See text for details.

‡ Determined in the exhypoxic polycythemic mouse; controls were (mean±SEM): saline, 0.3±0.1; 0.1 IU Ep, 2.4±0.2; 0.4 IU Ep, 8.7±0.8.

§ Numbers in parentheses indicate the number of surviving fetuses in each group.

¶ Not determined.

The results presented in Fig. 1 (groups 10–12) demonstrate the pivotal role of Ep in the mediation of the erythropoietic effects of L-T4 and DT in nephrectomized fetuses. Near total inhibition of erythropoiesis was seen when circulating Ep in these fetuses was neutralized by the administration of anti-Ep. Control values shown in Fig. 1 were derived from fetuses in groups 7–9. These fetuses were given injections of normal rabbit serum using procedures similar to those described for the administration of the antibody to groups 10–12. We have previously reported (2) that the administration of similar amounts of normal rabbit serum to sheep fetuses did not appreciably affect any of the erythropoietic parameters reported here.

To assess the influence of the treatment of the fetus on maternal erythropoiesis, maternal plasma samples obtained at intervals shown for the fetuses were assayed for Ep activity in polycythemic mice. No detectable rise in maternal Ep activity was noted throughout the study period. Moreover, maternal reticulocyte and hematocrit values remained unchanged.

DISCUSSION

The results here demonstrate that the administration of DT and L-T4 to fetal sheep during the last trimester of gestation results in increased production of erythrocytes. This was evident not only from increased incorporation of radioiron into the circulating erythrocytes, but also from elevated numbers of circulating reticulocytes and generally increased hematocrit values. The expression of the erythropoietic effects of DT and L-T4 in these fetuses was dependent upon the presence of Ep and was mediated, at least in part, through increased production of Ep. In this regard, plasmas from DT- and L-T4-treated fetuses exhibited significant erythropoiesis-stimulatory activity in exhypoxic polycythemic mice. That this effect of the fetal plasma was caused by the presence of elevated Ep levels was demonstrated by the fact that pretreatment of the plasma with anti-Ep completely neutralized its erythropoietic activity. Moreover, complete inhibition of in vivo erythropoiesis was achieved in control, as well as DT- and

TABLE III

*Effect of DT and L-T4 on Ep in Normal and Bilaterally Nephrectomized Sheep Fetuses**

Fetus	Treatment	Reticulocytes‡	Hematocrit‡	Erythrocyte-^{59}Fe incorporation‡
		%	%	%
Normal (5)§	Vehicle	3.2±0.3	32.8±2.3	24.6±3.7
Normal (4)	L-T4	7.3±0.6‖	43.4±4.2	34.8±2.8‖
Normal (4)	DT	8.1±0.6‖	50.2±5.1‖	42.3±4.3‖
Nephrectomized (3)	Vehicle	2.2±0.2	31.2±2.0	20.5±2.9
Nephrectomized (4)	L-T4	8.1±0.4‖	41.8±3.0	36.7±3.8‖
Nephrectomized (4)	DT	7.6±0.7‖	48.6±4.2‖	39.1±4.0‖

* See text for details.

‡ Each value represents mean±SEM of results from all fetuses in each group.

§ Numbers in parentheses indicate the number of surviving fetuses in each group.

‖ $P < 0.05$ when compared to respective vehicle-treated groups.

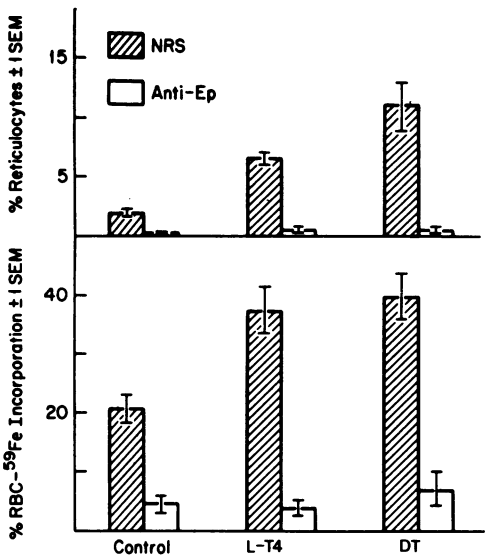

FIGURE 1 Suppression of erythropoiesis in control (vehicle-treated), DT- and L-T4-treated bilaterally nephrectomized sheep fetuses with anti-Ep. Each value represents the mean ±1 SEM of results obtained from either three (control) or four (testosterone and thyroxine groups) fetuses. NRS, normal rabbit serum. RBC, erythrocyte.

L-T4-treated, fetuses by the administration of anti-Ep. These results, once again, demonstrate the pivotal role of Ep in the regulation of erythropoiesis in the mammalian fetus (2), and point to the similarities in the regulation of erythropoiesis between the fetus and the adult (29). In these studies, despite the use of pharmacological doses of these hormones, the erythropoietic influences of DT and L-T4 were confined to the fetus; maternal erythropoietic status remained unaltered during the study period. This is in accord with reported observations that little, if any, transplacental passage of DT, L-T4, and Ep occurs in sheep (29, 30).

Stimulation of erythropoiesis in the adult by DT and L-T4 has been well documented (21–23, 31–34). Like the fetus, the erythropoietic response of the adult to these hormones is mediated by Ep (31, 33, 34). Furthermore, this effect of androgens in the adult is dependent on the presence of the kidney (24). The mechanism underlying the androgen effect on Ep production is not known. In the fetus, however, the manifestation of the erythropoietic effects of DT and L-T4 did not require the presence of the kidneys. Administration of DT and L-T4 to bilaterally nephrectomized fetuses resulted in the enhancement of erythropoiesis and Ep production similar to that observed in fetuses with intact kidneys; this response was totally abolished by injections of anti-Ep.

Since the early classic studies of Jacobsen et al. (3), the kidney has been acknowledged to be the primary site of Ep production in adult mammals. However, in most species, small but detectable amounts of Ep are produced in the absence of both kidneys (3, 4, 35). The overwhelming evidence suggests that the liver is the major source of this extrarenal Ep (5, 36). The cellular origin of extrarenal Ep is not known. Studies by Peschle et al. (37) have implicated the reticuloendothelial system (including the Kupffer cell) in extrarenal Ep formation, whereas Erslev et al. (38) suggest that the hepatocyte may be involved. Results described here, in agreement with earlier observations (6), demonstrate that the liver, rather than the kidney, is the primary source of Ep in the fetus. The switch to renal predominance occurs sometime during the early neonatal period and is associated with the degree of neonatal maturity (39, 40). Thus the liver, active during fetal life, becomes dormant in the adult when the kidney assumes the primary role in Ep production. However, the hepatic system may become reactivated during periods of renal nonfunction and/or absence to once again resume Ep production (5, 36).

Unlike the kidney in the adult and the liver in the fetus, which produce adequate amounts of Ep in response to normal day-to-day needs, hepatic reactivation does not occur in the absence of above normal levels of stimulation. In bilaterally nephrectomized laboratory animals, the activation of extrarenal Ep formation requires a greater intensity of hypoxia than the kidney needs for Ep formation (15). In the isolated cases of renoprival man where detectable Ep levels were noted, it was preceded by severe anemia (12, 41). In this regard, experiments by Ersley et al. (38) indicate that in the presence of severe hypoxic stress, extrarenal Ep production may reach levels comparable to those of renal formation. In a majority of clinical cases, however, hepatic reactivation does not occur, and an adequate rate of erythrocyte production is not maintained in most patients with renal failure (10, 11).

The results of the present study demonstrate that the extrarenal site of Ep formation is responsive to the same regulatory forces that affect renal Ep production. Thus DT and L-T4 are effective promoters of hepatic Ep formation in fetal sheep. Moreover, these results for the first time provide definitive experimental evidence in support of continuing the use of hormonal agents such as androgens in attempts to reactivate hepatic Ep production in anephric adults. It should be noted that in several clinical trials, androgens have not been particularly effective in anephric patients, perhaps because of the absence of optimal conditions.

Further studies are required to determine the optimal condition(s) under which maximal stimulation of hepatic Ep production in adults in response to androgens occurs. In our studies, the degree of DT- and L-T4-induced stimulation of Ep production in the fetus was generally comparable to that seen in intact adults (un-

published observations, based on studies in two adult female sheep). However, the relative increase in Ep formation in anephric adults in response to androgen therapy is significantly less than when the kidneys are present (18). Thus additional steps may be needed to maximize the hepatic response to these hormones in the adult. In this regard, it has been demonstrated that exposure of DT- and L-T4-treated adult animals to hypoxic stimuli results in a synergistic (rather than additive) effect on Ep formation (42).

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REFERENCES

1. Gordon, A. S. 1973. Erythropoietin. *Vitam. Horm.* **31:** 105–174.
2. Zanjani, E. D., L. I. Mann, H. Burlington, A. S. Gordon, and L. R. Wasserman. 1974. Evidence for a physiologic role of erythropoietin in fetal erythropoiesis. *Blood.* **44:** 285–290.
3. Jacobson, L. O., E. Goldwasser, W. Fried, and L. Plazak. 1957. Role of the kidney in erythropoiesis. *Nature (Lond.).* **179:** 633–634.
4. Jacobson, L. O., E. K. Marks, E. O. Gaston, and E. Goldwasser. 1959. Studies on erythropoiesis. XI. Reticulocyte response of transfusion-induced polycythemic mice to anemic plasma from nephrectomized mice and to plasma from nephrectomized rats exposed to low oxygen. *Blood.* **14:** 635–643.
5. Fried, W. 1972. The liver as a source of extrarenal erythropoietin production. *Blood.* **40:** 671–677.
6. Zanjani, E. D., J. Poster, H. Burlington, L. I. Mann, and L. R. Wasserman. 1977. Liver as the primary site of erythropoietin formation in the fetus. *J. Lab. Clin. Med.* **89:** 640–644.
7. Lucarelli, G., P. Howard, and F. Stohlman, Jr. 1964. Regulation of erythropoiesis. XV. Neonatal erythropoiesis and the effect of nephrectomy. *J. Clin. Invest.* **43:** 2195–2203.
8. Naets, J. P., and M. Wittek. 1968. Erythropoiesis in anephric man. *Lancet.* **1:** 941–943.
9. Kominami, N., E. G. Lowrie, L. E. Ianhez, A. Skaren, C. L. Hampers, J. P. Merrill, and R. D. Lange. 1971. The effect of total nephrectomy on hematopoiesis in patients undergoing chronic hemodialysis. *J. Lab. Clin. Med.* **78:** 524–532.
10. Desforges, J. F. 1970. Anemia in uremia. *Arch. Intern. Med.* **126:** 808–811.
11. Erslev, A. J. 1970. Anemia of chronic renal disease. *Arch. Intern. Med.* **126:** 774–780.
12. Erslev, A. J., P. J. McKenna, J. P. Capella, R. J. Hamburger, H. E. Cohn, and J. E. Clark. 1968. Rate of red cell production in two nephrectomized patients. *Arch. Intern. Med.* **122:** 233–235.
13. Erslev, A. J., J. Caro, E. Kansu, O. Miller, and E. Cobbs. 1979. Plasma erythropoietin in polycythemia. *Am. J. Med.* **66:** 243–247.
14. Caro, J., E. Kansu, S. Brown, T. Murray, O. Miller, and A. J. Erslev. 1978. Biologic and immunologic erythropoietin in uremic-nephric and anephric patients. *Blood.* **52** (Suppl. 1): 78. (Abstr.)
15. Schooley, J. C. and L. J. Mahlmann. 1972. Erythropoietin production in the anephric rat. I. Relationship between nephrectomy, time of hypoxic exposure and erythropoietin production. *Blood.* **39:** 31–38.
16. Caro, J. and A. J. Erslev. 1977. Erythropoiesis and response to erythropoietin in rats with chronic uremia. *Blood.* **50**(Suppl. 1): 123. (Abstr.)
17. DeGowin, R. L., A. R. Lavender, M. Forlano, D. Charleston, and A. Gottschalk. 1970. Erythropoiesis and erythropoietin in patients with chronic renal failure treated with hemodialysis and testosterone. *Ann. Intern. Med.* **72:** 913–918.
18. Eschbach, J. W., and J. W. Adamson. 1973. Improvement in the anemia of chronic renal failure with fluoxymesterone. *Ann. Intern. Med.* **78:** 527–532.
19. Fried, W., O. Jonasson, S. Lang, and F. Schwartz. 1973. The hematologic effect of androgen in uremic patients: study of packed cell volume and erythropoietin responses. *Ann. Intern. Med.* **79:** 823–827.
20. Williams, J. S., J. H. Stein, and T. F. Ferris. 1974. Nandrolone decanoate therapy for patients receiving hemodialysis: a controlled study. *Arch. Intern. Med.* **134:** 289–296.
21. Fried, W., and C. W. Gurney. 1965. Erythropoietic effect of plasma from mice receiving testosterone. *Nature (Lond.).* **206:** 1160–1161.
22. Mirand, E. A., A. S. Gordon, and J. Wenig. 1965. Mechanism of testosterone action on erythropoiesis. *Nature (Lond.).* **206:** 270–272.
23. Shahidi, N. T. 1973. Androgens and erythropoiesis. *N. Engl. J. Med.* **289:** 72–80.
24. Meineke, H. A., and R. C. Crafts. 1968. Further observations on the mechanism by which androgens and growth hormone influence erythropoiesis. *Ann. N. Y. Acad. Sci.* **149:** 298–307.
25. Zanjani, E. D., E. Horger, A. S. Gordon, L. N. Cantor, and D. L. Hutchinson. 1969. Erythropoietin production in the fetal lamb. *J. Lab. Clin. Med.* **74:** 782–788.
26. Zanjani, E. D., E. N. Peterson, A. S. Gordon, and L. R. Wasserman. 1974. Erythropoietin production in the fetus: Role of the kidney and maternal anemia. *J. Lab. Clin. Med.* **83:** 281–287.
27. Zanjani, E. D., J. D. Lutton, R. Hoffman, and L. R. Wasserman. 1977. Erythroid colony formation by polycythemia vera bone marrow in vitro dependence on erythropoietin. *J. Clin. Invest.* **59:** 841–848.
28. Mott, J. C. 1973. The renin-angiotensin system in feotal and newborn mammals. *In* Foetal and Neonatal Physiology. K. S. Comline, K. W. Cross, G. S. Dawes, and P. W. Nathanielsz, editors. Cambridge University Press, Cambridge, England. 166–180.
29. Zanjani, E. D., J. Poster, L. I. Mann, and L. R. Wasserman. 1977. Regulation of erythropoiesis in the fetus. *In* Kidney Hormones. J. W. Fisher, editor. Academic Press, Inc., New York. 463–493.
30. Thorburn, G. D., and P. S. Hopkins. 1973. Thyroid function in the foetal lamb. *In* Foetal and Neonatal Physiology. K. S. Comline, K. W. Cross, G. S. Dawes, and P. W. Nathanielsz, editors. Cambridge University Press, Cambridge, England. 488–507.
31. Peschle, C., E. D. Zanjani, A. S. Gidari, W. D. McLaurin, and A. S. Gordon. 1971. Mechanism of thyroxin action on erythropoiesis. *Endocrinology.* **89:** 609–612.
32. Malgor, L. A., C. C. Blank, E. Klainer, S. E. Irizar, P. R. Torales, and L. Barrios. 1975. Direct effects of thyroid hormones on bone marrow erythroid cells of rats. *Blood.* **45:** 671–679.
33. Popovic, W. J., J. E. Brown, and J. W. Adamson. 1977. The influence of thyroid hormones on in vitro erythro-

poiesis: mediation by a receptor with beta adrenergic properties. *J. Clin. Invest.* **60:** 907–913.
34. Dainiak, N., R. Hoffman, L. A. Maffei, and B. G. Forget. 1978. Potentiation of human erythropoiesis *in vitro* by thyroid hormone. *Nature (Lond.).* **272:** 260–262.
35. Mirand, E. A., and T. C. Prentice. 1957. Presence of plasma erythropoietin in hypoxic rats with or without kidney and/or spleen. *Proc. Soc. Exp. Biol. Med.* **96:** 49–54.
36. Schooley, J. C., and L. J. Mahlmann. 1974. Hepatic erythropoietin production in the lead-poisoned rat. *Blood.* **43:** 425–428.
37. Peschle, C., G. Marone, A. Genevese, C. Magli, and M. Condorelli. 1976. Hepatic erythropoietin: enhanced production in anephric rats with hyperplasia of Kupffer cells. *Br. J. Hematol.* **32:** 105–111.
38. Erslev, A. J., J. Caro, and E. Kansu. 1977. Renal and extra-renal erythropoietin production. *Blood.* **50**(Suppl. 1): 126. (Abstr.)
39. Carmena, A. O., D. Howard, and F. Stohlman. 1968. Regulation of erythropoietin. XXII. Production in the newborn animal. *Blood.* **32:** 376–382.
40. Wang, F., and W. Fried. 1972. Renal and extrarenal erythropoietin production in male and female rats of various ages. *J. Lab. Clin. Med.* **79:** 181–186.
41. Naets, J. P., and M. Wittek. 1968. Presence of erythropoietin in the plasma of one anephric patient. *Blood.* **31:** 249–251.
42. Gordon, A. S., E. A. Mirand, J. Wenig, R. Katz, and E. D. Zanjani. 1968. Androgen actions on erythropoiesis. *Ann. N. Y. Acad. Sci.* **149:** 318–335.