The brief description we have provided here of histological alterations in lower jaws of radiothyroidectomized trout differs in no important way from the more extensive descriptions already published by others(9,11, 12,13). These changes characteristically have been found to include: (a) progressive thyroid follicular epithelial pycnosis and cytolysis whose rates depend upon doses of the radioiodine, (b) leucocytic infiltration and removal of debris, (c) fibrous replacement and scar formation, and (d) sclerotic changes in the walls of adjacent blood vessels.

Summary. A simplified procedure for radiothyroidectomy of large numbers of juvenile steelhead trout (alevins or fry) is described. Complete destruction of thyroid tissue was achieved 8 to 14 weeks after immersion in a solution of radioiodine in lake water (initial concentration: 12.5 or 25 μc I-131/cc for 8 days).

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Effect of Erythropoietin and Oxygen Tension on *in vitro* Synthesis of Hemoglobin A and F by Adult Human Bone Marrow.* (30415)

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The hemoglobin found in the peripheral blood of the normal human adult is heterogeneous in structure. In addition to normal adult hemoglobin there are 2 other minor fractions, hemoglobin A_2 and hemoglobin F. The latter, which is the predominant hemoglobin in the newborn, falls to very low levels by the end of the first year of life and only in certain pathological conditions is it found increased. Pathological conditions include Thalassemia, some hemoglobinopathies, aplastic anemia, some cases of acute leukemia and some cases of vit. B_{12} deficiency. The factors, genetic and environmental, which control the rate of synthesis of the various hemoglobins are poorly delineated. Allen and Jandl (1), using reticulocyte suspensions obtained from the cord blood of newborns, found that conditions of hypoxia or glucose deficiency induced a *relative* increase in the synthesis of fetal hemoglobin (HbF) as compared with adult hemoglobin (HbA). Thomas *et al*(2), using various hematopoietic organs obtained from fetuses, reported essentially similar findings irrespective of the organ used. They also demonstrated that hematopoietic tissue

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in all of the organs studied, liver, spleen and bone marrow, synthesized both Hb A and F. Lucarelli *et al*(3) and Stohlman *et al*(4) have investigated the effects of nephrectomy in the neonatal rat and have shown that the immature animal does not show the depression of erythropoiesis following nephrectomy found in the adult animal. This finding suggests that erythropoiesis in the neonatal rat is not dependent upon erythropoietin. However, there exist no previous studies on the effects of erythropoietin specifically on the synthesis of hemoglobin A and F. The studies reported here attempt to elucidate several of the factors which might differentially influence the rate of synthesis of hemoglobins A and F.

Normal adult human bone marrow was obtained by aspiration from the posterior iliac crest. The nucleated cells were concentrated by differential centrifugation at 4°C, (first for 5 minutes at 500 rpm and then at 2000 rpm for 10 minutes) and resuspended in a media composed of two-thirds Hanks' solution and one-third autologous plasma. The final suspension contained about 5000 nucleated cells/cm^3 in a volume of 3.2 ml. Penicillin G was added to a final concentration of 50 units/ml. Glycine 2-C^{14} was used to label total hemoglobin, isoleucine U-C^{14} was used to measure rate of synthesis of hemoglobin F; whereas glycine is incorporated into both hemoglobin A and F, isoleucine is incorporated only into hemoglobin F. Incubations were carried out at 37°C, without agitation, in siliconized 10 ml Erlenmeyer flasks under a gas phase of 5% CO_2 in air. Samples were removed at 2, 24, 48 and 72 hours and total nucleated cells, normoblasts and reticulocytes were determined by standard methods. Cellular morphology remained recognizable up to at least 48 and usually 72 hours. The washed cells were exposed to shock lysis and total hemoglobin was isolated by acid-acetone precipitation. The isolated globin was purified several times by reprecipitation and then counted in a Nuclear Chicago automatic model-gas flow counter.† The results are expressed as millimoles amino acid incorporated per 10^{12} nucleated erythroid precursors. Control experiments demonstrated that the rate of synthesis over the period studied was unaffected by (1) number of cells in the incubation media (up to about 20,000 cells/cm^3), (2) amount of carrier amino acid added, (3) added glucose, and (4) time at which radioactive amino acid was added; addition of the label 2 hours before the cells were harvested gave the same *rate* of synthesis as was observed when the isotope was added at the time incubations were begun.

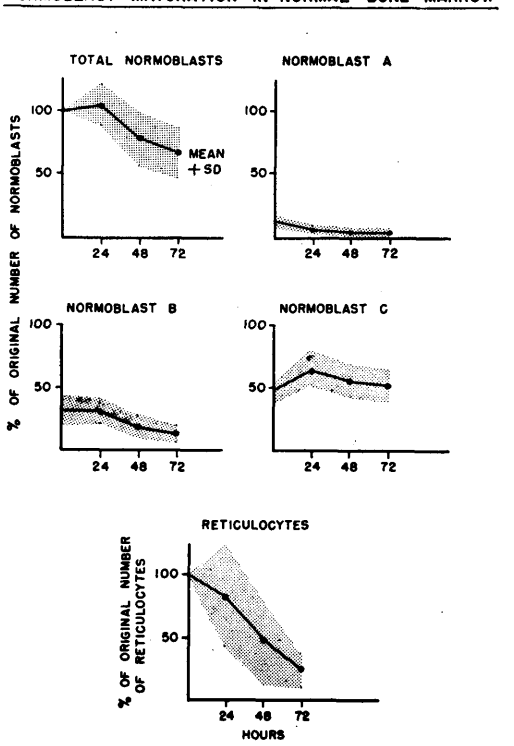

FIG. 1. Normoblast maturation in normal bone marrow cultures. Solid lines represent mean of 8 experiments; shaded area, one standard deviation. Normoblast A = basophilic normoblasts; B = polychromatophilic normoblasts; C = orthochromatophilic normoblasts.

Differential counts of the nucleated erythroid precursors (Fig. 1) showed a disappearance of basophilic normoblasts by 48 hours with a definite increase in the total number

† The method used for separation of globin is an adaption of that described by Lingrel, J. B., Borsook, H., Biochem., 1963, v2, 309 and will appear shortly in Necheles, T. F., Steiner, M., Baldini, M., Blood, in press.

TABLE I. Glycine and Isoleucine Incorporation into Hemoglobin by Bone Marrow Nucleated Erythroid Cells.

	Glycine-2-C^{14}				Isoleucine-U-C^{14}			
	mMoles amino acid incorporated/nucleated erythroid precursor × 10^{12}							
Time (hr) →	2	24	48	72	2	24	48	72
Exp 13	.60	8.1	9.6	9.0	.05	.48	.43	.34
14	.65	6.9	7.2	7.3	.02	.15	.12	.11
16	.31	4.0	4.9	5.1	.03	.24	.22	.17
20	.40	3.2	5.1	5.8	.06	.42	.54	.71
23	.37	—	8.9	7.9	.09	.71	.60	.41
24	.36	4.9	6.3	—	.14	1.34	1.39	—
Avg	.45	5.4	7.0	7.0	.06	.56	.55	.35
S.D.	.14	2.0	1.8	1.6	.04	.43	.45	.24

of orthochromatic cells. There was an initial increase in the total number of reticulocytes which then decreased linearly with time. Concomitantly, the incorporation of glycine-2-C^{14} into globin continued up through 48 to 72 hours whereas significant isoleucine-C^{14} incorporation occurred only during the first 24 hours (Table I). Similar experiments using reticulocytes obtained from the peripheral blood of patients with marked reticulocytosis showed that the synthesis of total hemoglobin per reticulocyte was only 1-2% of that found in the nucleated erythroid precursors and that isoleucine-C^{14} incorporation comprised a very small proportion of total hemoglobin synthesis in these cells.

Partially purified human erythropoietin‡ was added to cell suspensions to a final concentration of about 1 unit/ml. Previous studies on the *in vitro* effect of erythropoietin have demonstrated a stimulating effect upon (1) the incorporation of Fe^{59} into cells and into hemoglobin(5), (2) the incorporation of glycine-C^{14} into heme(6), and (3) the incorporation of glucosamine into bone marrow cells(7). There have, as yet, been no reported studies on the effects of erythropoietin on the differential synthesis of human hemoglobins. In each of the experiments reported here (Table II) a significant increase in glycine-2-C^{14} incorporation into globin was noticed whereas isoleucine incorporation showed no significant change. Simultaneous cell counts showed an increase in total normoblasts, reflecting primarily an increase in orthochromatic cells. No significant increase in basophilic normoblasts was seen.

In parallel preliminary experiments, the effect of oxygen tension on globin synthesis was also measured. At levels between 25 and 5% O_2 in the gas phase, there was a moderate progressive depression in total globin synthesis but no significant change was noted in isoleucine incorporation. This essentially confirms the results reported by Allen and Jandl(1) and Thomas and coworkers(2).

These experiments provide further evidence that hemoglobins A and F may well respond differently to environmental stimuli. Differential staining of smears of peripheral red cells of most individuals with elevated levels of hemoglobin F appears to demonstrate that the bulk of this hemoglobin is confined

TABLE II. The *in vitro* Effect of Erythropoietin on Glycine and Isoleucine Incorporation.

	mMoles amino acid incorporated/nucleated erythroid precursor × 10^{12}							
	#20		#23		#24		Avg	
	Control	Erythropoietin	Control	Erythropoietin	Control	Erythropoietin	Control	Erythropoietin
Glycine-2-C^{14}								
2 hr	.40	.35	.37	.63	.36	.53	.38	.50
24 "	3.2	4.2	—	7.1	4.9	7.5	4.1	5.8
48 "	5.1	7.1	8.9	9.9	6.3	8.5	6.9	8.5
72 "	5.8	5.6	7.9	7.6	—	—	6.8	6.6
Isoleucine-U-C^{14}								
2 hr	.06	.06	.09	.07	.14	.14	.10	.09
24 "	.42	.45	.71	.44	1.34	1.65	.82	.85
48 "	.54	.46	.60	.70	1.39	1.10	.84	.75
72 "	.71	.58	.41	.45	—	—	.56	.51

‡ Obtained from Dr. F. Stohlman, St. Elizabeth's Hospital, Boston.

to a limited number of cells(8), presumably the progeny of stem cells which have retained the ability to synthesize this "primitive" form of hemoglobin. Two explanations of these and other observations are possible. There may exist a "clone" of cells in all individuals capable of synthesizing fetal hemoglobin. Normally non-proliferating, this clone can, under periods of "stress," be called into renewed activity. As a consequence, the amount of fetal hemoglobin in the peripheral blood will increase. This "fetal clone" of cells would be assumed not to be responsive to the effects of erythropoietin and would be relatively insensitive to the effects of hypoxia. Stohlman(4) has suggested that the fetal erythropoietic apparatus is unresponsive to the effects of erythropoietin and it may be the persistence of small numbers of these cells which form the "fetal clone" in the adult. The data obtained in this series of experiments permit an alternative explanation. It is probable that the messenger RNA for both fetal and adult hemoglobin is synthesized in the basophilic normoblast, and it is possible, as has previously been suggested, that the messenger RNA for fetal hemoglobin is less stable than that for adult hemoglobin. Thus, in adult bone marrow, a relative increase in the basophilic normoblasts with shortened maturation time will lead to a relative increase in fetal hemoglobin in the mature cell(9). On the other hand, situations which induce proliferation or maturation in the polychromatophilic stages with relative increases in the orthochromatophilic cells will lead to increased synthesis of adult hemoglobin. In the present series of experiments, the rate of incorporation of isoleucine-C^{14} closely parallels the number of basophilic cells present in the incubation mixture. Erythropoietin, which under these conditions leads primarily to a relative increase in the number of orthochromatophilic cells, stimulates the synthesis of total globin but does not significantly change the rate of isoleucine incorporation. Hypoxia, which depresses the rate of proliferation, reduces total globin synthesis without significant effect on the synthesis of Hb F.

At present, the data from these and other workers' studies fit both hypotheses equally well, and there are no data available which enable us to choose between them. Indeed, it is conceivable that both may be valid, and under various pathological conditions one or the other mechanism may be of paramount importance in determining the level of Hb F in the peripheral blood.

These studies cover but a few of the possible environmental factors which may influence the rate of synthesis of the various globins in the intact animal.

Summary. Hemoglobin F and A synthesis was studied in *in vitro* suspensions of normal adult human bone marrow. Hb A synthesis continued throughout the 72-hour period whereas Hb F synthesis ceased after 24 hours. Erythropoietin, added *in vitro,* stimulated total hemoglobin synthesis without affecting Hb F synthesis. Hypoxia depressed total globin synthesis without affecting Hb F synthesis. Thus the synthesis of Hb A and F appears to respond independently to environmental stimuli.

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