Erythropoiesis in Hypothyroidism (37531)

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Alteration in thyroid function influences the synthesis and life span of the red blood cell. Erythropoietic decompensation occurs in experimental animals and man following thyroid ablation and has been reversed by the administration of thyroidal and nonthyroidal metabolic stimulants (1). This anemia has been attributed to an adaption to diminished total tissue oxygen requirements, with a resultant decrease in the number of erythrocytes needed for oxygen transport (2–4).

There is no uniformity of opinion about the characteristic picture of the anemia in hypothyroid patients. The three morphologic types include: normocytic–normochromic; hypochromic–microcytic; and macrocytic. The variety of anemias, the incomplete incidence of anemia in hypothyroid subjects, and the lack of correlation between the severity of the anemia and the severity of the hypothyroidism suggest that several mechanisms are operative in the pathogenesis of this anemia (1). The hypochromic–microcytic anemia may be the result of abnormal iron absorption; however, iron lack is not the only mechanism, since thyroid replacement therapy is needed for complete repair of the anemia.

The purpose of the present study was to attempt to clarify the role which disordered iron metabolism and alterations in homeostatic regulation of red blood cell production play in the erythropoietic decompensation observed in hypothyroidism.

Methods and Materials. Female Sprague–Dawley rats (Holtzman Strain) were used in all experiments. The rats were 6–8 weeks old at the beginning of each experiment. Rats were fed a diet of Purina Laboratory Chow,[1] allowed tap water *ad libitum* and maintained in a controlled environment. Animal room illumination was controlled by automatic cycling, the light period extending from 6 AM to 6 PM. The Principles of Laboratory Animal Care as promulgated by the National Society for Medical Research were followed.

Each rat was anesthetized with an intraperitoneal injection of pentobarbital sodium. The ventral neck area was shaved, a vertical incision made, the thyroid glands identified and thyroidectomy carried out. Control animals were treated similarly without thyroid removal. In order to insure complete ablation of the thyroid, two weeks following thyroidectomy 0.5 mCi of ^{131}I Na was administered to each rat intraperitoneally. Total body radioactivity was measured in each rat weekly thereafter in a small animal whole body counter.[2] Subsequent experiments were performed when the whole body radioactivity had reached background levels.

Oxygen utilization was determined by means of a Warren E. Collins oxygen spirometer. Oxygen utilization was measured before and 10 weeks following thyroidectomy or sham operation.

Hematocrits were measured by standard methods and red blood cell mass was determined by dilution of ^{59}Fe tagged red blood cells after the manner of Reichlin and Harrington (5).

Groups of thyroidectomized and intact rats were given ip injections of 0.5 ml of saline, 0.5 ml of phenylhydrazine anemic rabbit plas-

[1] Iodine content 1.18 ppm, iron content 275 ppm.

[2] Packard Armac.

ma, 250 μg of dextro-triiodothyronine or 250 μg levotriiodothyronine 72 and 48 hr prior to sacrifice. Eighteen hours prior to sacrifice, 1 μCi ^{59}Fe/1 μg ^{56}Fe citrate was administered via tail vein. At sacrifice, the 18 hr RBC ^{59}Fe incorporation was determined as previously described (6). Separate groups of thyroidectomized and intact rats were exposed to 0.5 atm pressure for 6 hr, removed to an ambient atmosphere and immediately given 1 μCi ^{59}Fe/1 μg ^{56}Fe iv and sacrificed 18 hr later and the erythrocyte iron incorporation measured.

Groups of thyroidectomized or sham operated animals were starved for a period of 18 hr and then received 1 μCi ^{59}Fe citrate/20 μg ^{56}Fe citrate in 1 ml volume via stomach tube. Prior to the administration of radioiron, the thyroidectomized and intact animals were divided into four groups of 6 and 4 rats each, respectively. Each animal in the intact or thyroidectomized group received an ip injection of 0.5 ml 0.9% saline solution, 250 μg levo-triiodothyronine in a 0.5 ml volume, 0.5 ml of anemic rabbit plasma or 50 μmole of $CoCl_2$ in a 0.5 ml volume 48 and 24 hr prior to the administration of radioiron. Following the intragastric administration of radioiron the total radioactivity of each animal was determined immediately and at daily intervals thereafter by means of a small animal total body detector.[3] The daily determinations of radioactivity continued until the radioactivity remained constant, thus obviating group differences in gastrointestinal motility. Appropriate corrections were made for radioactive decay. The percent radioiron absorbed was calculated using the initial radioactivity of each animal as a measure of the administered dose.

Groups of intact rats were either given 0.5 ml 0.9% saline, 250 μg of levo-triiodothyronine or 0.5 ml of anemic rabbit plasma ip. Thyroidectomized rats were given saline alone. They were maintained over the next 24 or 48 hr in metabolic cages and their urine collected in containers maintained in dry ice. The frozen urine samples were thawed and lyophilized. Urinary erythropoietin was assayed by a modification of the method of Cotes and Bangham (7). Each 24-hr urine sample was reconstituted in a volume of 5 and 1 ml of urinary concentrate which contained 20% of the total 24 hr urinary excretion was administered in divided 0.5 ml doses to each of 5 exhypoxic mice 48 and 72 hr post-hypoxia. Twenty-four hours following the last injection, 0.2 μCi ^{59}Fe/0.2 μg ^{56}Fe citrate was injected iv via the tail vein. Forty-eight hours thereafter, the erythrocyte radioiron incorporation was determined.

Statistical evaluation was carried out by comparisons of standard error distributions and by means of Students t test. A probability value of $p < 0.05$ was accepted as significant.

Results. The hypothyroid state was verified by the diminished oxygen consumption observed in thyroid ablated rats. The oxygen consumption was 125.7 ± 46.0[4] cc/100 g/hr in 32 intact sham operated rats and 90.7 ± 23.0[5] cc/100 g/hr in 28 thyroid ablated rats ($p < 0.005$).

The development of anemia was verified by the decrease in hematocrit 44.2 ± 0.9[6] vol.% in 32 sham operated rats to 33.8 ± 1.4[7] vol.% in 28 thyroid ablated rats, as well as a decrease in red blood cell mass (2.23 ± 0.08[8] ml/100 g in 8 sham operated rats and 1.68 ± 0.14[9] ml/100 g in 8 thyroid ablated rats).

The 18-hr ^{59}Fe red blood cell incorporations are indicated in Fig. 1. In the saline treated animal the erythrocyte iron incorporation was decreased in the thyroidectomized animal as compared to the intact animal. The administration of erythropoietin caused an increase over the saline control for both the thyroidectomized and intact animal. Of additional note is the observation that the administration of erythropoietin produced an increase in both intact and thyroidectomized animals to a similar level of red blood cell ^{59}Fe incorporation. Exposure to 6 hr of hypoxia produced the expected increase in the

[3] See footnote 2.

[4] Standard error.

[5] See footnote 4.

[6] See footnote 4.

[7] See footnote 4.

[8] See footnote 4.

[9] See footnote 4.

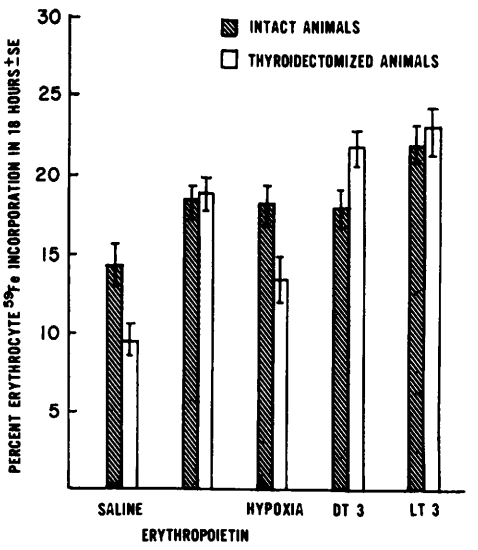

FIG. 1. Erythrocyte radioiron incorporation: Erythropoietin: Laboratory standard anemic rabbit plasma harvested from phenylhydrazine-treated rabbits.

intact animal and also increased the erythrocyte radioiron incorporation in the thyroidectomized animal, although not to the same level as the intact animal. Both dextrotriiodothyronine (DT3) and levo-triiodothyronine (LT3) produced increases in both the thyroidectomized and the intact animal to similar levels of erythrocyte radioiron incorporation. The thyroidectomized animals were capable of response to both erythropoietic stimulants and thyroid hormones.

The results of the 9-day retention of ^{59}Fe in the total body of the animal as a measurement of iron absorption are presented in Table I. The amount of iron absorbed in a 9-day period by the intact animal was greater than that of the thyroidectomized animal. Stimulation of erythropoiesis produced by the administration of anemic rabbit plasma resulted in a significant increase in iron absorption in the intact animal and also in the thyroidectomized animal. However, iron absorption increased to a greater level in the intact animal following administration of anemic rabbit plasma than in the thyroidectomized animal. Levo-triiodothyronine produced an increase in iron absorption in both the intact and the thyroidectomized animals. There was, however, no significant difference between the level of iron absorption in the intact animal given L-triiodothyronine and the thyroidectomized animal given L-triiodothyronine. At the levels of cobaltous chloride administered, there was no significant difference between the intact and thyroidectomized animals absorption of radioiron. There was a significant difference between the absorption of radioiron by the intact animal and the thyroidectomized animal. However, daily body retention of radioiron plateaued in the intact group earlier than in the thyroidectomized group presumably due to alterations in gastrointestinal motility.

The results of the urinary erythropoietin bioassay are presented in Table II. The urine of intact sham operated rats contained low but measurable levels of erythropoietic ac-

TABLE I. Gastrointestinal Radioiron Absorption.

Material administered	Percent ^{59}Fe retention at 9 days ± SE		
	Intact rats	Thyroidectomized rats	Significance[a]
Saline	9.61 ± 2.55	6.10 ± 2.62	$p < 0.05$
Erythropoietin[b]	27.23 ± 4.13	13.54 ± 3.66	$p < 0.05$
LT3[c]	24.99 ± 7.76	20.66 ± 3.04	N.S.
$CoCl_2$	10.90 ± 3.10	6.72 ± 2.45	$p < 0.05$

[a] Determination by Student's t test. A p value < 0.05 was accepted as significant. Intact and thyroidectomized rats are compared for each treatment.

[b] Anemic rabbit plasma laboratory standard harvested from anemic acetyl phenylhydrazine treated rabbits.

[c] LT3: Levotriiodothyronine.

N.S.: Not significant.

TABLE II. Urinary Erythropoietin Bioassay.
(18 Hr RBC 59-Fe Incorporation in Exhypoxic Mice)

	% RBC ^{59}Fe Inc. ± SE	No. 24-hr urine samp.	No. mice
Saline control	0.81 ± 0.37	—	25
Erythropoietin[a]	32.35 ± 4.42	—	25
Intact urine control	1.83 ± 0.67	5	25
Thyroidectomized urine	0.67 ± 0.35	6	30
Thyroidectomized post-LT3	1.94 ± 0.62	4	20

[a] Anemic rabbit plasma laboratory standard harvested from anemic acetyl phenylhydrazine treated rabbits.

tivity when compared with saline controls. The erythropoietin levels in urine from thyroidectomized rats was not significantly different from the saline control but was significantly lower than levels found in normal urine. The administration of levo-triiodothyronine to thyroidectomized rats returned the diminished urinary erythropoietin level to normal.

Discussion. The concept of the humoral regulation of erythropoiesis is generally accepted. Clinical and experimental studies have demonstrated that the fundamental modulator of red blood cell production is the elaboration of the erythropoietic hormone, erythropoietin, in response to diminished oxygen supply. The basic need is the maintenance of the balance between oxygen supply and the capacity for oxygen transport. Anemia or hypoxemia stimulate the production of erythropoietin, which in turn accelerates erythropoiesis resulting in repair of the anemia. This basic mechanism has been questioned when applied to certain hormonal erythropoietic interrelationships (8, 9) and the clinical and physiologic implications are being investigated further. The complex effect of calorigenic hormones on erythropoiesis has raised questions as to whether calorigenic hormones influence erythropoiesis through the single mechanism of erythropoietin production.

The association of anemia with hypothyroidism has been long recognized. This anemia may be a simple anemia, an anemia of the iron deficiency type, an anemia similar to Addisonian pernicious anemia, or a complex multifactorial anemia. There is no uniformity about the characteristics of the clinical picture in the hypothyroid anemias. The lack of correlation between the severity of the anemia and the severity of the hypothyroidism, the nonuniformity of the morphologic expression, and the variability of response to differing treatment modalities support the hypothesis that several mechanisms are clinically operative in the pathogenesis of this anemia.

The results of the present study suggest that thyroid ablation produces an alteration in iron metabolism characterized by diminished erythropoiesis accompanied by a decrease in the gastrointestinal absorption of radioiron. In addition, urinary erythropoietin levels fall following thyroid ablation and may be returned to normal by the administration of exogenous triiodothyronine. These results further suggest that whereas the erythropoietic decompensation can be corrected acutely by the administration of erythropoietic stimulants, the defect in iron absorption can only be fully repaired by the administration of thyroid hormone. The decrease in iron absorption may provide an explanation of the iron deficiency type of anemia observed in some instances of hypothyroidism. The relative influence of these factors in producing the erythropoietic abnormalities observed in hypothyroidism is not clear. However, it appears obvious that many factors are involved, and that thyroid hormone affects erythropoiesis by other means in addition to the erythropoietin homeostatic regulatory mechanism.

Summary. The purpose of this study was to clarify the role which disordered iron me-

tabolism and alterations in the homeostatic regulation of red cell production play in hypothyroid anemia. Following the measurement of the oxygen consumption, rats were thyroidectomized and given an ablative dose of sodium ^{131}iodine. The oxygen consumption was again measured and the experiments initiated 8 weeks thereafter. The 18-hr red cell radioiron incorporation was used as an index of erythropoiesis. The gastrointestinal absorption of radioiron and the 24-hr urinary erythropoietin levels were assessed. Hypothyroid rats demonstrated a decreased red cell radioiron incorporation and a diminished GI iron absorption. The diminution in erythropoiesis was reverted toward normal by the administration of erythropoietin, D-triiodothyronine, L-triiodothyronine or exposure to hypobarbaric hypoxia. Erythropoietin, which completely corrected the erythropoietic depression in the hypothyroid rats, did not correct the decreased GI radioiron absorption whereas L-triiodothyronine restored both to normal. Urinary erythropoietin was decreased in the hypothyroid rat and reverted to normal following L-triiodothyronine administration. These data suggest that the mechanism producing the erythropoietic decompensation in hypothyroid states is complex and involves the lack of erythropoietic stimulation by erythropoietin. Acute erythropoietic compensation appears possible by the administration of nonspecific erythropoietic stimulants; however, the defect in gastrointestinal iron absorption can only be repaired by the administration of thyroid hormones.

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