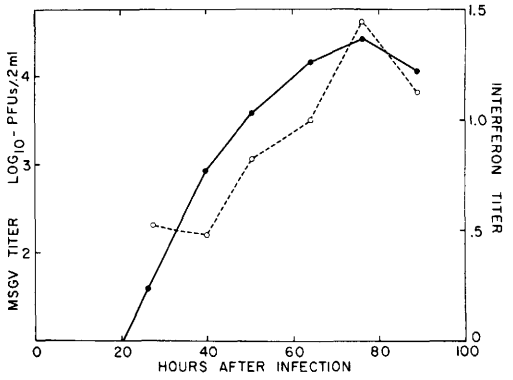

FIG. 1. Increase of interferon activity with increasing titer of MSGV. Solid line—MSGV titer, dashed line—interferon titer. Interferon was assayed as described in Materials and Methods. Interferon titer is expressed as the $\log_{10}$ of the ratio of the EMC titer obtained on control medium to the EMC titer obtained on MSGV medium.

plate. After 18 hours, the media were removed and the plates infected with approximately 40 plaque-forming units of EMC in 0.2 ml of Hanks' balanced salt solution. After 20 minutes of adsorption, the monolayers were covered with 5 ml of agar overlay medium containing 100 μg/ml DEAE, stained 36 hours later, and the plaques counted at 48 hours. The results are presented in Table III. In addition to the marked reduction in count, plaques in the experimental group were approximately half the diameter of those in control plates.

TABLE III. Reduction in Number of EMC Plaques in "L" Cell Monolayers Pretreated for 18 Hours with MSGV Culture Medium.

	Plaques per plate		
Plate No.	I	II	III
Control medium	35	44	40
MSGV "	12	10	12

Summary. Using a heterologous virus-cell system, an EMC virus interfering substance having many properties similar to those of interferon(3,4) was detected in the growth medium from mouse embryo cell cultures infected with the murine salivary gland virus. The interfering material was non-dialyzable, heat labile at 60°C for one hour, stable at pH 2, not affected by MSGV antiserum, not sedimented at 90,000 × *g*, and was active in a cell system unable to support MSGV replication. In infected cultures, the rise in MSGV titer was associated with a corresponding rise in the interferon activity. Efforts to relate interferon production to latency *in vivo* are in progress.

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Evidence for a Non-Calorigenic Effect of Thyroxin on Erythropoiesis as Judged by Radioiron Utilization.* (29626)

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It seems certain that the thyroid plays a role in the process of erythropoiesis. Most workers have correlated this effect with the well known role of the thyroid gland in regu-

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lating general metabolic rate and, thereby, oxygen need(1-5).

On several occasions data in our laboratory indicated that oxygen utilization and erythropoiesis did not increase to a similar degree in animals treated with thyroxin. Furthermore, Donati, Warnecke, and Gallagher(6) have shown that D and L triiodothyronine have quite different effects on oxygen utilization in rats but, at the same time, stimulate erythropoiesis to a like degree.

The present experiments were done in an attempt to determine whether or not thyroxin can play a non-calorigenic role in erythropoiesis in addition to that based on stimulation of oxygen need.

Methods. Adult Sprague-Dawley (Holtzman) female rats of 2-3 months of age were used in these experiments. All rats were fed Purina Chow *ad libitum* plus lettuce once a week. Completeness of hypophysectomies and thyroidectomies were checked carefully at autopsy with a binocular dissecting scope.

Oxygen consumptions were performed according to the method previously described (1). Rate of erythropoiesis was judged by the Fe59 incorporation method. Intravenous injections of 1 μc of Fe59 were given at 4:00 P.M. the day before autopsy. Eighteen hours later 0.5 ml of blood was removed *via* heart puncture and its activity determined in a well-type scintillation counter. This figure was doubled to represent activity in 1.0 ml of blood. Red cell utilization was calculated on the basis of radioactivity recovered related to the amount given; the following formula was used.

$$\% \text{ Incorporation} = \frac{\text{Counts/min} \times \text{total blood vol}}{\text{Total counts/min injected}}$$

Total blood volume was considered to be 4.87% of body weight unless the animal was given a transfusion, in which case it was considered to be 5.23%(7).

All differences claimed here were verified statistically.

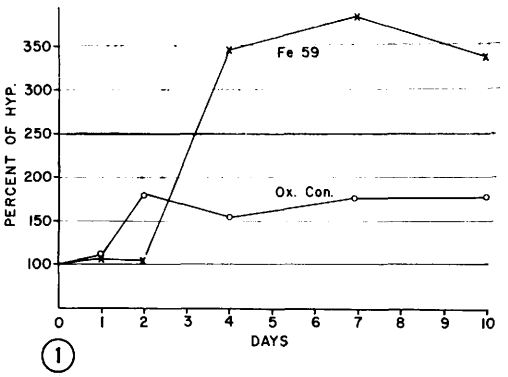
Procedure and results. The first part of this experiment was a comparison of responses in oxygen consumption and in Fe59 uptake to injections of 0.05 mg of D, L thyroxin per day for a 10-day period. Thirty animals were hypophysectomized and allowed to rest for a 2-week period before injections were instituted; such rats are quite sensitive to erythropoietic agents. Studies were made on separate groups of rats after 1, 2, 4, 7, and 10 days of treatment; values obtained were compared to data from hypophysectomized untreated controls.

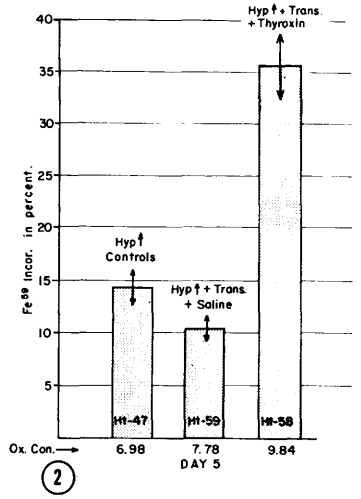
The results are depicted in Fig. 1. The hypophysectomized control rats exhibited an average Fe59 uptake of 13.5 $\pm$ 2.56% and an average oxygen consumption of 7.02 $\pm$ 0.26 liters per square meter of body surface per hour. Thyroxin injections induced increases in Fe59 uptake (248, 278, and 242% above the control level on days 4, 7, and 10 respectively) which were considerably higher than could be accounted for by the responses in oxygen consumption (52, 74, and 75% above control values on days 4, 7, and 10).

As a further check rats were treated with thyroxin after being made polycythemic, the thought being that the increased number of erythrocytes would adequately take care of any increased need for oxygen transport, leaving any response obtained as due to something other than oxygen need. Twenty rats were divided into 3 groups—1) hypophysectomized controls; 2) hypophysectomized followed 2 weeks later by a transfusion of 3.4 ml of blood, in which the packed cell volume had been concentrated to 70% by removing plasma, and then injected daily with normal saline for 5 days; and 3) same as group #2 except for daily injections of 0.05 mg of thyroxin in place of the saline.

The results obtained are shown in Fig. 2. The hypophysectomized, transfused, saline treated rats exhibited iron uptakes which averaged 10.38 $\pm$ 1.10%. Those animals transfused and treated with thyroxin exhibited values for iron incorporation averaging 35.33 $\pm$ 4.35%. This is a 240% increase over the response of the transfused rats treated with saline only.

Oxygen consumptions of the 3 groups of rats were 6.98 $\pm$ 0.46 liters for hypophysectomized controls, 7.78 $\pm$ 0.65 liters for transfused saline treated rats, and 9.84 $\pm$ 0.26 liters for thyroxin treated animals.





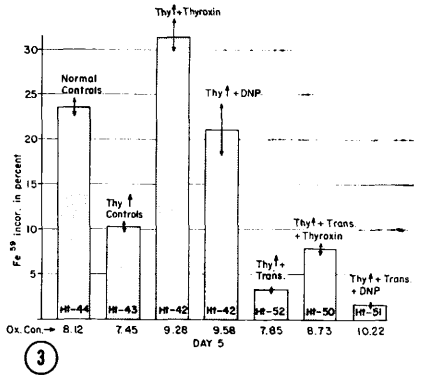


FIG. 1. Effects of 10 daily injections of 0.05 mg of thyroxin on Fe59 uptake and oxygen consumption in hypophysectomized adult female rats. Injections were started 2 weeks after surgery. Percent of hyp. = % of values for hypophysectomized untreated control animals.

FIG. 2. Effects of 5 daily injections of 0.05 mg of thyroxin on Fe59 incorporation in hypophysectomized rats transfused with 3.4 ml of blood in which the packed cell volume was concentrated to 70%. Injections were started 2 weeks after hypophysectomy. Ht = hematocrit level; Ox. Con. = oxygen consumption in liters per square meter body surface per hour; arrows indicate standard error of mean.

FIG. 3. Effects of 5 daily injections of 0.05 mg of thyroxin or dinitrophenol (15 mg/kg B.W. 3 × daily) on Fe59 incorporation in thyroidectomized adult female rats or on thyroidectomized rats previously transfused with 3.2 ml of blood in which packed cell volume was concentrated to 70%. Treatments were started 2 weeks after surgery. These values are compared to normal control, thyroidectomized control, and thyroidectomized transfused rats. (Abbreviations as in Fig. 2.)

While oxygen consumption in the thyroxin treated rats increased 26% over transfused saline treated controls, iron uptake increased 240%, a response much greater than can be accounted for by oxygen need alone.

The third part of this experiment was based on the fact that 2,4 dinitrophenol is known to be a calorigenic agent but one which does not possess the other characteristics of thyroxin. It was felt that the erythropoietic effect of this drug would be based entirely on its stimulation of oxygen need, and that any response to thyroxin in excess of that to dinitrophenol could correctly be assigned to a non-calorigenic effect of thyroxin on erythropoiesis; this would depend upon a stimulation of oxygen need by the two drugs of a similar nature.

Sixty rats were thyroidectomized, this surgery being utilized because hypophysectomized rats are quite sensitive to dinitrophenol, and divided 2 weeks later into the following groups—1) thyroidectomized untreated controls, 2) treated daily with 0.05 mg of thyroxin for 5 days, 3) treated 3 times daily (every 8 hours) for 5 days with dinitrophenol in amounts of 15 mg/kg body weight each injection, 4) transfused with 3.2 ml of concentrated blood, 5) transfused as above and treated with daily injections of 0.05 mg of thyroxin for 5 days, and 6) transfused and treated with daily injections of dinitrophenol as described for group #3. Another group of 7 rats served as normal controls.

The results are presented in Fig. 3. The normal control animals exhibited an iron uptake of 24.0 ± 1.50% and an oxygen consumption of 8.12 ± 0.70 liters per square meter of body surface per hour. Thyroidectomy, as might be expected, induced a sig-

nificant decrease in iron uptake to 10.3 ± 0.63%; the oxygen consumption in this group of rats averaged 7.45 ± 0.40 liters. Dinitrophenol treatment increased iron utilization to near normal levels (21.6 ± 3.10%) and simultaneously induced an increase in oxygen consumption to 9.58 ± 0.35 liters. Thyroxin, on the other hand, increased iron uptake to levels above normal (31.4 ± 2.40%) a figure 45% higher than obtained with the dinitrophenol. And this greater effect was obtained in the face of an oxygen consumption (9.28 ± 0.57 liters) no higher than obtained with the dinitrophenol (9.58 ± 0.35 liters).

Transfusion (hemat. 52%) decreased Fe59 uptake in the thyroidectomized rats to levels (3.68 ± 0.17%) even lower than in the thyroidectomized controls (10.3 ± 0.63%); oxygen consumption averaged 7.85 ± 0.51 liters. Dinitrophenol injections for 5 days in transfused rats (hemat. 51%) had no effect on erythropoiesis, the iron uptake averaging 2.38 ± 0.28%, while stimulating oxygen consumption to a relatively high figure of 10.22 ± 0.51 liters; this lack of response in iron uptake was expected since the transfused erythrocytes provided the necessary oxygen transport. In contrast, injections of thyroxin into transfused thyroidectomized rats (hemat. 50%) elevated iron uptake to an average of 8.86 ± 0.86%, a 141% increase over the iron uptake exhibited by the thyroidectomized transfused controls and a 272% increase over that of the dinitrophenol treated transfused rats. This increase occurred in spite of the fact the oxygen consumption in these animals was elevated to only 8.73 ± 0.52 liters in contrast to an average of 10.22 liters for the dinitrophenol treated animals.

Discussion. There seems no doubt that the thyroid gland plays a regulatory role in blood cell formation. This role, however, has been relegated to a secondary position due to its influence on general metabolism which, in turn, influences oxygen need. We have contributed to this over-simplified concept but have always been careful to leave room for a more direct effect(8). Judging erythropoiesis by the Fe59 uptake method and oxygen need by oxygen consumption, the present experiment indicates that thyroxin influences erythropoiesis in some yet unknown non-calorigenic fashion in addition to its known role in influencing general metabolism.

Fisher and Crook(9) treated hypophysectomized rats with triiodothyronine (50 μg/kg/d) for 14 days. They obtained a 61% increase in total red cell volume with only a 28% increase in oxygen consumption. They attributed this discrepancy to the toxicity of the drug. In the light of the present work, and that of Donati *et al*(6) these data can be interpreted as evidence for a direct effect of triiodothyronine on erythropoiesis in addition to its role in creating oxygen need.

Evans *et al*(10) treated thyroidectomized female rats with low doses of thyroxin and triiodothyronine. Although the low non-calorigenic doses had little effect upon total red cell volume, it was noted that the highest dose of triiodothyronine (0.02 μg) did increase red cell volume to near normal values. The authors explained this on the basis of a less rapid decline in metabolic rate in the thyroidectomized rats treated with this dose. The present work would explain this effect upon erythropoiesis in the absence of an effect upon calorigenisis as a normal function for this material.

We have no suggestion as to the exact manner in which thyroxin exerts this direct effect upon erythropoiesis. The Fe59 uptake method of determining rate of erythropoiesis actually measures delivery of red cells to the peripheral circulation and does not indicate where or how thyroxin acts.

Summary. Thyroxin has been shown to have an effect on erythropoiesis, as judged by Fe59 uptake, which is greater than would be expected from its influence upon oxygen consumption. This non-calorigenic effect on erythropoiesis has been shown in 4 ways—1) by observing a greater percentage increase in Fe59 incorporation than in oxygen consumption in hypophysectomized thyroxin treated rats; 2) by observing an increase in Fe59 uptake in thyroxin treated polycythemic hypophysectomized rats where the increased number of erythrocytes should take care of any erythropoietic response to oxygen need; 3)

by finding that thyroxin induced a greater erythropoietic response in thyroidectomized rats than did dinitrophenol even when the latter drug induced a higher oxygen consumption, and 4) by noting that thyroxin treated thyroidectomized polycythemic rats responded by an elevation in Fe59 uptake while dinitrophenol in similar rats had no effect.

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Development of Hepatic Phosphorylase in the Chick Embryo.* (29627)

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Experiments performed during the past few years on the relationship between the embryologic development of insulin and glucagon and the synthesis and breakdown of glycogen have provided evidence that, in the rat embryo, glucagon-like phosphorylase-activating substances and phosphorylase activity develop in a parallel fashion reaching maximum concentrations shortly before birth. Approximately at the same time, hepatic glycogen concentration decreases abruptly(1-6) and blood glucose concentration increases(7). Nemeth and collaborators(8) reported that, in the guinea pig, hepatic phosphorylase may be demonstrated several days before the beginning of glycogen deposition. On the other hand, Bot and collaborators(9) and Grillo (10,11) have reported that, in some tissues of the chick embryo, glycogenesis antedates the appearance of demonstrable phosphorylase activity. These results suggest that the phosphorylase-mediated pathway may not be directly related to glycogen synthesis in these embryonic tissues. Ballard and Oliver(12) found measurable phosphorylase activity in the liver of chick embryos as early as the 11th day of incubation, but did not examine younger embryos. Leibson(13) reported that epinephrine injections cause a significant decrease in liver glycogen before day 14, while, according to Thommes and Firling(14), glucagon is effective as early as day 6. Since glycogenolysis induced by epinephrine or by glucagon requires the activation of phosphorylase, these results suggest that this enzyme may be present in the liver of the chick embryo earlier than suggested by previous studies. The purpose of this work was to reinvestigate the problem of the ontogeny of hepatic phosphorylase in the chick embryo.

Materials and methods. Fertile chicken eggs (White Leghorn, Carruthers Farm, Bancroft, Mich.) were incubated at 37.5 ± 0.3°C in a commercial farm incubator. When

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