thelial System, B. N. Halpern, ed., Blackwell Scientif. Publ., Oxford, Eng., 1957, 52.

14. Frank, E. D., Frank, H. A., Jacob, S. W., Fine, J., Am. J. Physiol., 1962, v202, 7.

15. Holden, W. D., Di Palma, R. G., Drucker, W. R., McKalen, A., Ann. Surg., 1965, v162, 517.

16. Fine, J., Frank, E. D., Ravin, H. A., Rutenburg, S. H., Schweinburg, L.B., N. Eng. J. Med., 1959, v260, 214.

17. Hershey, S. G., Anesthesiol., 1960, v21, 303.

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Effect of Isomeres of Triiodothyronine on Erythrokinetics. (31359)

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Accelerated erythropoiesis occurs in hyperthyroid humans(1) and animals with drug induced hyperthyroidism(2-4). This augmentation of erythropoiesis has been attributed to hormonal calorigenesis with a resultant imbalance between total tissue oxygen requirement and oxygen transport capacity of the red blood cell(2-3). In prior studies levo-triiodothyronine and its calorigenically inactive dextroisomere, dextro-triiodothyronine, produced equal increases in erythrocyte radioiron incorporation. Oxygen utilization, however, was significantly greater in the LT_3 treated animals(4). This has suggested that the erythropoietic acceleration following triiodothyronine administration was not completely dependent upon alterations in oxygen need. The present study constitutes an attempt to characterize further the effect of thyroactive substances on erythrokinetics and explore the mechanisms involved.

Methods and materials. All animals utilized in these experiments were maintained on a diet of Purina Laboratory Chow* with tap water allowed *ad libitum*. Homologous red blood cells were obtained by cardiac puncture during light ether anesthesia from female Sprague-Dawley rats weighing 250-300 g. The blood was centrifuged, the plasma removed, and the red blood cells resuspended in 0.9% saline solution sufficient to produce a hematocrit of 70%. Female Sprague-Dawley rats, 180-200 g, were transfused on 2 successive days with red blood cell suspension equal to one-half of the recipient animals' red blood cell mass. Forty-eight and 96 hours following the last transfusion LT_3† or DT_3‡ in doses of 100 or 250 μg was administered I.P. Control animals were given 0.9% saline solution. Six hours following the last injection of T_3, 1 μc ^{59}Fe/1 μg ^{56}Fe citrate was given *via* the tail vein and the 18-hour erythrocyte radioiron incorporation determined(5).

Female Sprague-Dawley rats, 180-200 g, were given a single I.P. injection of 25, 100 or 250 μg LT_3 or DT_3 or 0.9% saline solution. Groups of rats were given 1 μc ^{59}Fe/1 μg ^{56}Fe citrate I.V. either 24, 48, 72 or 96 hours following the injection of triiodothyronine and the 18-hour erythrocyte radioiron incorporation determined. Pooled plasma obtained either 6, 12, 24, 48 or 72 hours following I.P. administration of a single dose of 100 μg of LT_3 or DT_3 was bioassayed for erythropoietic stimulating activity by modification of the bioassay of Cotes and Bangham (6). Virgin female CF #1 mice weighing 20-25 g were maintained in an altitude chamber at 0.5 atmosphere pressure for a period of 2 weeks. The mice were removed one hour daily for routine care. Four days following removal from the chamber and return to ambient conditions, 0.4 ml saline, 0.4 ml ane-

* Iron content 275 p.p.m.

† Supplied as Cytomel® sodium (Lot #BS-0207) Smith Kline and French Research Laboratories, Philadelphia.

‡ Prepared by Sigma Chemical Co., St. Louis, Mo., by iodination of tyrosines after the manner of Chalmers, Dickson, Elks and Hems(7). Impurity was determined to be less than 1% by single chromatographic spots and a rotation value of + 21.5°.

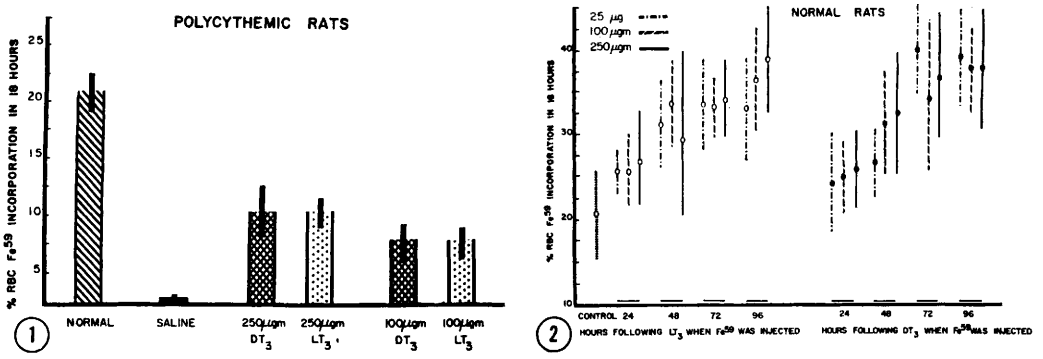

FIG. 1. Effect of LT_3 and DT_3 on 18-hr erythrocyte radioiron incorporation in polycythemic rats. Each bar represents results obtained in 15 rats. Standard deviation is indicated by vertical line.

FIG. 2. Effect of a single dose of LT_3 or DT_3 on 18-hr erythrocyte radioiron incorporation in normal rats. Each point represents results obtained in 10 animals. Standard deviation indicated by vertical line.

mic rabbit plasma(7), or 0.4 ml plasma from T_3 treated rats was injected I.P.; 48 hours later, 0.2 μc ^{59}Fe/0.2 μg ^{56}Fe citrate was administered *via* the tail vein, and the 72-hour red blood cell incorporation of radioiron was determined(6).

Results and comment. The effect of LT_3 and DT_3 on erythrocyte radioiron incorporation in polycythemic rats is presented in Fig. 1. Normal rats incorporated 21.4% of an injected dose of radioiron into their red blood cells in 18 hours, whereas transfused polycythemic rats incorporated less than 1%. LT_3 and DT_3 in both dosage schedules augmented erythrocyte radioiron incorporation to similar degrees in transfused polycythemic rats when presumably the increased red blood cell mass with its increased potential for oxygen transport has obviated response to further tissue oxygen need.

The effect of a single dose of LT_3 or DT_3 on erythrocyte radioiron incorporation in normal animals is presented in Fig. 2. Both LT_3 and DT_3 produced comparable increases in the erythrocyte radioiron incorporation at similar dose levels. There was no essential difference in erythrocyte radioiron incorporation at the 3 dose levels. The red blood cell ^{59}Fe incorporation was slightly increased at 24 hours and was further increased at 48 and 72 hours.

The results of plasma erythropoietin bioassay are presented in Table I. The base line ^{59}Fe incorporation was less than 1% in saline injected rats. Anemic rabbit plasma produced an increase to 24%. Plasma obtained from rats 6, 12, 24, 48 and 72 hours following administration of T_3 demonstrated no erythropoietic stimulating activity. If triiodothyronine produced the acceleration of erythropoiesis by increasing erythropoietin elaboration or production, increased plasma levels would have been expected at these time intervals particularly since the maximum response occurred at 72 hours.

These data suggest that the acceleration in erythropoiesis secondary to triiodothyronine administration is not completely due to alterations in calorigenesis since both LT_3 and DT_3 produced comparable increases in

TABLE I. Erythropoietin Bioassay of Plasma from DT_3- and LT_3-Treated Rats.

Material bioassayed	72 hr RBC ^{59}Fe inc., % ± S.D.*	Material bioassayed	72 hr RBC ^{59}Fe inc., % ± S.D.*
Saline	.8 ± .3	Saline	.6 ± .2
6 hr DT_3 plasma	.3 ± .2	6 hr LT_3 plasma	.4 ± .2
12 " " "	1.8 ±1.6	12 " " "	.9 ± .4
24 " " "	.9 ± .4	24 " " "	1.2 ± .9
48 " " "	.4 ± .1	48 " " "	.6 ± .3
72 " " "	.4 ± .1	72 " " "	.4 ± .2

* S.D. = Standard deviation of values from 5 mice.

erythrocyte radioiron incorporation. In addition, the increase in erythrocyte radioiron produced by LT_3 and DT_3 in transfused polycythemic rats, when the oxygen carrying capacity of the increased red cell mass would have been expected to obviate an erythropoietic response to oxygen need, further supports the contention that the mechanism of enhanced erythropoiesis is independent of changes in calorigenesis. The rate of response to a single injection of triiodothyronine suggests that the erythropoietic effect is not mediated through erythropoietin elaboration. Furthermore, plasma from T_3 treated animals did not stimulate erythropoiesis in a highly sensitive assay system. Triiodothyronine stimulates erythropoiesis as determined by augmented erythrocyte radioiron incorporation. This stimulation is apparently not due to the calorigenic properties of the hormone alone and is apparently not mediated through augmented erythropoietin production.

Summary. Both dextro-triiodothyronine and levo-triiodothyronine produced similar increases in RBC ^{59}Fe incorporation in polycythemic rats when the increased red blood cell mass would have been expected to obviate the response to oxygen need. Single doses of 25, 100 or 250 μg LT_3 and DT_3 produced similar increases in RBC ^{59}Fe incorporation in normal rats. The RBC ^{59}Fe incorporation was minimally increased at 24 hours and continued to increase 48 and 72 hours following a single dose of LT_3 or DT_3. Plasma obtained from rats following administration of LT_3 or DT_3 failed to demonstrate erythropoietic activity on bioassay in the plethoric mouse, suggesting that increased erythropoietin production is not the mechanism for the augmented erythropoiesis.

1. Donati, R. M., Warnecke, M. A., Gallagher, N. I., Ann. Int. Med., 1965, v63, 945.

2. ———, Proc. Soc. Exp. Biol. and Med., 1964, v115, 405.

3. Cline, M. J., Berlin, N. I., Am. J. Physiol., 1963, v204, 415.

4. Evans, E. S., Rosenberg, L. L., Simpson, M. E., Endocrinology, 1961, v68, 517.

5. Meineke, H. A., Crafts, R. C., Proc. Soc. Exp. Biol. and Med., 1964, v117, 520.

6. Gallagher, N. I., Hagan, D. Q., McCarthy, J. M., Lange, R. D., ibid., 1961, v106, 127.

7. Chalmers, J. R., Dickson, G. T., Elks, J., Hems, B. A., J. Am. Chem. Soc., 1949, 3424

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Phosphoglyceric Kinase in Human Vascular Tissue.* (31360)

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The reaction catalyzed by the phosphoglyceric kinase enzyme is an integral part of the glycolytic pathway. Since glycolysis is the main source of biological energy in human aortic tissue(1) it was considered important to determine the phosphoglyceric kinase (PGK) activity in various types of human blood vessels. The PGK measurements were made essentially as described by Axelrod and Bandurski(2) in which procedure the tissue is incubated with 3-phosphoglycerate (3-PGA) and adenosine triphosphate (ATP) in the presence of hydroxylamine. This reverse reaction is especially suitable for PGK activity determinations in crude tissue extracts(3). Colorimetric assay of 1,3-diphosphoglycerate formed was made by the Lipmann-Tuttle(4) ferric hydroxamic method.

Materials and methods. Vascular samples were obtained fresh at autopsy from subjects ranging in age from 0 to 86 years and were immediately frozen. A total of 445 specimens was included in the study. The PGK assays were made separately on normal and arteriosclerotic tissue portions.

Aqueous 2% homogenates of intima-media layers of the vascular samples were prepared at 0°C with a Kontes Duall type tissue

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