

Thyroxine-Enhanced Susceptibility of Mice to *Klebsiella pneumoniae** (33986)

LOREN G. MARTIN¹ AND ROBERT W. BULLARD
(Introduced by Howard H. Rostorter)

Department of Anatomy and Physiology, Indiana University, Bloomington, Indiana 47401

The role of thyroid function in resistance to infection with a variety of procaryotic pathogens has been examined by several researchers. The results obtained vary with the species of both the host and the infecting agent. While moderate hyperthyroid conditions would seem to favor host survival during bacterial infections apparently because of enhanced hemic defense factors (1-8), it has been postulated that more severely hyperthyroid animals might be less resistant presumably as a result of severe overtaxing of the total metabolic processes of the host (9-11). The present study was undertaken to learn if indeed a relatively small increase in metabolic rate induced by small amounts of exogenous L-thyroxine would enhance host resistance while larger quantities would result in greater susceptibility.

Materials and Methods. Animals. Young female Swiss albino mice weighing 15-20 g were purchased from commercial suppliers and were given Wayne Lab-Blox and water *ad libitum*.

Hormonal treatment. L-Thyroxine (TH) (K&K Laboratories, Inc., Hollywood, California) and 6-propyl-2-thiouracil (PTU) (National Biochemicals Corporation, Cleveland, Ohio) were injected intraperitoneally each day into eight groups of 35 mice each for a period of 20 days. The PTU was administered as a suspension with gum arabic (Fisher Scientific Company, Fair Lawn, New Jersey) in physiological saline (2:1). The eight groups were treated as follows: (1) control animals, no injections; (2) 2.0 mg PTU; 0.00 μ g TH; (3) 2.0 mg PTU, 0.50 μ g

TH; (4) 2.0 mg PTU, 1.00 μ g TH; (5) 2.0 mg PTU, 2.00 μ g TH; (6) 2.0 mg PTU, 5.00 μ g TH; (7) 2.00 mg PTU, 10.00 μ g TH; (8) 2.00 mg PTU, 20.00 μ g TH. The period of 20 days was chosen so that endogenous stores of thyroxine would have become depleted, leaving only the exogenous thyroid hormone in the animals at the time of infection.

Thyroid autopsy. At the termination of the 20 days of hormone treatment, 10 animals from each group were killed with ether, and their thyroids were weighed (wet wt) to permit calculation of the "normal" level of thyroid hormone in these PTU-blocked mice.

Bacteria, culture medium, and infective dose. *Klebsiella pneumoniae* (strain L979) was obtained from the Stock Culture Collection, Department of Microbiology, Indiana University, Bloomington, Indiana. Cultures were grown at 37° for 24 hr in Bacto Nutrient Broth (Difco Laboratories, Detroit, Michigan) enriched with FeSO₄ (3 μ g/ml). An inoculum of 3×10^5 viable organisms was employed for intraperitoneal injection, and survival times were measured for 1 week.

Results. In order to compute the normal level of thyroid function in this group of PTU-blocked mice, the thyroid wet weights were plotted against the amount of injected TH/day. This assay procedure for thyroid hormone was based upon the maintenance or restoration of normal thyroid weight by the administration of L-thyroxine to mice simultaneously treated with the goitrogen, PTU (12). However, this dose which produces restoration of the thyroid to its original weight in the PTU-blocked mouse cannot be precisely related to the "normal" thyroid secretion rate in the untreated animal, for PTU also increases renal clearance of iodide, increases fecal conjugation of thyroxine,

* Supported by Contract F-44620-68-C0014 of the Office of Aerospace Research, U.S. Air Force.

¹ Work done during tenure as a trainee on U.S. Public Health Service Training Grant Program, GM-1233.

and depresses peripheral degradation of thyroid hormones to "intracellularly active" compounds (13, 14). This block in the conversion of thyroxine to "active" thyroid hormone must be only partial, however, for physiological quantities of exogenous hormone have been repeatedly confirmed to inhibit goiters produced by antithyroid drugs (14). Since one cannot then equate the effectiveness of a given dose of exogenous hormone in such a goitrogen-blocked animal to endogenous TH levels in a nonblocked animal as a measure of euthyroidism, the blood levels of TH were not used as an index of thyroid function. Instead, the dosage which was able to restore the TSH release to normal and hence to restore the thyroid to its original weight was used as such a measure of normal thyroid function in the blocked animal. Hence, while the estimated TH level calculated by this method cannot be precisely related to the "normal" control animal's circulating TH level, this method does give an index as to whether the various groups of treated mice may be considered euthyroid, hyperthyroid, or hypothyroid.

As the "normal" control thyroid wet weight has been experimentally determined as 65.5 $\mu\text{g/g}$ body weight (point N on Fig. 1), the estimated normal level of TH in the blocked animal can be read (point Q on Fig. 1) as 2.6 $\mu\text{g/day}$. Thus, the normal or euthyroid level in these blocked animals was reached at 2.6 $\mu\text{g/day}$. Hence, groups 2, 3, 4, and 5 may be considered hypothyroid, whereas 6, 7, and 8 are hyperthyroid.

After hormone treatment for 20 days, the eight groups of mice were injected intraperitoneally with the test bacteria, and the survivors were tabulated during the following week; all deaths occurred within the first 48 hr. These data are entered on Figure 2.

As shown in Fig. 2, the hypothyroid groups (2, 3, 4, and 5) of animals were not killed by *K. pneumoniae*, while the hyperthyroid groups (6, 7 and 8) had a higher death rate than did the "normal" euthyroid animals. The most hypothyroid (nearly athyroid) group of animals, group 2, had the highest survival rate, and each small addition

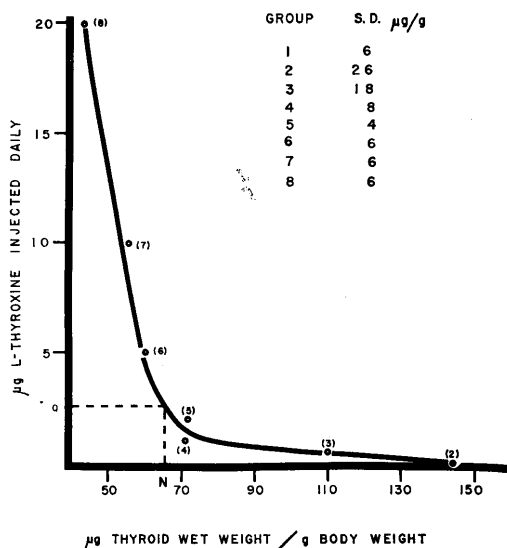


FIG. 1. Graphic determination of estimated normal TH level (point Q) in PTU-blocked mice with a "normal" average thyroid wet weight/g body weight of 65.5 μg (point N); numbers in parentheses represent group number designations.

of L-thyroxine lowered the animals' chance of survival even below the euthyroid state.

Discussion. Milzer *et al.* (9) first noted that the incidence and severity of the viral infection, poliomyelitis, could be increased by

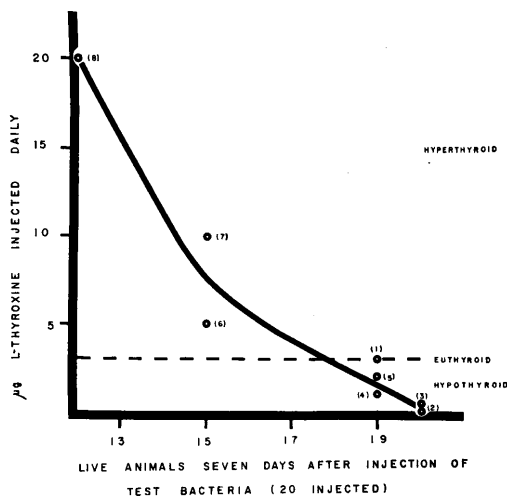


FIG. 2. Survival of mice injected with graded doses of L-thyroxine and a blocking dose of PTU for a period of 20 days after inoculation with *K. pneumoniae*; Numbers in parentheses represent group number designations.

fatigue and chilling in the rhesus monkey, while the incubation time of the same disease in mice is shortened after acclimation of the host to increased environmental temperatures but lengthened by thyroxine administration (10, 11). Holtman (11) also observed that thiouracil shortens survival periods and enhances paralytic effects, while Gollan (15) found no such differences.

Smith *et al.* (16), noting the seasonal variation and predilection of lower age groups for poliomyelitis, proposed that perhaps the thyroid does play an important role in natural resistance. They postulated that perhaps the increased incidence of death without paralysis in hyperthyroid mice results from sufficient stress imposed by the viral infection upon an already overtaxed metabolism to cause death in a large percentage of the animals before signs of paralysis are evident.

On the other hand, changes in blood defense factors induced by hyperthyroid states should increase host protection during microbial infection. Leukocytes from hyperthyroid animals have an increased metabolic rate (5, 8), and monocytes from such animals inhibit intracellular multiplication of tubercle bacilli as well as show an increased rate of phagocytotic activity (4, 5). Thyroidectomy decreases and repeated thyroxine injections increase the numbers of circulating neutrophils, lymphocytes, and eosinophils (2, 3, 6). Thyroxine treatment of mice (1) and guinea pigs (7) induces a true hyperplasia of lymphoid elements. Antibody and complement production rates are increased by hyperthyroidism (4, 6), but muramidase activity of rat kidney cells (17) and serum antiplasmin activity (18) are decreased.

Thus, the majority of the hemic defense factors seem to be augmented by hyperthyroidism and decreased by hypothyroidism. However, Nutter *et al.* (19) found that hyperthyroidism decreases the survival of mice treated with tubercle bacilli, while Lurie (4) and Solkanki and Junnarkar (5) found increased resistance in the rabbit and guinea pig. Nutter, however, gave large intravenous or intraperitoneal inocula, while Lurie gave much smaller dosages via the respiratory pas-

sages. Thus, these divergent results may in part be attributable to the acute septicemia obtained in the mouse experiments contrasted with the chronic infiltrative process in the rabbit studies.

The lethal action of endotoxins from cultures of *Escherichia coli*, *Salmonella typhosus*, and *Brucella abortus* is enhanced by acutely treating mice for 24 hr with 80 μ g of triiodothyronine before injecting the test material (20). The authors postulated that while the endotoxins cause severe vasoconstriction, the thyroid hormone increases the metabolic demand of certain organs and tissues to such an extent that the hypoxic state falls below tolerable levels.

Our results, obtained with mice on a much longer hormonal treatment with thyroxine, are in agreement with this study, perhaps because in both investigations acute gram-negative agents were employed. Thus, while a chronic infective process might be better fought by the enhanced hemic defense factors elicited by hyperthyroidism, the potentiating effect of the hormone upon the endotoxins as well as the severe metabolic stress imposed upon the organism may actually increase host damage initiated by the acute infective process. Since even the very small increase in L-thyroxine brought about a greater death rate in our study, it is hard to see how a "severe" metabolic stress played a real role in the lower dosages (hypothyroid), although, of course, it certainly could at the higher levels of hyperthyroidism. At no level of thyroxine injection does it appear that the thyroid hormone aids host survival by augmentation of these hemic defense factors, but, rather, it seems that the acute *Klebsiella* infection is better able to cause host damage with even minute additions of exogenous hormone.

Summary. By injecting mice with PTU to block synthesis of endogenous thyroid hormone, and by injecting graded dosages of L-thyroxine for a 20-day period, the estimated normal TH level of these blocked animals was determined. Using *Klebsiella pneumoniae* as an acute infective agent, it was demonstrated that the hypothyroid state increases

host resistance, whereas any increase in L-thyroxine decreases resistance. Several possible mechanisms are discussed.

The authors gratefully acknowledge the advice and assistance of Dr. E. D. Weinberg, Department of Microbiology, Dr. H. H. Rostorfer, Department of Anatomy and Physiology, and Dr. G. E. Wertenberger, Department of Anatomy and Physiology, Indiana University, Bloomington, Indiana.

1. Marder, S. N., J. Natl. Cancer Inst. **11**, 1153 (1951).
2. Larizza, P. and Chirico, G., Haematologica **38**, 771 (1954).
3. Aschkenasy, A., Sang **27**, 97 (1956).
4. Lurie, M. B., Ann. N. Y. Acad. Sci. **88**, 83 (1960).
5. Solkanki, B. R. and Junnarkar, R. V., Indian J. Med. Res. **49**, 1063 (1961).
6. Debry, G., Berger, H., and Jolibois, C., Rev. Tuberc. (Paris) **26**, 311 (1962).
7. Ernström, U., Acta Pathol. Microbiol. Scand. **59**, 145 (1963).
8. Homolsky, M. W., Michel, R., Carnicero, H., and Roche, J., Biochim. Biophys. Acta **69**, 420 (1963).
9. Milzer, A., Levin, P., and Levinson, S., J.

Bacteriol. **45**, 78 (1943).

10. Holtman, F. D., Science **103**, 137 (1946).
11. Holtman, F. D., Science **104**, 50 (1946).
12. Dempsey, E. W. and Astwood, E. B., Endocrinology **32**, 509 (1943).
13. Purves, H. D. in "The Thyroid Gland" (R. Pitt-Rivers and W. R. Trotter, eds.) Vol. 2, p. 18. Butterworths, London and Washington, D. C. (1964).
14. Greer, M. A., Kendall, J. W., and Smith, M., "The Thyroid Gland" (R. Pitt-Rivers and W. R. Trotter, eds.), Vol. 1, pp. 362-363. Butterworths, Washington, D. C. (1964).
15. Gollan, F. Proc. Soc. Exptl. Biol. Med. **67**, 362 (1948).
16. Smith, S. C., Rasmussen, A. F., Elvehjem, C. A., and Clark, P. F., Proc. Soc. Exptl. Biol. Med. **82**, 269 (1953).
17. Litwack, G., Biochim. Biophys. Acta **67**, 501 (1963).
18. Wabner, C. I., Gray, J. F., and Blatt, W. F., U. S. Army Med. Res. Lab. Rept. **672**, 1(1966).
19. Nutter, J. E., Gemmil, C. L., and Myrvik, Q. N., Federation Proc. **17**, 528 (1958).
20. Melby, J. C. and Spink, W. W., Proc. Soc. Exptl. Biol. Med. **101**, 546 (1959).

Received Dec. 16, 1968. P.S.E.B.M., 1969, Vol. 131.