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The Influences of Thyroid and Antithyroid Substances on Murine Leprosy

I. Comparison of Host-Parasite Relationship within Liver Lesions[1,2]

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The role of thyroid hormone in resistance to human leprosy infection is not clear. O'Byrne (12) and Rojas (14) have described favorable results in leprosy patients treated with methimazole (Tapazole) and propylthiouracil, both of which are potent antithyroid substances. However, Levy *et al.* (9) and Browne and Hogerzeil (5) have not found methimazole to be useful in the treatment of human leprosy. Because of the obvious difficulties that would be encountered in a comprehensive and controlled study of thyroid and antithyroid substances in human leprosy, it was decided to determine the effect of these substances in murine leprosy. The concept of this experiment derived from one of us (HG), who gained experience and knowledge of a similar project (7) while in Colombia, South America.

METHODS AND MATERIALS

One hundred and sixteen female albino Swiss Webster mice weighing 20-25 gms. were divided into five groups, each comprising 22 to 24 individual mice. These groups were treated with either radioactive Iodine (I^{131}), Tapazole (TAP), L-tetraiodothyronine (T4) or L-triiodothyronine (T3), or retained as a control group receiving only saline injections. Table 1 presents details of treatment schedules.

On the same date all mice were given, intraperitoneally, the same dose of *M. lepraemurium* (approximately 10^6) Hawaiian strain organisms in an infected liver homogenate. Individual mice from each group were selected at random 75, 103, or 125 days postinfection, given an intramuscular traced dose of I^{131} (0.5 μc), sacrificed 24 hours later and autopsied. The thyroid gland was removed and its radioactivity determined on a crystal scintillation counter following digestion with 5N NaOH. The liver and other internal organs were removed, fixed, sectioned and stained with hematoxylin and eosin and with Kinyoun's acid-fast stain (11).

In comparing liver sections from the various groups, the absence or presence and number of granulomas (per three fields at one hundred power magnification), cellular composition of the individual granulomas, and the relative number and staining characteristics of intracellular acid-fast organisms was evaluated.

RESULTS

Because statistically significant differences in results were not noted in animals sacrificed after 75, 103 and 125 days respectively, the data were combined. The percentage of mice surviving to complete the experiment and their average thyroid I^{131} uptake are shown in Table 2. The thyroid hormone treated groups had a considerable mortality prior to the days selected for sacrifice. Twenty-one mice not infected with *M. lepraemurium* were given similar doses of T^4 and T^3 with 70 per cent and 72

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TABLE 1. *Treatment schedule.*

Group	Treatment
Control	No treatment (only saline IM)
I^{131}	40 μc I^{131} [a]
TAP	100 μgm. IM[b] (freshly prepared)
T^4	10 μgm. IM[b] (freshly prepared in alkaline saline).
T^3	10 μgm. IM[b] (prepared weekly)

[a] Given IM at 2 and 4 weeks prior to infection.
[b] Started 1 week prior to infection and given every other day, throughout the course of the experiment.

TABLE 2. *Group survival rates and I^{131} uptake studies.*

Group	Surviving mice	I^{131} up take		
	Per cent	Per cent	SE[a]	Significance
Control	95.8	7.4	0.6	
I^{131}	95.8	6.4	0.8	
TAP	95.8	4.0	0.4	$p<0.01$
T^4	79.2	0.9	0.3	$p<0.01$
T^3	70.8	1.9	0.5	$p<0.01$

[a] Standard error.

TABLE 3. *Relative numbers of hepatic granulomas per 3 high powered fields.*

Group	Average number of granulomas	Standard error
Control	19.38	4.66
I^{131}	16.15	2.49
TAP	8.26	1.47
T^4	5.22	1.20 ($p<0.01$)
T^3	2.12	0.51 ($p<0.01$)

per cent respectively surviving at 60 days. Significant differences in the combined I^{131} uptake studies were noted when the TAP, T^4 and T^3 groups were compared to the control group. The dose of radioactive I^{131} initially given to the I^{131} groups did not cause significant hypothyroidism as assessed by tracer uptake studies. The exogenous T^4 and T^3 depressed normal endogenous thyroid function. Therefore the animals so treated showed a low tracer uptake.

The relative number of granulomas in the liver sections is shown in Table 3. The T^4 and T^3 groups had a significantly decreased number of granulomas as compared with the control groups.

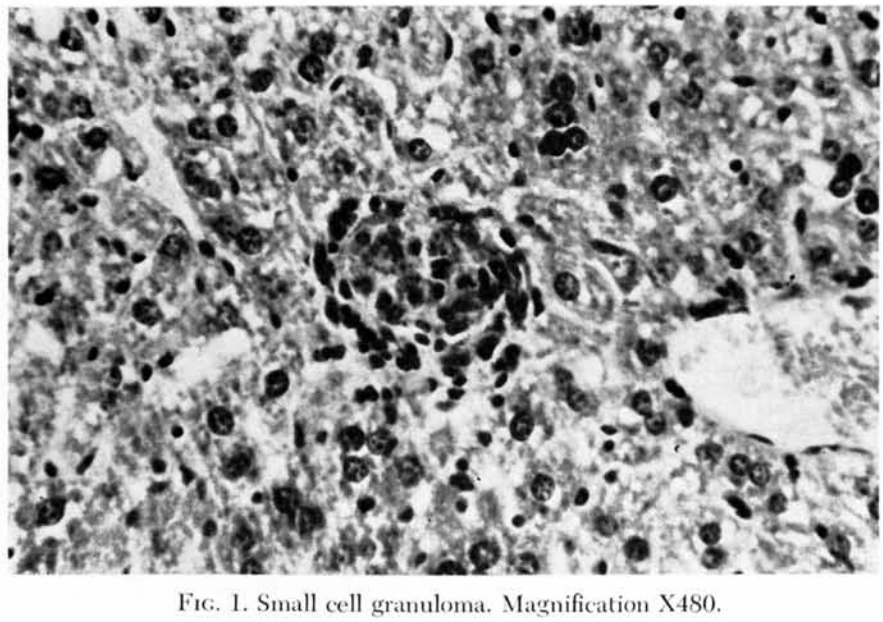

FIG. 1. Small cell granuloma. Magnification X480.

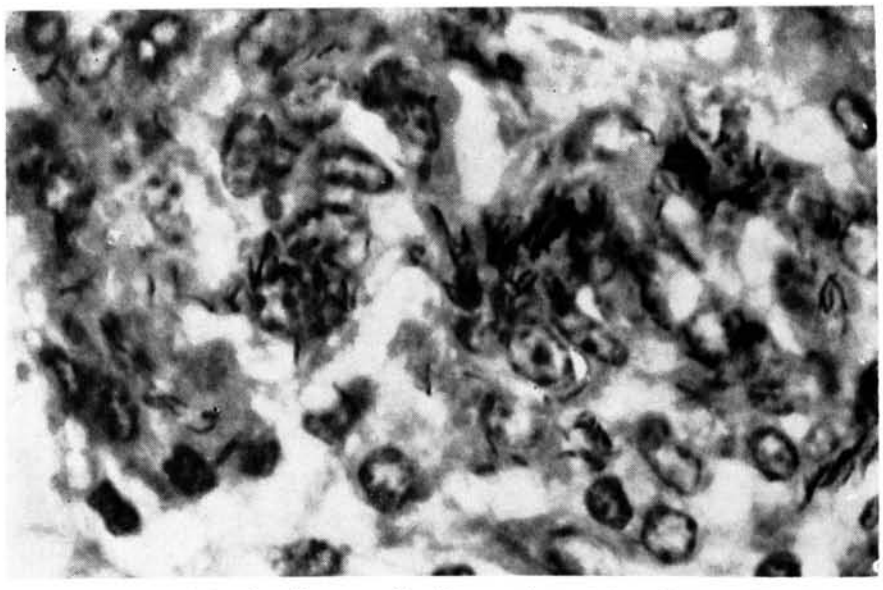

FIG. 2. Acid-fast bacilli in small cell granuloma. Magnification X1,000.

The character of the granulomatous response differed in the various experimental groups. Two distinct histopathologic types of granuloma formation could be discerned. Table 4 presents a comparison of these granulomas. The small cell granuloma was composed of collections of well-defined, discrete cells each with a centrally placed single nucleus, occupying approximately two-thirds of the cell, and a slightly eosinophilic cytoplasm which often contained brown to black, irregular, small to large clumps of pigment (Fig. 1). A surrounding mantle of discrete small mononuclear cells, mainly lymphocytes, was often observed at the periphery of this type of granuloma. Giant cells were rare. Acid-fast staining revealed irregularly stained, long, narrow, sparse intracellular bacilli (Fig. 2).

The second type of granuloma was composed of large multinucleated cells with either centrally, peripherally, or eccentrically placed nuclei (Fig. 3). On hematoxylin-eosin staining the cytoplasm was granular and lacked pigment. Acid-fast staining revealed that the large cell granulomas contained numerous fully stained short bacilli (Fig. 4).

The number of acid-fast staining bacilli in 100 small and 100 large cell granulomas was determined under oil immersion and was recorded by the following semilogarithmic coding scheme. Each granuloma was scored from zero to four depending

TABLE 4. *Comparative features of small and large cell granulomas.*

	Small cell granulomas	Large cell granulomas
Cell type	Mononuclear cells (histiocytes and Kupffer's cells)	Multinuclear cells
Presence of giant cell	Unusual	Usually present
Number of intracellular acid-fast bacilli	Few	Many, clumped
Acid-fast staining	Irregular and long bacilli	Solid and short bacilli
Mantle of peripheral mononuclear cell	Usually present (mainly lymphocytes)	Occasionally present
Presence of pigment	Usual	Unusual

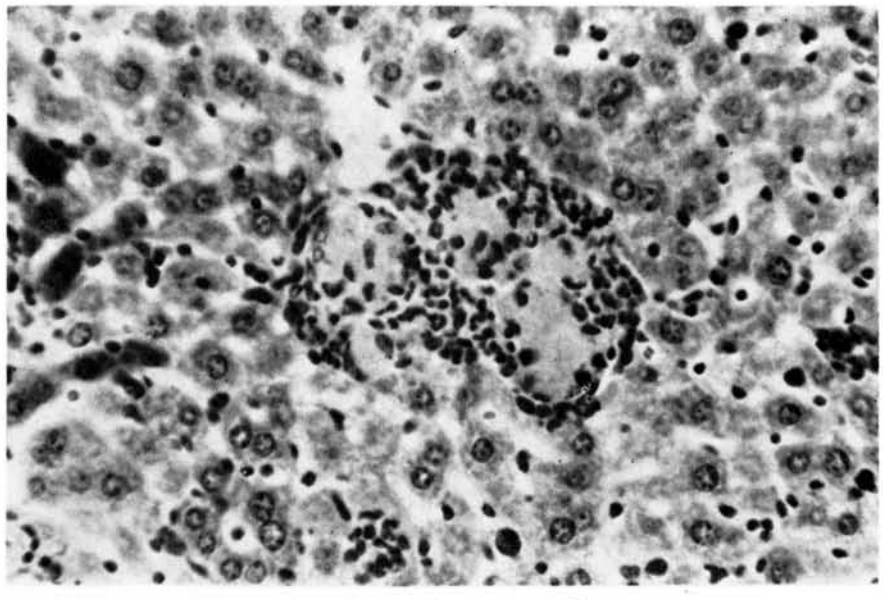

FIG. 3. Large cell granuloma. Magnification X480.

on the number of stained bacilli present. The scoring was done as follows:

0 = no bacilli seen in the granuloma
1 = 1 to 10 bacilli per granuloma
2 = 11 to 100 bacilli per granuloma
3 = 101 to 1,000 bacilli per granuloma
4 = over 1,000 bacilli per granuloma

The small cell granuloma had an average score of 1.63 while that of the large cell granuloma was 3.21. The difference between the two groups is significant ($p<0.001$).

TABLE 5. *Comparison of incidence and type of granulomatous response in liver.*

Group	No. granuloma Per cent	Small cell granuloma Per cent	Large cell granuloma Per cent
Control	8.7	52.2	39.1
I^{131}	4.3	43.5	52.5
TAP	16.7	62.5	20.8
T^4	10.5	68.4	21.1
T^3	29.4	64.7	5.9

The quantitative aspects of the granulomatous liver infiltrate are shown in Table 5. The T^3 group, and to a lesser extent the T^4 and TAP groups, demonstrated no granulomas or predominately the small cell type, in contrast to the control group.

DISCUSSION

Experimental murine leprosy is caused by *M. lepraemurium* and is a chronic progressive disease in mice. The causative organisms of murine leprosy and human leprosy share many similar characteristics as both are intracellular acid-fast bacilli which cannot be cultured on ordinary media and are susceptible to many similar drugs, including the sulfones ([8]). Because of these and other similarities, as well as previous lack of other suitable *in vivo* procedures, murine leprosy has been used in the past as an experimental model to study human leprosy.

From a study of liver pathologic changes alone it is difficult to draw definitive conclusions as to the efficacy of thyroid hormone therapy in murine leprosy. However, there is an apparently changed host-parasite relationship demonstrated in the thyroid groups especially T^3. These animals had a qualitative as well as a quantitative decrease in the severity of their disease as measured by strictly morphologic means.

Two distinct types of granuloma formation were found, designated as large and small cell granulomas. The large cell granuloma has multinucleated giant cells with scant pigmentation and numerous intracel-

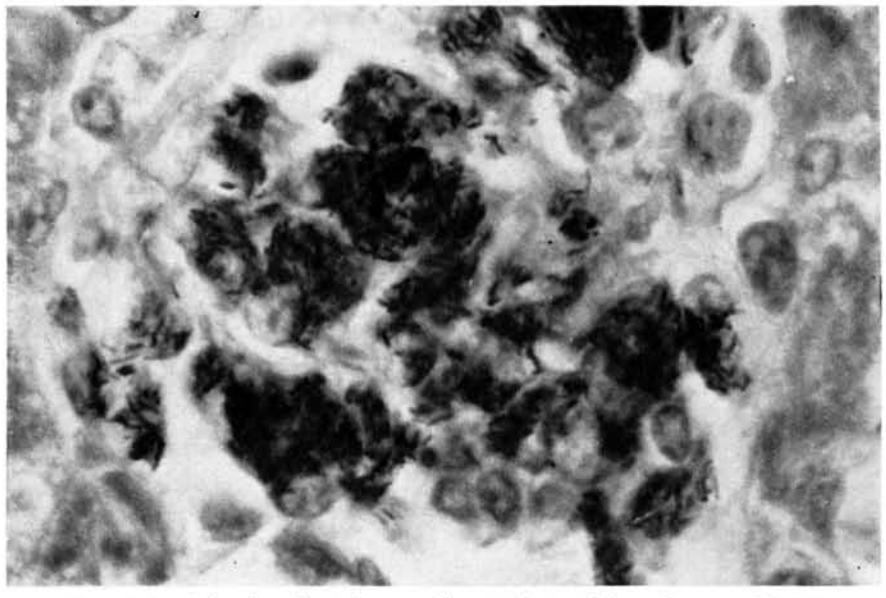

FIG. 4. Acid-fast bacilli in large cell granuloma. Magnification X1,000.

lular acid-fast organisms. It occurs in hepatic granulomas of mice with murine leprosy ([6, 8]). The second type, the small cell granuloma, consists primarily of either histiocytic or Kupffer's cells with abundant pigment formation and scant intracellular acid-fast organisms; a surrounding mantle of lymphocytes was often present. The T^3 groups demonstrated a predominantly small cell type of hepatic granulomatous response at one extreme, with the T^4 and TAP groups intermediate in terms of the character of their granulomatous response.

In a preliminary report Gutierrez ([7]) noted a decreased number of granulomas in the livers of the mice given T^3 as compared to similarly infected controls. Interestingly, only six of his 20 T^3 animals survived the treatment period. Thyroid hormones, both T^3 and T^4, are known to potentiate pressor amines, to have calorigenic effects and to increase the susceptibility of animals to various bacterial products including endotoxins ([10]). It is postulated that the latter susceptibility led to the premature deaths in both infected and noninfected groups of mice treated with T^3 and T^4. Gutierrez also found that treatment with a single dose of 85 μc of I^{131} as given in the present study did not result in a decreased uptake of tracer I^{131} given up to five months after the iniitial I^{131}. We are hesitant to speculate on the significance of the liver granulomas results obtained in the group.

Although the literature on the role of thyroid hormones and antithyroid drugs in leprosy is sparse, there is abundant evidence regarding the effect of these substances in tuberculosis. Briefly, it has been found ([10]) that thyroid hormones, T^3 and T^4, result in an increased resistance to tuberculosis infection in rabbits having moderate innate resistance. Hypothyroidism and antithyroid drugs have the opposite effect. Although there is some species variation, thyroid hormone administration generally results in activation of lymphatic tissue, increased mononuclear phagocytic activity and decreased accumulation of intracellular organisms within granulomas. However, Backman ([1]) stated that both hyperthyroid and hypothyroid states enhanced the multiplication of the tubercle bacilli in lung and spleen in mice.

Bergel has reported ([4]) that when rodents fed pro-oxidant diets high in unsaturated fats and low in vitamin E, susceptibility to experimental infection with various mycobacterial organisms occurs, including *M. leprae* and *M. lepraemurium*. He postulated that pro-oxidant diets alter lysosomes which may be involved in the ultimate degradation of intracellular organisms such

as the mycobacteria. Sulfone drugs such as diaminodiphenyl sulfone ([3]), some antithyroid drugs ([14]), and thyroid hormone ([2]) have active antioxidant activity. This may account for the diminished intracellular accumulations of organisms, in T^3, T^4, and TAP groups. T^3 is known to cross cell membranes at a faster rate than T^4 and is considered the active intracellular thyroid hormone ([10]). On the other hand, T^4 is thought to be the transport form of the thyroid hormone in the blood. Had the TAP groups been given a higher dosage of TAP, the antioxidant effects might have been more evident.

The aforementioned may be important in the apparent reduction in liver pathologic changes in the thyroid groups as compared to the controls. However, an additional factor may be the influence of the calorigenic and hypermetabolic properties of thyroid hormone upon the body temperature of the T^3 and T^4 treated groups, being detrimental to propagation of the intracellular organisms. Many mycobacterial infections including leprosy may show temperature dependence ([15]).

The morphology of mycobacteria as revealed by acid-fast staining is thought to reflect their viability ([13]). Nonviable bacilli stain irregularly yielding a beaded or fragmented appearance. Those which stain uniformly are considered viable. By these criteria, the small cell granulomas of our mice generally contained a majority of nonviable organisms while the large cell granulomas contained a majority of viable organisms.

SUMMARY

Exogenous thyroid hormone adequate to suppress endogenous thyroid activity in the mouse resulted in qualitatively and quantitatively diminished hepatic granulomatous response to *Mycobacterium lepraemurium*. These effects are discussed in relation to the antioxidant and calorigenic effects of thyroid hormones.

RESUMEN

Adecuada cantidad de hormonas tiroídeas exógenas para suprimir la actividad tiroídea endógena en el ratón resultó en una disminución cualitativa y cuantitativa a la respuesta granulomatosa hepática al *Mycobacterium lepraemurium*. Estos efectos se discuten en relación con los efectos antioxidantes y calorigénicos de la hormona tiroídea.

RÉSUMÉ

L'utilisation d'une hormone thyroïdienne exogène susceptible de supprimer l'activité endogène de la thyroïde chez la souris, a entraîné une diminution qualitative et quantitative de la réaction granulomateuse du foie à l'égard de *Mycobacterium lepraemurium*. Cette action est discutée à la lumière de ce que l'on sait des effets anti-oxydants et calorigènes des hormones thyroïdiennes.

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REFERENCES

1. Backman, A. The influence of induced hyperthyroidism on experimental tuberculosis in mice. Am. Med. Exper. et Biol. Fenniae, Suppl. 3 (1960) 38.
2. Bartfield, H. and Siegel, S. M. Antioxidant activity of thyroxine and related substance. Exper. Cell Res. **49** (1968) 25-30.
3. Bergel, M. Lysosomes. Their relationship with vitamin E and leprosy. Leprosy Rev. **38** (1967) 189-192.
4. Bergel, M. The effect of pro-oxidant diets on some experimental mycobacterial infections. Leprosy Rev. **39** (1968) 15-21.
5. Browne, S. G. and Hogerzeil, L. M. Methimazole in the treatment of leprosy. Leprosy Rev. **33** (1962) 190-192.
6. Fite, G. L. The pathology of experimental rat leprosy. U.S. National Institute of Health Bulletin (leprosy) No. 173 (1940) 45-76.

7. GUTIERREZ, R. Preliminary report on the effects of L-triiodothyronine, radioactive iodine^{131}, and methimazole on the experimental murine leprosy. Leprosy Rev. **38** (1967) 31-33.
8. HADLER, W. A., FERREIRA, A. L. and ZITI, L. M. An attempt to stimulate and depress the function activity of the inflammatory cells from lesions experimentally induced by *M. leprae* and *M. lepraemurium*. Leprosy Rev. **36** (1965) 163-170.
9. LEVY, L., MURRAY, L. P. and FASAL, P. The lack of effect of methimazole therapy in lepromatous leprosy. Reassessment by examination of bacterial morphology. Internat. J. Leprosy **35** (1967) 149-153.
10. LURIE, M. B. Resistance to Tuberculosis. Cambridge, Harvard University Press, 1964, pp. 265-301.
11. [MANUAL OF HISTOLOGIC AND SPECIAL STAINING TECHNICS.] New York, McGraw-Hill Book Co., 2nd ed., 1960, p. 175.
12. O'BYRNE, A. Antithyroid substance in the treatment of leprosy. Internat. J. Leprosy **28** (1960) 401-407.
13. REES, R. J. W. and VALENTINE, R. C. The appearance of dead leprosy bacilli by light and electron microscopy. Internat. J. Leprosy **30** (1962) 1-9.
14. ROJAS, B. Antithyroid substance and lepropsy. Leprosy Rev. **34** (1963) 203-208.
15. SHEPARD, C. C. Stability of *Mycobacterium leprae* and temperature optimum for growth. Internat. J. Leprosy **33** (1965) 541-550. (Part 2)