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Impairment of Innate Resistance by Triiodothyronine.* (24299)

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Lurie and Ninos(1) observed that rabbits treated with triiodothyronine displayed an enhanced resistance to chronic infection by tubercle bacilli. This paper reports the effect of triiodothyronine on experimental infection of mice by a strain of group A streptococci, by *Candida albicans*, and on transplantable mouse leukemia.

Materials and methods. L-3,3',5 triiodothyronine (Smith, Kline & French Co.) was prepared for inoculation of mice by dissolving 4 mg in 1.3 ml 0.01 N NaOH, 90 mg of NaCl added, and total volume of solution brought to 10 ml with distilled water free of carbonate. Triiodothyronine solution (T3) after sterilization by autoclaving for 30 minutes at 110°C., was stable for 6 days at 4°C. *Streptococcus pyogenes*, type 18, and *Candida albicans*, strain 780, were grown and prepared for inoculation of mice as described elsewhere (2,3). Swiss albino mice, Tumblebrook strain, and BALB/C mice, purchased from Jackson Memorial Laboratory, were used as test hosts for *Candida albicans* and *Streptococcus pyogenes* respectively. *Strain C58 mice and transplantable leukemia line I_b* originated with MacDowell and have been maintained in our laboratory since 1952. Young C58 mice moribund from growth of I_b cells served as donors of malignant leukocytes used for tests. Spleens harvested aseptically from leukemic mice were transferred to a sterile Petri dish and minced with Bard-Parker blades until particles were approximately 2 mm^3. Minced tissue flooded with Scherer's (4) maintenance solution (MS) was agitated gently and the supernatant fluid discarded. Tissue was minced further until particles approximated 1 mm^3, supernatant fluid transferred aseptically to a sterile tube, centrifuged to sediment gross particles, and supernate saved as the source of leukemic cells. White blood cells were separated from erythrocytes by cyclic differential centrifugation at low speed(5); line I_b leukocytes comprising the sedimented fraction were suspended in MS and counted in a hemocytometer. Per cent viability of cell suspensions was determined by incubation of an aliquot of cells with an equal volume of 0.2% trypan blue (dissolved in distilled water containing NaCl 0.8%, KCL 0.04%, glucose 0.1%); unstained cells were considered viable(6). Cell suspensions prepared as described above did not contain more than 5% dead cells.

Results. Accelerative effect of triiodothyronine on streptococcal infection of mice. The extraordinary sensitivity of male BALB/C mice to infection by *Streptococcus pyogenes*, type 18, provided an ideal experimental system to assess the effect of T3 on infection. Triiodothyronine, 0.1 ml (40 μg) was inoculated subcutaneously (SQ) into each of 6 test mice 24 hours before they were infected by intraperitoneal inoculation with streptococci as described previously(2). After inoculation, mice received 0.1 ml injections of T3 at 48 hour intervals during the 6-day observation period; control mice were inocu-

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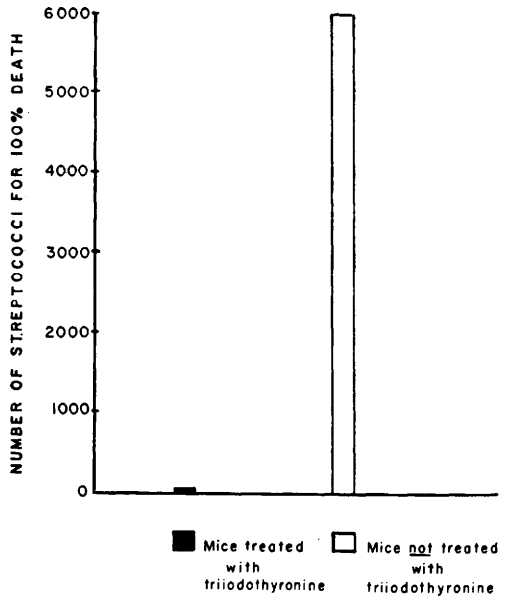

FIG. 1. Effect of triiodothyronine (40 μg) given SQ every 48 hr on time for death of male BALB/C mice infected intraper. by *Streptococcus pyogenes*, type 18.

lated only with streptococci since tests revealed that T3 was not lethal for mice nor did its vehicle influence host response to infection. Representative results from 3 replicate experiments (Fig. 1) show that mice treated with T3 died within 24 hours after inoculation with 6 to 10 organisms whereas approximately 6000 organisms were required to kill untreated mice under the same experimental conditions. Because male BALB/C mice were uniformly susceptible to lethal infection by the test organism, the total number of deaths in test and control groups was the same at the end of the 6 day observation period. *Effect of triiodothyronine on susceptibility of Swiss and BALB/C mice to infection by Candida albicans.* The high innate resistance of mice to infection by *Candida albicans* contrasted sharply with the extraordinary susceptibility of BALB/C mice to test streptococci. Thus, further characterization of the influence of T3 on innate resistance was accomplished by assaying its effect on the course of experimental infection of Swiss albino and inbred BALB/C mice by *Candida albicans.* Procedure for growth of organisms and inoculation of mice has been reported(3). Twenty mice in each test group were inoculated with 0.5 ml of cell suspension containing 35 million organisms. Test mice received 40 μg of T3 subcutaneously 24 hours before inoculation with cells, and every 48 hours thereafter; control mice were inoculated with *Candida albicans* alone. Experiments with each strain of mice were conducted in duplicate. Data (Fig. 2) representative for experiments that employed both BALB/C and Swiss mice reveal that T3 increased per cent mortality and accelerated rate of death. *Effect of triiodothyronine on rate of death from transplanted leukemia.* The possible effect of T3 on innate resistance to neoplastic growth was surveyed by study of its influence on progression of spontaneous leukemia in C58 mice. Results of such experiments were subject to broad variation because it was impossible to allocate control and test mice into equivalent groups comprised of animals at the same stage of leukemia. Analysis of the dose-response curve of C58 mice to line I_b cells (7) afforded an opportunity to assess influence of T3 on host response to transplanted leukemic cells. Three groups of 10 test and 10 control animals per group were employed for comparative purposes; mice of each group received a graded dose of I_b cells. Forty μg

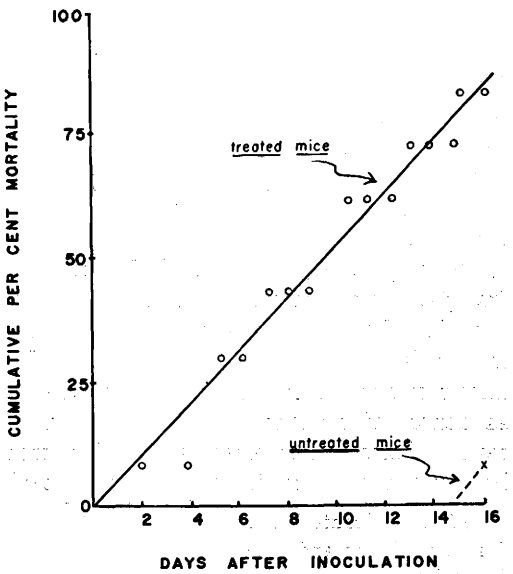

FIG. 2. Effect of triiodothyronine (40 μg) given SQ every 48 hr on time for death of BALB/C mice infected intraper. by *Candida albicans*.

TABLE I. Effect of Triiodothyronine on Time for Death of C58 Mice to Transplantable Leukemia, Line I_b.

Dose of cells per mouse	Avg time in days for death*	
	Treated mice	Control mice
$10^{4.7}$	6.0	7.0
$10^{3.2}$	7.0	9.0
$10^{0.7}$†	12.0	13.3

* Data are representative of 3 replicate experiments; 10 mice were used in each test and control group for each exp.

† Time for death of animals (*ca.* 50% of test subjects) that received leukemic cells.

of T3 was inoculated SQ into mice 24 hours before intraperitoneal inoculation of I_b cells and every 48 hours thereafter; control mice did not receive T3. In 3 replicate experiments a small but consistent accelerative effect of T3 on time for death from transplanted leukemic cells was observed (Table I) although the magnitude of the change was not remarkable.

Discussion. The ready suppression of innate immunity by a variety of experimental procedures contrasts with the lack of suitable methods for enhancement of native resistance. Lurie and Ninos(1) demonstrated that triiodothyronine augmented innate resistance of rabbits to chronic tuberculosis. Long and Sewell(8) observed that injection of thyroxine into guinea pigs increased immunity to diphtheria toxin and resulted in increased production of circulating antitoxin. These findings served as the basis for the study reported here which had as its objective a further survey of the effect of triiodothyronine (T3) on innate immunity. Contrary to anticipated results, it was found that mice treated with T3 were more susceptible to infection by a strain of group A streptococci and by *Candida albicans* than untreated controls, whereas time for death of mice from transplanted leukemia was only slightly accelerated. In experimental candidiasis both threshold of resistance and time for death were reduced. These observations are consistent with those of Nutter and coworkers(9) and Melby, *et al.*(10), who reported suppression by triiodothyronine of innate resistance to infectious agents. Three factors seem to be important to an understanding of the divergent results concerning the effect of T3 on innate immunity: 1) the test systems employed in the various investigations serve to illustrate the individuality of each host-parasite system; 2) the dose of test organisms influenced the magnitude of the observed effect in at least one case(10), and 3) the dose of T3 may augment resistance providing that it enhances the physiologic and metabolic activity of cells concerned in resistance to infection, or it may subvert innate immunity if used in doses that are thyrotoxic.

Summary. Studies were carried out to provide additional information on the effect of triiodothyronine on innate resistance. Mice treated with the drug were significantly more susceptible to infection by *Candida albicans* and by a strain of *Streptococcus pyogenes*, type 18, than untreated controls. Treatment of mice with triiodothyronine failed to enhance significantly time for death of C58 mice to transplanted leukemia, line I_b.

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