Total Acid Phosphatase Content of Peritoneal Leucocytes Obtained from l-Triiodothyronine Treated Guinea Pigs.* (27521)

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Recently, *in vitro* experiments by Hsu(1) demonstrated a greater than normal bactericidal activity of peritoneal macrophages obtained from guinea pigs previously treated with l-triiodothyronine. Older observations of Lurie(2) also suggest that there is a heightened bactericidal activity of rabbit pulmonary macrophages, *in vivo*, during experimental hyperthyroidism induced by this drug. The studies of Cohn and Hirsch(3,4), dealing with the bactericidal mechanisms of polymorphonuclear leucocytes strongly implicate a role for lysosomal-bound enzymes in this process. Their experiments revealed that many of the acid-hydrolases, first identified by de Duve (5) as lysosomal enzymes (acid phosphatase, ribonuclease, cathepsin, and others), are abundantly present in the specific cytoplasmic granules of phagocytic cells. Moreover, it was shown that phagocytosis of bacteria by such cells causes these enzymes to become solubilized and to participate in the digestion of the engulfed microorganisms. We have become interested in the possibility that administration of l-triiodothyronine to animals leads to an increase in the quantity of the lysosomal enzymes present in reticuloendothelial cells. In this way, the observed increase in cytopeptic activity of macrophages of triiodothyronine treated animals may be explained. The present report deals with an investigation of one of the known lysosomal enzymes, non-specific acid phosphatase, in peritoneal leucocytes harvested from guinea pigs treated with this hormone.

Methods. The animals used in these experiments were male, albino guinea pigs of the Hartley strain and weighed 500 g at the beginning of treatment. Half of the animals received daily intramuscular injections of l-triiodothyronine (Smith, Kline and French L-2623-Z) in doses ranging from 10 μg daily at the outset to 25 μg daily at the end of treatment. Control animals received daily intramuscular injections of the vehicle alone (isotonic saline at pH 10.0). Treatment was continued for 4 weeks, during which time all control animals gained weight and a final average increase of 25% of starting weight was recorded in this group. During this same interval, however, treated animals either showed no change in weight or underwent varying degrees of weight loss up to a maximum of 20% of starting weight in some cases. Three days before the end of the treatment all animals were injected intraperitoneally with 10 ml of sterile, heavy mineral oil, to produce an inflammatory exudate. Peritoneal leucocytes were harvested 72 hours later by irrigating the peritoneal cavity with a warmed solution of tissue culture medium 199 (Microbiological Associates, Bethesda, Md.) adjusted to pH 6.9. The exudates thus collected were sampled for differential and total white cell counts, after which a known volume of each exudate was centrifuged at low speed in order to collect the cells. Cells were resuspended in 5 ml of ice-cold saline and treated for 10 minutes in a Raytheon sonication apparatus (model 9KC-OSC, Raytheon Manufacturing Co., Waltham, Mass.) at an output voltage of 170 V. The lysates thus prepared revealed few intact cells when examined under phase contrast microscopy and appeared to contain fully released enzyme activity. That is, additional lytic procedures such as freezing and thawing did not increase the release of enzyme from disrupted cells. Aliquots of these lysates (0.1 ml) were assayed for acid phosphatase according to the method of Bessey, Lowry, and Brock(6) using disodium paranitrophenyl phosphate in 0.00375 M final concentration as substrate. Reactions were carried out in 0.1 M citrate buffer at pH 4.8, containing 0.001 M Mg^{++}. Acid phosphatase activity was expressed as millimoles of paranitrophenol released per hour at 37°C per

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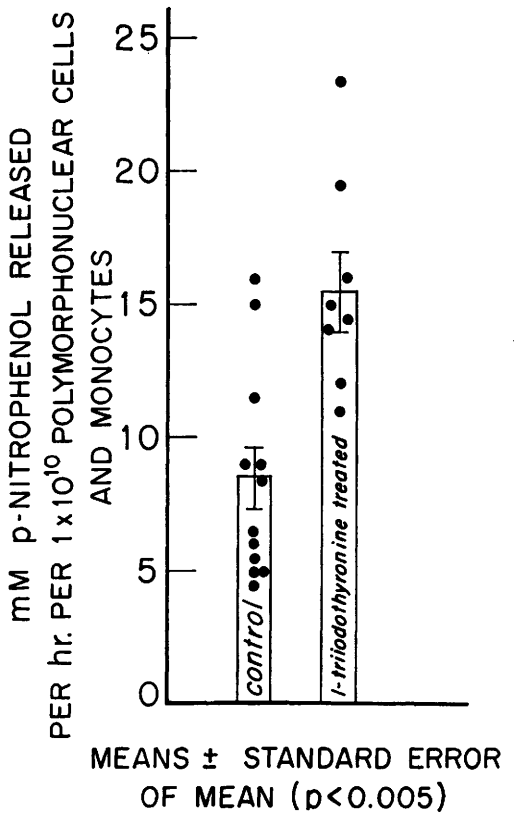

FIG. 1. Effect of l-triiodothyronine on acid phosphatase content of guinea pig peritoneal leucocytes. Enzyme activity extracted from a standard number of exudate cells is expresssed as millimoles of product formed at 37°C.

1×10^{10} phagocytic cells (polymorphonuclear cells and monocytes).

Results. Fig. 1 summarizes the results obtained in 2 separate experiments involving a total of 12 control and 8 hormone-treated animals. Prolonged treatment of guinea pigs with l-triiodothyronine caused a significant elevation in acid phosphatase content of the macrophage and polymorphonuclear leucocyte population of peritoneal fluid, as determined by biochemical assay. The hormone treatment also caused an increase in total numbers of white cells which migrated into the peritoneal cavity after injection of mineral oil, although the proportion of polymorphonuclear cells and monocytes did not change appreciably (Table I). The average number of lymphocytes in exudates obtained from both controls and treated animals was 30% of the total white cell count, but these cells were excluded from the expression of enzyme/10^{10} leucocytes. This was done to avoid errors resulting from individual variations in per cent lymphocytes, the acid phosphatase content of which is low as compared to the other 2 cell types. It should be emphasized that enzyme activity was expressed per standard number of phagocytic cells, and that the data shown in Fig. 1 are therefore unaffected by the greater numbers of cells available in hormone-treated animals.

Discussion. The work of Cohn and Hirsch (3,4) indicates that acid phosphatase and other lysosomal enzymes play some role in the intracellular digestive functions of polymorphonuclear cells. Our results demonstrate increased acid phosphatase content of peritoneal leucocytes in triiodothyronine treated guinea pigs. We therefore suggest that the heightened bactericidal activity of the macrophage-system observed after treatment with this hormone(1,2) may be due to increased amounts of lysosomal hydrolases. In this connection, a recent study by Allison provides similar evidence to support the view that lysosomal content of macrophages is linked to the bactericidal activity of these cells. Allison found that macrophages of rabbits bred for increased genetic resistance to tuberculosis also contain greater than normal amounts of acid phosphatase(7) although the significance of this change was not discussed by the author.

Our suggestion that thyroid secretions may exert some degree of regulatory influence upon the quantity of lysosomal enzymes is further supported by the following observation. The resorption of larval structures during the process of amphibian metamorphosis is known to be initiated by thyroid secretions in these organisms. These same anatomic

TABLE I. Effect of l-Triiodothyronine on Numbers of Guinea Pig Peritoneal Leucocytes.

Guinea pig group	Total wbc/mm³ (avg)	% pmn (avg)	% mnc (avg)
Control	5300	6	64
l-triiodothyronine treated	7400	8	63

pmn = polymorphonuclear cells.
mnc = monocytes.

changes are coincidentally associated with large increases in total and free activity of cathepsin, a lysosomal-bound enzyme, in the affected tissues(8).

Cortisone, which depresses reticuloendothelial cell function and lowers resistance to infection, has been shown to inhibit the cytopeptic activity of liver Kupffer cells(9). This latter observation, together with the report that this hormone effectively inhibits solubilization of lysosomal enzymes in a number of *in vitro* situations(10,11), suggests an interesting parallelism between l-triiodothyronine and cortisone. On the one hand, l-triiodothyronine appears to increase cytopeptic activity of macrophages, and, in our experience, also increases macrophage-content of at least one lysosomal enzyme. On the other hand, cortisone appears to decrease cytopeptic activity of liver macrophages, and this may indeed be correlated with an inhibiton of free-intracellular activity of lysosomal enzymes.

It should be noted that these 2 hormones also have opposite effects upon the resistance of animals to the lethal effects of bacterial endotoxins. In this case, however, the actions of these agents are reversed; that is, experimental hyperthyroidism aggravates susceptibility to endotoxin(12), while cortisone protects animals from the lethal action of this agent(13). One wonders, therefore, whether uncontrolled increases in free lysosomal enzyme activity occur in the tissues of endotoxin-treated animals and are, in fact, responsible for the pathogenetic alterations leading to death in endotoxin shock.

The principal suggestion presented here also has some bearing on a recent finding by Calvert(14), that increased susceptibility to carbon-tetrachloride hepatotoxicity occurs during hyperthyroidism. De Duve has already demonstrated that increased free lysosomal enzyme activity occurs in rat liver very soon after carbon-tetrachloride administration, and has suggested that release of lysosomal enzymes by this chemical in liver cells may be the responsible mechanism for damage to that organ(5). The findings of Calvert might readily be explained, therefore, by the concept that thyroid hormone increases content of lysosomal enzymes in liver cells.

Summary. Guinea pigs were injected with l-triiodothyronine in doses ranging from 10 μg to 25 μg daily over 4 weeks of treatment. Assay of acid phosphatase content of peritoneal leucocytes performed at the end of that time revealed a significantly greater quantity of this lysosome-bound enzyme in peritoneal exudate cells of treated animals as compared with controls. It is suggested that the enhanced bactericidal activity of reticuloendothelial cells in l-triiodothyronine-treated animals may be related to an increased cell content of lysosomal enzymes, including acid phosphatase, with resultant functional increases in the cytopeptic activity of these cells.

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