Thyroidal Hormones Influence Oil Red O Staining of Lipids in Cultured Human Kidney Cells[1] (36349)

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We have reported previously that the T-1 cell line, derived from the normal human kidney by Van der Veen *et al.* (1), responds to small additions of the iodinated thyroidal hormones, L-thyroxine (T_4) and 3,5,3′-L-triiodothyronine (T_3), introduced into the growth medium (2). Thus, alterations of morphology, proliferation, dimensions, and the metabolism of proteins and nucleic acids result from the exposure to 1.78×10^{-9}–1.78×10^{-5} *M* T_4 or T_3. Extending further the utility of this *in vitro* model in studying the peripheral fate of these hormones, we recently reported that the behavior of the T-1 cells generally conforms to *in vivo* patterns when exposed to two thyroxine analogs, diiodothyropropionic acid and triiodothyroacetic acid (3). Our earlier studies pointed to the synthesis of RNA by the nucleus, particularly that associating with the nucleolus, as an initial response by these cultured cells to the presence of the thyroidal hormones. Using antibiotics and labeled T_4, the genome and the nuclear membrane were implicated as primary interacting foci, with the latter structure presumably regulating the transfer of the synthesized ribosomal and messenger RNA and their precursors across the nuclear–cytoplasmic interface (4). More recently, it has been shown that a concomitant rise in nascent membrane phospholipids should likewise be induced by the thyroidal hormones (5, 6).

That the thyroidal hormones affect the cellular content of lipids as a general response has been widely encountered (7). We are reporting now that the presence of certain lipids in the T-1 cell cytoplasm is likewise influenced by T_4 and T_3 and can be followed with oil red O staining.

Materials and Methods. As has been described (2), the T-1 cells were cultured in Eagle's MEM supplemented with 10% fetal bovine serum and antibiotics, utilizing 37° incubators gassed with a mixture of 95% air and 5% CO_2. About 3×10^5 cells, released with trypsin (0.05%) and dispersed by gentle agitation, were overgrown onto pairs of microscope slides placed in glass petri dishes. Following 4–6 days incubation with T_4 (1.78×10^{-7}–8.90×10^{-6} *M*), the cells were fixed (10% neutral formalin), stained with oil red O, counterstained with Harris' hematoxylin and aqueous fast green, and mounted in glycerin jelly (8).

Results. Droplets in the cultured kidney cells visualized after oil red O staining vary in size and hue, and are largely confined to the cytoplasm. The addition of thyroxine to the growth medium markedly increases the intensity of the lipid stain. While this reaction to T_4 is evident qualitatively by inspection (Fig. 1), scoring the number of dye droplets per treated cell relative to that of the corresponding control population (Table I) makes evaluation of the effect more sensitive. By this means, a biphasic response (9) to T_4 over the range of concentrations studied is revealed, with the optimal effect elicited by

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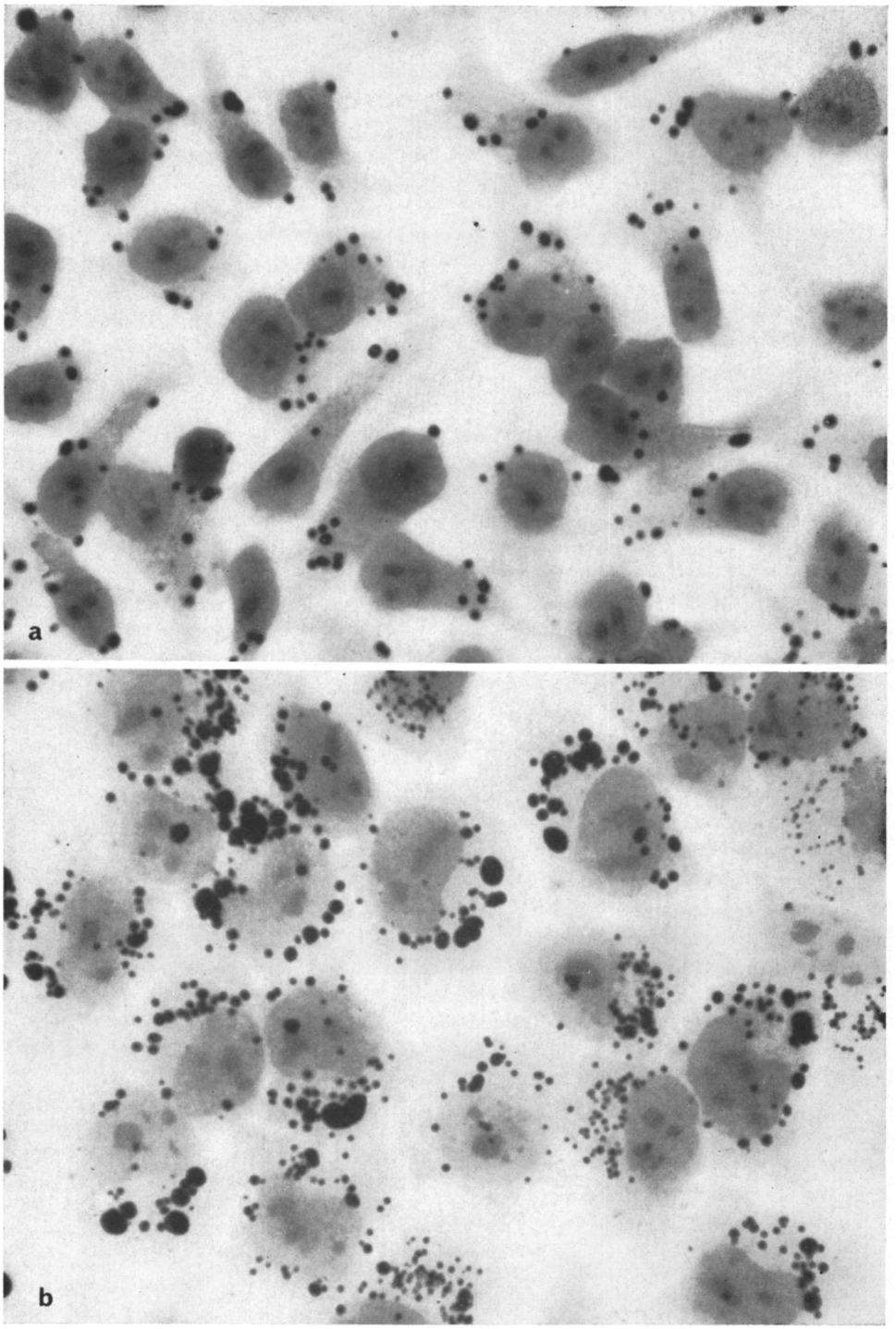

FIG. 1. Appearance of T-1 human kidney cells following oil red O staining (×686): (a) control; (b) after treatment with T_4 (1.78×10^{-6} *M*) for 6 days; note the increase in the number of lipid droplets.

TABLE I. Relative Increase in Oil Red O Droplets per T-1 Cell Induced by Exposure to L-Thyroxine (T_4) for 4–6 Days.

T_4 Conc (M)	Mean relative increase[a] (%)	Range (%)
8.90×10^{-6}	20.9 (2)	12.0–29.7
1.78×10^{-6}	48.1 (7)	23.6–71.2
8.90×10^{-7}	36.0 (2)	23.0–49.0
1.78×10^{-7}	18.2 (2)	16.6–19.8

[a] Results are expressed relative to control obtained for each of the experiments; the number of determinations is indicated in parentheses. For each experiment, duplicate microscope slides were overgrown and at least 200 cells were scored.

1.78×10^{-6} M. Fewer tests made with triiodothyronine suggest a similar pattern, the relative peak in the staining reaction of 56% being produced by 1.78×10^{-7} M (2 experiments); even a T_3 concentration as low as 8.90×10^{-9} M (1 experiment) produced a relative increase of 24% in the number of dye droplets.

Discussion. These findings made with oil red O imply that the thyroid hormones bring about in the T-1 cells an increase in the cytoplasmic content of globular unsaturated hydrophobic lipids, *e.g.*, triglycerides, cholesterol esters, and fatty acids (10), similar to the response patterns displayed by many other systems. Thus, fatty acid synthesis is depressed in hypothyroid (11) and hypophysectomized animals (12), but the administration of T_4 promoted incorporation of ^{14}C-acetate into hepatic fatty acids (13). Analogous to the biphasic behavior evinced by the T-1 cells treated with the thyroidal hormones are the experiments performed on rat liver slices by Fletcher and Myant (14), indicating that less than 25 μg/day of T_4 stimulated fatty acid synthesis, while more inhibited its formation. In normal human erythrocytes incubated with T_4 or T_3, the levels of 2,3-diphosphoglyceric acid likewise varied biphasically (15). In the mouse, the thyroidal hormones markedly enhanced the rate of oxidation of glycerophosphate and glycerophosphate dehydrogenase activity in mitochondria of the kidney and liver, but not of the brain, lung, and spleen; no changes in the activity of several other enzymes were observed (16). The absence of staining by oil red O in the nucleus is surely not because such lipids are absent (17, 18); possibly, it is necessary first to "unmask" them by the use of organic acids, successful for nuclear lipid staining with the closely related dye, Sudan black B (19).

It appears, then, that these actions evoked by the thyroidal hormones in the T-1 line merit more intensive exploration; identification of the subcellular sites participating and determination of the roles played by the responding lipids should yield leads for discovering the corresponding mechanisms that prevail *in vivo*.

Summary. L-Thyroxine (1.78×10^{-7}–8.90×10^{-6} M) added to the growth medium enhances lipid staining by oil red O in the T-1 human kidney cell line. By counting dye droplets as a function of hormone concentration, a biphasic response pattern is disclosed, the optimal effect resulting from a titer of 1.78×10^{-6} M. Fewer experiments made with L-triiodothyronine suggest similar biphasic behavior and that the most active concentration is about one-tenth less than T_4; as little as 8.90×10^{-9} M of T_3 increases lipid visualization. These findings further substantiate the validity of the T-1 cell line as an *in vitro* model for investigating thyroidal hormone interactions.

1. Van der Veen, J., Bots, L., and Mes, A., Arch. Gesamte Virusforsch. **8**, 230 (1958).

2. Siegel, E., and Tobias, C. A., Nature (London) **212**, 1318 (1966).

3. Siegel, E., and Morse, N. M., Proc. Soc. Exp. Biol. Med. **137**, 595 (1971).

4. Siegel, E., and Tobias, C. A., Science **153**, 763 (1966).

5. Tata, J. R., Biochem. J. **116**, 617 (1970).

6. Nicholls, E., and Matty, A. J., J. Endocrinol. **48**, lviii (1970).

7. Wolff, E. C., and Wolff, J., *in* "The Thyroid Gland" (R. Pitt-Rivers and W. R. Trotter, eds.), Vol. 1, p. 237. Butterworths, Washington, DC (1964).

8. Luna, L. G. (ed.), "Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology." McGraw-Hill, New York (1968).

9. Kerkof, P. R., and Tata, J. R., Biochem. J. **112**, 729 (1969).

10. Adams, C. W., *in* "Advances in Lipid Metabol-

ism" (R. Paoletti and D. Kritchevsky, eds.), Vol. 7, p. 1. Academic Press, New York (1969).

11. Dayton, S., Dayton, J., Drimmer, F., and Kendall, F. E., Amer. J. Physiol. **199**, 71 (1960).

12. Bates, M. W., Zomzely, C., and Mayer, J., Endocrinology **57**, 505 (1955).

13. Gompertz, D., and Greenbaum, A. L., Biochim. Biophys. Acta **116**, 441 (1966).

14. Fletcher, K., and Myant, N. B., J. Physiol. (London) **144**, 361 (1958).

15. Snyder, L. M., and Reddy, W. J., J. Clin. Invest. **49**, 1993 (1970).

16. Lee, Y.-P., and Hsu, H.-T., Endocrinology **85**, 251 (1969).

17. Dounce, A. L., *in* "The Nucleic Acids" (E. Chargaff and J. N. Davidson, eds.), Vol. 2, p. 93. Academic Press, New York (1955).

18. Stoneburg, C. A., J. Biol. Chem. **129**, 189 (1939).

19. Ackerman, G. A., Science **115**, 629 (1952).

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