actually were new cells derived from the orginally explanted cells through cell division. The brown metachromasia consistently observed in vitro in MLD was not seen to any significant degree in the normal cultured white matter and cerebral cortex.

The value of tissue culture for the study of hereditary metabolic disorders was demonstrated by Danes and Bearn (*7*), who were able to maintain (for periods up to 8 months) viable cultures of skin fibroblasts from patients with Hurler's disease (an inborn error of mucopolysaccharide metabolism). When stained for mucopolysaccharides with toluidine blue O, 60 to 100 percent of the cultured cells contained pink or red metachromatic granules. On the other hand, similar cultures of normal controls showed that only 0.1 percent of the cells contained metachromatic granules.

Although more definite proof is needed, our study suggests that the biochemical alterations of metachromatic leukodystrophy (intracellular accumulation of cerebroside sulfate) may be transmitted from cell to cell, at least under our conditions in vitro. If this hypothesis is substantiated by other techniques, such as thymidine incorporation, subcultures, and development of a cell line, the use of cultured cells from patients with metachromatic leukodystrophy for the further study of this disease or even for therapeutic trials will be valuable.

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22 December 1966

Thyroxine Interaction with Actinomycin D and Possible Biological Implications

Abstract. *Actinomycin D and thyroxine interact in solution* (p*H 8 to 10*), *as revealed by changes in the absorbance of actinomycin D. Thyroxine can prevent the growth-inhibitory effect of actinomycin D on* Bacillus subtilis *if it is present in a molar ratio of 3000 (thyroxine to actinomycin D).*

Actinomycin D has been extensively used for investigating RNA metabolism and protein synthesis in biological systems (*1*). While a few anomalous effects of actinomycin D have been reported (*2*), the general interpretation of its effect is that it inhibits DNA-dependent RNA synthesis. This inhibition is the specific result of binding of actinomycin D to DNA.

Studies involving subcellular binding of thyroxine (*3*) and actinomycin D (*4*) in tadpole liver nuclei showed that thyroxine is capable of interacting relatively strongly with actinomycin D. To define more clearly the nature of this interaction, we studied it in relation to its biological effect.

The concentration of crystalline actinomycin D was calculated from its extinction coefficient (E) at 440 mμ with a molar extinction coefficient value of 2.48×10^4 (*5*).

The absorption maximum of actinomycin D was shifted toward the higher wavelengths (Fig. 1), and absorption at 475 mμ increased while that at 440 mμ decreased after thyroxine was added.

Interaction between the two compounds was tested at *p*H 8, 9, and 10 (Fig. 2). Thyroxine is insoluble at a lower *p*H. To minimize any experimental error resulting from volume changes, the ratio of $E_{475\,m\mu}$ to $E_{440\,m\mu}$ was plotted against the molar ratio of actinomycin D to thyroxine. Smaller changes in the spectrum were observed in the system containing deoxyguanosine and actinomycin D. Deoxyguanosine has been used as a model compound in support of the hypothesis that the binding sites of actinomycin C (*6*) in DNA were the guanosine residues. The high affinity of guanine for actinomycin C in comparison with other constituents of the nucleic acids has been explained by the fact that it is a better electron donor than the pyrimidines and that its reactive center is different from that of adenine (*7*).

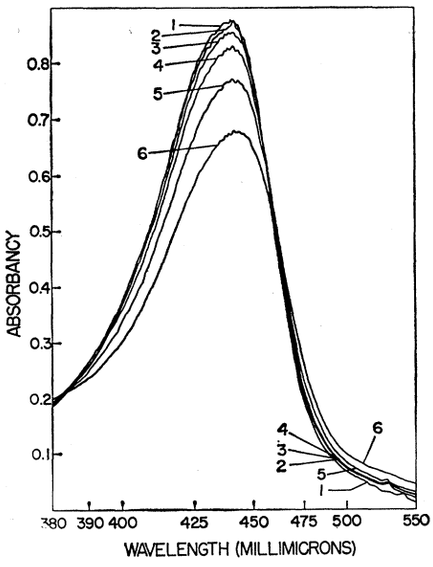

Fig. 1. Thyroxine binding to actinomycin D. Spectral changes of actinomycin D (20 μg/ml) after the addition of thyroxine solution were recorded by a Beckmann DK-2 spectrophotometer. The experiment was performed by successively adding 10, 10, 20, 50, and 100 μg of thyroxine to a solution of actinomycin D in 0.1*M* tris buffer, *p*H 8.0. Curve 1, actinomycin D; curve 2, actinomycin D and 10 μg of thyroxine; curve 3, as above and additional 10 μg of thyroxine; curve 4, as above and additional 20 μg of thyroxine; curve 5, as above and additional 50 μg of thyroxine; curve 6, as above and additional 100 μg of thyroxine.

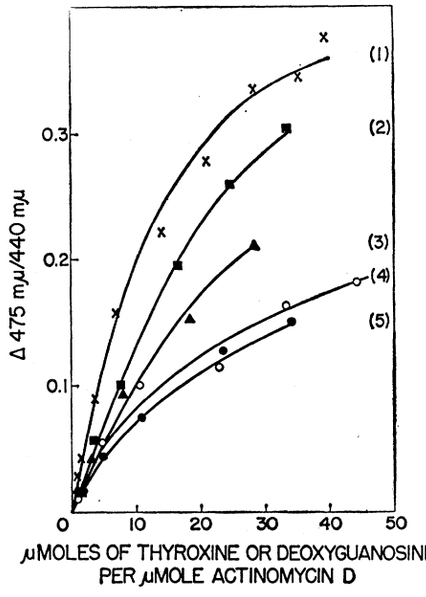

Fig. 2. Actinomycin D binding to thyroxine and deoxyguanosine. Ratios of the absorption at 475 and 440 mμ are plotted against the molar ratios of thyroxine to actinomycin D and deoxyguanosine to actinomycin D at different *p*H values. Curve 1, thyroxine and actinomycin D at *p*H 8; curve 2, thyroxine and actinomycin D at *p*H 9; curve 3, thyroxine and actinomycin D at *p*H 10; curve 4, deoxyguanosine and actinomycin D at *p*H 8; curve 5, deoxyguanosine and actinomycin D at *p*H 7.

The chemical nature of the binding between actinomycin D and thyroxine (or that between actinomycin D and deoxyguanosine) is not known. The absorption spectrum of actinomycin D maximally bound to deoxyguanosine was not altered by the addition of thyroxine. This suggests that actinomycin D binds thyroxine and deoxyguanosine in a similar manner. Spectrophotometry indicates that there is no interaction between thyroxine and deoxyguanosine.

The spectrophotometric data support the observation that thyroxine can reverse the growth-inhibitory effect of actinomycin D on *Bacillus subtilis*. Actinomycin D (0.1 μg/ml) inhibited the growth of bacteria completely, whereas the culture inoculated with medium containing thyroxine (200 μg/ml) and actinomycin D (0.1 μg/ml) grew normally (Fig. 3). However, the same concentration of thyroxine had no effect when we used 0.25 μg of the antibiotic per milliliter. No further significant changes were observed in the spectrum of actinomycin D solution at a molar ratio (approximately 50) of thyroxine to actinomycin D. The reason or reasons for the requirement of a molar ratio greater than 50 to reverse the growth-inhibitory effect of actinomycin D are not clear, although the binding to other compounds and degradation of thyroxine by bacteria are probably major factors. Delayed administration of actinomycin D to a thyroxine-treated culture produced a lesser degree of inhibition. Thyroxine had no effect on cultures treated with actinomycin D. This agrees with the observation that DNA binds actinomycin D about 10 to 20 times more effectively than it binds deoxyguanosine (*1*). Therefore, any actinomycin D bound to cellular DNA is not available for binding with thyroxine. Thyroxine exerts no effect on the growth of this bacterium. Apparently, the complex of actinomycin D and thyroxine is unable to penetrate the cell membrane.

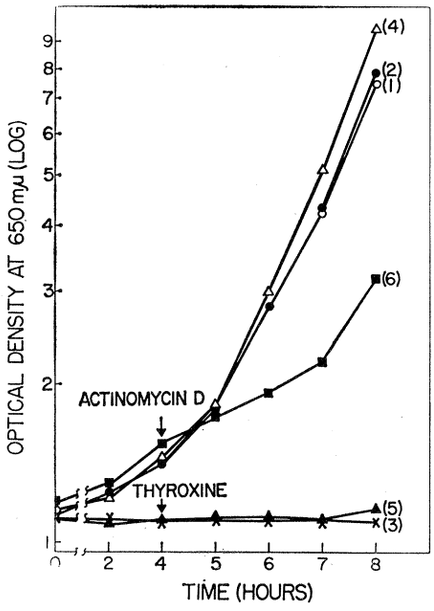

Fig. 3. Effect of actinomycin D and thyroxine on the growth of *Bacillus subtilis*, which was grown (at 35°C in a shaker) to 1 optical-density unit at 650 mμ in a minimal medium composed of salts and glucose. Stock culture (2 ml each) was then inoculated into the following media, and the growth followed. Curve 1, minimal medium; curve 2, thyroxine (200 μg/ml) in minimal medium; curve 3, actinomycin D (0.1 μg/ml) in minimal medium; curve 4, actinomycin D and thyroxine in minimal medium; curve 5, actinomycin D in minimal medium (thyroxine added after 1 hour of incubation of the culture); curve 6, thyroxine in minimal medium (actinomycin D added after 1 hour of incubation of the culture).

Our study may explain the observation that uptake of I^{125}-L-thyroxine by human kidney epithelial cells (T-1) was decreased by actinomycin D (*8*). Hence, it is important to examine the degree of interaction between thyroxine and actinomycin D in such a system.

A major portion of the labeled actinomycin D administered to tadpoles was associated with the nuclei, and most of the measurable activity was recovered in the chromatin fraction. The binding of actinomycin D to tadpole liver chromatin in vitro was not inhibited by thyroxine, or vice versa. This suggests that their binding sites are not the same (*9*); thyroxine binds mainly with the nuclear membrane fraction, whereas actinomycin D binds to DNA (*10*).

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11. Supported in part by grant C-3571 from NIH, and by grant G-21237 from NSF. We thank Dr. B. Weisblum, Department of Pharmacology, for the culture of *B. subtilis*, and Dr. H. Weissbach, NIH, for the gift of H^3-labeled actinomycin D.

28 December 1966

Heliacus (Gastropoda: Architectonicidae) Symbiotic with Zoanthiniaria (Coelenterata)

Abstract. *Eight species of* Heliacus *are obligate symbionts with* Zoanthus *and* Palythoa, *colonial zoanthid sea anemones. The gastropods live among* Zoanthus *polyps and under the edges of* Palythoa *incrustations, on which they feed and to which they and their egg masses are attached with sturdy but elastic mucous threads.*

Eight species in the marine gastropod genus *Heliacus* are symbiotic with *Zoanthus* and *Palythoa*, colonial zoanthiniarian sea anemones of the family Zoanthidae. *Heliacus* comprises about 30 Recent tropical and warm temperate species in the Architectonicidae (*1*, *2*), a family of mesogastropod prosobranchs with opisthobranch affinities. Hitherto, obligate symbiosis was unknown in the Architectonicidae. Other prosobranch symbionts with coelenterates are the families Epitoniidae, Janthinidae, Ovulidae, and Coralliophilidae, whose post-larval stages all live with and feed on coelenterates (*3*). Little is known as to why these prosobranchs are not deterred by nematocysts.

In the tropics, *Zoanthus* and *Palythoa* are abundant, sessile animals on rock substrata in shallow water. *Palythoa* lives where wave action is strong, such as reefs. At some places, *Palythoa* incrusts as much as 90 to 95 percent of the substratum (*4*). Architectonicids are seldom observed *in situ*, and population densities of *Heliacus* with zoanthids are usually low (Table 1). The abundance of *Heliacus* must be regulated by factors other than the abundance of zoanthids because *Heliacus* can be scarce where zoanthids are luxuriant. Also, the symbiosis with *Zoanthus* and *Palythoa* cannot occur in all species of *Heliacus* because there are several that live in the Mediterranean (*2*), where the Zoanthidae do not occur but where other zoanthiniarian families do (*5*).