thyroid-hypophyseal histology and physiology in the opossum appears to be well worth the undertaking, for the host of questions raised by the afore-mentioned association indicates a fundamental problem of singular importance. Is it possible that there is a low level of hypophyseal-thyroid interaction but that the tissues of this animal are adapted to small amounts of thyroxine so that their efficiency is maintained? (The opossum is sedentary but not sluggish.) Are the oxidative metabolic and enzymatic rates of the tissues of this marsupial different from those of other mammals? Experiments are being designed to elucidate these points regarding the hypothyroid animal with the hope that clues may be provided that might be important in human thyroid physiology.

References and Notes

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Synthesis of Oligosaccharides from Maltose by Rat Liver

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Enzymes that can synthesize oligosaccharides from maltose are known to be present in molds and bacteria. As far as we are aware, no such enzyme system has been shown to occur in animal tissues. Recently, however, it has been shown that an enzyme that can transfer a glucose residue from maltose to flavins (*1*) is present in rat liver. We have observed that the rat liver contains an enzyme system that can convert maltose by transglucosidation into oligosaccharides with simultaneous liberation of glucose.

The enzyme was obtained in an amorphous powder form by fractional precipitation of the liver homogenate with alcohol after the amylase had been removed by adsorption on starch. The fraction obtained between 50 and 80 percent alcohol concentration was centrifuged and was dried with cold acetone and ether. The enzyme (20 mg) was incubated at 37° ± 2°C with 1 ml of 20-percent maltose in 0.02*M* acetate buffer at *p*H 5.0. The progress of the reaction was followed by circular paper-chromatographic technique, as is described by Giri and Nigam (*2*). Figure 1 shows the formation of oligosaccharides from maltose at varying periods of incubation.

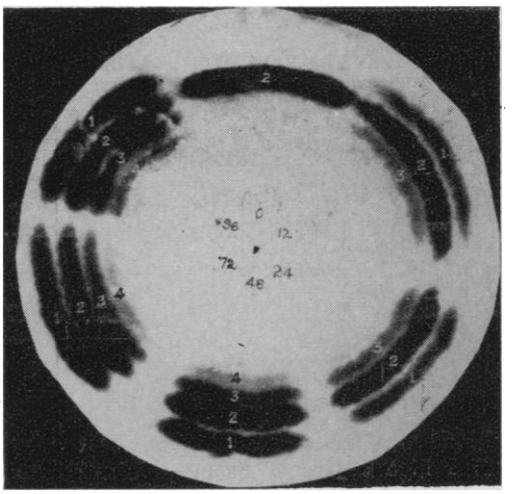

Fig. 1. Circular paper chromatogram, showing the synthesis of oligosaccharides from maltose by the enzyme prepared from rat liver. The numbers on the inner circle indicate the time of incubation in hours. Whatman No. 1 filter paper (18 cm in diameter) was used. Developing solvent: *n*-butanol, pyridine, and water (6: 4: 3); double development. The reagent used was 2-percent triphenyltetrazolium chloride in water-saturated butanol mixed with an equal volume of N alcholic potash. Band 1 indicates glucose; band 2, maltose; band 3, maltotriose; and band 4, maltotetraose.

The two oligosaccharides formed have been identified as maltotriose and maltotetraose; they were identified by our running chromatograms with known reference substances in different solvents and by determining the papergram mobilities. The oligosaccharides yield only glucose on hydrolysis, thereby showing that they contain only glucose units. The two oligosaccharides were isolated by charcoal-celite column chromatography, as is described by Whistler and Durso (*3*), and confirmed as maltotriose and maltotetraose by (i) optical rotation, (ii) partial hydrolysis with 0.1*N* sulfuric acid, (iii) preparation of acetyl derivatives, (iv) periodate and hypoiodite oxidations, (v) hydrolysis by β-amylase, and (vi) priming capacity for phosphorylase (green gram) action. Other disaccharides—lactose, sucrose, cellobiose, and isomaltose—did not show the formation of oligosaccharides. The rat brain homogenate also was found to contain the enzyme.

It is interesting to note that rat liver contains an enzyme system that brings about the synthesis of oligosaccharides containing 1 : 4 glucosidic links only, whereas the mold enzyme systems synthesize oligosaccharides having both 1 : 4– and 1 : 6– glucosidic links. It is probable that the formation of 1 : 4 and 1 : 6 links are caused by the action of two different enzymes.

A more detailed report of this investigation is in preparation.

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Chemical and Biological Studies on 1,2-Dihydro-*s*-triazines. XI: Inhibition of Root Growth and Its Reversal by Citrovorum Factor

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Previous communications have described the synthesis (*1, 2*) of a new family of 1,2-dihydro-*s*-triazines and the microbiological (*3*), antitumor (*4*), and antimalarial (*1, 5*) activity exhibited by the derivatives of this series. Antimalarial activity (*6*) and antiprotozoan activity (*7*) have also been reported from other laboratories. The present report (*8*) concerns the activity of a representative compound—4,6-diamino-1-(3′,4′-dichlorophenyl)-1,2-dihydro-2,2-dimethyl-*s*-triazine (D-54 · HCl) (*1, 2*)—in a turnip seedling system similar to the "Kressewurzel growth test" (*9*).

Seeds of *Brassica rapa* L (*10*) were washed, soaked 2 hr in distilled water at 25°C, and placed in petri dishes on filter paper moistened with 6 ml of distilled water. The dishes were then slanted in racks providing a 45° angle and incubated in the dark at 25°C for 22 to 24 hr. Twenty seedlings with straight roots of equal length (as measured by calipers under magnification) were arranged in one or two rows in the upper two-thirds of petri dishes on filter paper moistened with 5 ml of 6.7 mM phosphate buffer (*p*H 5.9) that contained the various compounds to be tested. Identical control seedlings in dishes with buffer alone were included in each experiment. The tests were incubated as before for 24 hr, and root growth was then measured by placing the seedlings on millimeter graph paper.

The results of a typical experiment with D-54 · HCl and 4-aminopteroylglutamic acid (4-APGA), an analog of pteroylglutamic acid (PGA) known to inhibit plant growth (*11*), are illustrated in Fig 1. The addition of 100 μg/ml of D-54 · HCl to the buffer resulted in marked inhibition of root growth, and lesser concentrations were progressively less effective. The inhibitory activity of D-54 · HCl was considerably less than that of 4-APGA, as is indicated by the inhibition obtained with 2 μg/ml of 4-APGA. The data derived from several such experiments are summarized in Fig. 2, where the mean growth increment of treated seedlings is plotted as a percentage of the mean growth increment of control seedlings, which is designated as 100 percent. The inhibition of root growth by D-54 · HCl, as with 4-APGA, exhibited a linear increase in response to logarithmic increases in concentration of inhibitor.

The 1,2-dihydro-*s*-triazines, like 4-APGA, interfere with the conversion of PGA to citrovorum factor (CF) in bacterial (*3*) and mammalian liver systems (*12*). However, the mechanisms of action of the two classes of inhibitors differ, the 1,2-dihydro-*s*-triazines inhibiting a coenzyme I-dependent system concerned with the biological reduction of PGA (*13*). Thus, in appropriate microbiological systems, reduced PGA (*14*), coenzyme I or its precursors, CF, thymine or thymidine, but not unaltered PGA, reversed D-54 · HCl inhibition (*3, 13*). Accordingly, analogous reversal experiments were conducted with these various metabolites in the seedling system. These experiments were identical with those previously described, except that the metabolites were added to the buffer simultaneously with a constant concentration of inhibitor (10 μg/ml of D-54 · HCl) that resulted in about 50-percent inhibition of the mean growth increment. The extent of reversal was computed by determining the mean growth increments resulting from the addition of a given metabolite to such inhibited systems.

Inhibition by D-54 · HCl (Table 1), like that of 4-APGA in plant systems, was not reversed by PGA (*11*) or a precursor (PABA) but was reversed inconsistently by reduced PGA, partially by nicotinic acid or coenzyme I, and more effectively by CF, thymine or thymidine, as has been observed in bacterial systems. This pattern of reversal is consistent with previous observations, suggesting that, as is the case with certain bacteria, the conversion of PGA to CF in this seedling system involves a coenzyme I—

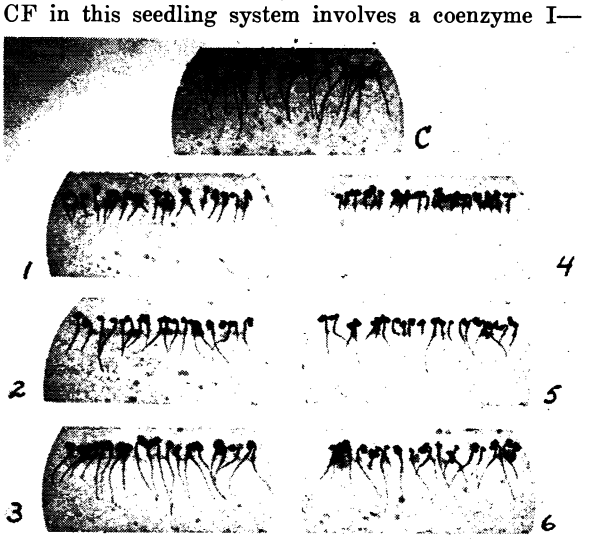

Fig. 1. Inhibition of root growth of seedlings of *Brassica rapa* L by D-54 · HCl and 4-APGA; (1, 2, 3) 2.0, 0.2, and 0.02 μg/ml of 4-APGA, respectively; (4, 5, 6) 100, 10, and 1 μg/ml of D-54 · HCl, respectively; *C*—untreated control.