tion of the ABO into the borehole, (ii) full envelopment of the bulb by the ABO secretory epithelium, and (iii) withdrawal of the ABO from the hole (Fig. 4). The period of the ABO in the borehole generally corresponded to that recorded with microhydrophones in earlier studies (*8*). Recording *II* (Fig. 4) is representative of the four *p*H curves. At the start of the observations the *p*H of the sea water in the dish was 8.0. As the snail withdrew its proboscis from the borehole, we inserted the microelectrode (Fig. 4, *e*) and the *p*H dropped to 6.9. In 1.3 minutes the ABO was extended into the borehole and made contact with the bulb (Fig. 4, *a*). In the ensuing minute the *p*H dropped rapidly to 4.7 as the secretory epithelium completely enveloped the bulb. During the subsequent 46 minutes the *p*H continued to drop slowly to a minimum of 3.9. Then the ABO was withdrawn (Fig. 4, *a*–) into the foot, which remained closely appressed over the borehole. As the ABO was retracted, it drew sea water from the cavity of the model across the glass-shell juncture to replace it, and the *p*H rose within 1 minute to 6.2, a *p*H lower by 0.7 than that of the sea water in the borehole when the ABO had first entered. During the steep rise of the *p*H curve, the proboscis was typically inserted into the borehole, exploring and rasping. Two minutes later we moved the microelectrode from the borehole to the sea water around the model (Fig. 2, *s*), and the *p*H rose to 7.5; during the following minute, while we flushed the viscid secretion from the bulb, the *p*H rose again to 8.0—that of the sea water. Full envelopment of the bulb by the secretory epithelium would correspond to full appression of the secretory epithelium to the bottom of the borehole during penetration, and thus the *p*H of the secretion, except for the neutralizing action of the $CaCO_3$ of the shell, should be comparable under both circumstances.

In a number of recordings made during the spring (Fig. 4, *III* and *IV*), the shell material within and in the vicinity of the borehole was impregnated with hot paraffin to eliminate ionization of the $CaCO_3$; in these recordings the minimum *p*H when the ABO was fully around the microelectrode bulb was 3.8, lower by 0.1 *p*H than the minimum values obtained in the unparaffined boreholes.

The *p*H of the fluid on the surface of ABO's, extruded from the amputated feet of snails pinned upside down, ranged from 6.3 to 7.2. When the microelectrode was pressed into the ABO, the *p*H dropped to a minimum of 6.0, but results varied widely, probably as a function of the physiology of the ABO at the time of extrusion.

A snail frequently extends a portion of the propodium of the foot into the the borehole in an exploratory manner after withdrawal of the ABO and before insertion of the proboscis. Contact of the propodial epithelium with the microelectrode did not alter the *p*H appreciably; nor did contact with the integument of the proboscis. On the other hand, when four different snails on separate occasions took the bulb of the microelectrode into their buccal cavities for periods ranging from several seconds to 1 minute, the *p*H fell from 7.2 to 6.7, from 6.7 to 6.1, from 7.2 to 6.7, and from 7.1 to 6.4, respectively—falls varying from *p*H 0.5 to 0.7. This finding suggests that secretions from the salivary glands or the accessory salivary glands, or both, which empty into the buccal cavity, may be slightly acidic.

We conclude that secretion of the normally functioning ABO in the borehole is distinctly acid, a finding contrary to earlier reports (*2–4, 6, 9*) but consistent with the earliest hypothesis (*7*), and acidifies sea water in the borehole over a short radius around the ABO. The acid, still uncharacterized, may play a part in the weakening of shell during boring; active excised ABO's etch polished shell when appressed to it (*4*), though bubbles of CO_2 have not been observed in such preparations. The presence of acid does not necessarily rule out the possibility that enzymes or chelating substances, or both, also may be involved in shell destruction. The failure of most earlier attempts to detect convincing acid reactions with *p*H papers was due to the insensitivity of the method and the fact that forcefully extruded and excised ABO's produce no, or minimal quantities of, acid. Apart from slight acidity in the buccal cavity, no acid was detected elsewhere in the snail.

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Carotenoid Biosynthesis in Rhodospirillum rubrum: Effect of Pteridine Inhibitor

Abstract. *A known inhibitor of pteridine utilization (4-phenoxy,2,6-diamino pyridine) blocks the synthesis of colored carotenoids in the photosynthetic bacterium* Rhodospirillum rubrum. *In many ways the effect is similar to the inhibition of the synthesis of colored carotenoids by diphenylamine. This inhibition is probably independent of other effects of pteridine on photosynthetic electron transport since it is not as readily reversible as the total inhibition of photosynthetic activity by pteridine analogs.*

Purple photosynthetic bacteria normally contain unsaturated methoxylated carotenoids such as spirilloxanthin or spheroidene (*1*). In a wide variety of microorganisms, including photosynthetic bacteria, diphenylamine inhibits the synthesis of the dehydrogenated colored carotenoid pigments, causing the accumulation of the more saturated phytoene with lesser amounts of

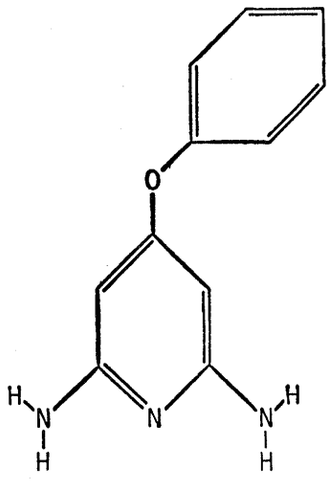

Fig. 1. 4-Phenoxy,2,6-diamino pyridine (PDAP).

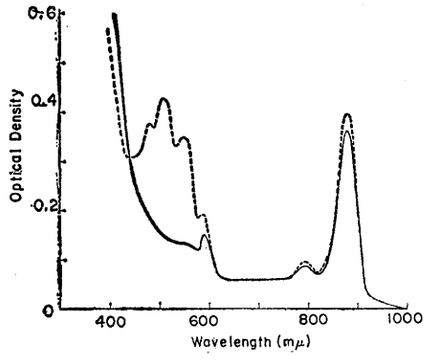

Fig. 2. The absorption spectra of two suspensions of *Rhodospirillum rubrum* grown anaerobically in the light. Spectra were determined through opal glass to minimize the effect of light scattering. Dotted line, normal cells. Solid line, cells grown in the presence of $10^{-4}M$ PDAP.

phytofluene, ζ-carotene, and neurosporene (*2*). It appears from these studies that diphenylamine may block the stepwise dehydrogenation of phytoene in some organisms.

Recent studies have indicated that 2-amino-4-hydroxy pteridines with substitutions in the six position, such as biopterin, are present and apparently associated with the photochemical apparatus in a number of photosynthetic organisms (*3–5*). 4-Phenoxy, 2,6-diamino pyridine (PDAP) (Fig. 1) and similar pteridine analogs inhibit the utilization of pteridines by aerobic microorganisms (*6*). These compounds also inhibit photosynthetic phosphorylation and CO_2 fixation in the anaerobic photosynthetic bacteria (*4*).

Rhodospirillum rubrum grown anaerobically in the dark contain no photosynthetic pigments or photosynthetic capacity. When transferred to the light in the presence of $10^{-4}M$ PDAP they develop a normal photosynthetic capacity, but lack colored carotenoids and show a spectral pattern very similar to that of cells grown in the presence of diphenylamine. Spectra of the suspensions (Fig. 2) show loss of the normal carotenoid absorption bands between 450 and 550 mμ. Methanol extracts of cells grown on PDAP show absorption bands corresponding closely to those of phytoene and ζ-carotene (Fig. 3).

Since PDAP inhibits the utilization of pteridines this finding suggests that a pteridine may function in carotenoid dehydrogenation. As a test of this possibility, cells of *R. rubrum* grown in the dark and devoid of all pigments were placed in a medium without pteridine in four separate vessels containing 0, 1, 10, 100 μM PDAP, respectively. After 48 hours of growth in the light, the chlorophyll, carotenoid, and pteridine concentrations decreased in those cells in the higher concentrations of PDAP (Fig. 4). It appears that pteridine synthesis was retarded in 1 μM PDAP, carotenoid synthesis was retarded in 10 μM PDAP, and the development of a functional photosynthetic apparatus was completely inhibited by 100 μM PDAP as indicated by the absence of both chlorophyll and carotenoids.

A fraction containing pteridine, prepared from the purple sulfur bacterium *Chromatium* according to the procedure of Forrest, Van Baalen, and Myers (*7*), was added to the vessel containing 100 μM PDAP, resulting in total recovery of photosynthetic activity as indicated by the reappearance of chlorophyll and the concomitant evolution of hydrogen (see Fig. 4). However, only carotenoids in the more saturated state were present. The dramatic reversal of the inhibitory effect of PDAP on the overall photosynthetic development could also be brought about by the addition of $10^{-4}M$ biopterin [pure synthetic 2-amino, 4-hydroxy, 6-(2,3,dihydroxypropyl) pteridine]. Again the carotenoid pattern was unaltered by the addition of $10^{-4}M$ pteridine.

It would seem that PDAP, a structural analog of pteridines, having a suggested structural relationship to diphenylamine (Fig. 1), has a twofold differential effect on the metabolism of photosynthetic bacteria. Clearly, PDAP prevents the conversion of pteridine precursors to pteridines (Fig. 4), as indicated by the lowered cellular concentration of pteridines in the presence of PDAP. It may also block the enzymatic dehydrogenation of phytoene to lycopene as indicated by the accumulation of phytoene under these conditions. The latter process could be either an indirect one preventing the synthesis of a pteridine cofactor for the dehydrogenation process, or a direct one blocking the enzymatic site for dehydrogenation. An alternative, of

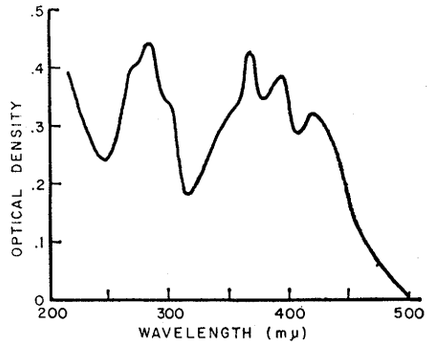

Fig. 3. Spectrum of methanol extract of *Rhodospirillum rubrum* cells grown in presence of $10^{-4}M$ PDAP. Absorption maxima would suggest the presence of phytoene and ζ-carotene.

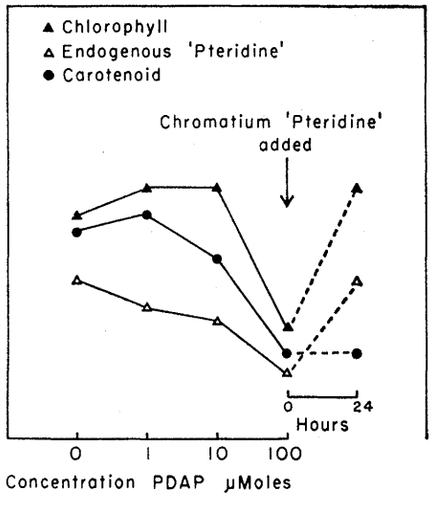

Fig. 4. The effect of PDAP concentration on chlorophyll, carotenoid, and pteridine concentrations in cells of *Rhodospirillum rubrum*. Chlorophyll (–▲–) determined by optical density at 880 mμ in vivo. Carotenoid (–●–) determined by optical density at 550 mμ in vivo and endogenous pteridine (–△–) determined by fluorescence yield at 450 mμ of an ammoniacal extract of harvested cells when excited at 365 mμ. The reversal of total pteridine and chlorophyll content by the addition of added pteridine is shown by the dashed lines over a 24-hour period. During this time there was no recovery of colored carotenoid inhibition.

course, is that pteridine is not a cofactor for synthesis of carotenoids and the action of PDAP on carotenoid dehydrogenation is independent of pteridines.

Both photosynthetic phosphorylation and CO_2 fixation in bacteria are inhibited by PDAP (*4, 5*). Pteridines can stimulate these processes (*8*). It seems probable that the effect of this pteridine inhibitor on other photosynthetic processes in bacteria (H_2 evolution, chlorophyll synthesis, CO_2 fixation, photophosphorylation, and so forth) is not related to the effect described above on carotenoid biosynthesis—unless the carotenoid pigments function more directly in photosynthetic electron transport than is presently envisaged.

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Calcitonin from Ultimobranchial Glands of Dogfish and Chickens

Abstract. *Acid extracts of thyroid glands from a small shark* Squalus suckleyi *and domestic fowl* Gallus domestica *contained no detectable calcitonin activity, while very potent hypocalcemic responses were obtained in rats with similar extracts from the ultimobranchial glands of these two species. The calcitonin concentration was 4 to 40 times that present in hog thyroid, which, as in most other mammals, contains ultimobranchial tissue. The evidence suggests that calcitonin is a fundamental calcium-regulating hormone present in all higher vertebrates and that it is an ultimobranchial rather than a thyroid hormone. It also indicates an important and hitherto unrecognized function for the ultimobranchial glands.*

In 1962 Copp *et al.* (*1*) presented evidence for a hypocalcemic hormone released when thyroid and parathyroid glands were perfused with high-calcium blood. They named the hormone calcitonin, since it was apparently involved in regulating the level or "tone" of calcium in body fluids. Originally thought to be of parathyroid origin, it was soon found that the hormone is present in (*2*), and released by (*3*), cells present in the mammalian thyroid. For this reason, the name thyrocalcitonin has been suggested for the hormone (*2*). Using immunofluorescent techniques, Bussolati and Pearse (*4*) showed that calcitonin is not present in the regular thyroid cells, but is present in the parafollicular C cells (also called "light" cells). Pearse and Carvalheira (*5*) also demonstrated by histological specific staining reactions that these are probably ultimobranchial cells. In other classes of vertebrates (fish, amphibians, reptiles, and birds) these exist as separate glands, but in mammals they become imbedded in the thyroid and inferior parathyroid (see *6*).

Inspired by these observations, we have investigated the hypocalcemic activity of acid extracts of thyroid and ultimobranchial glands in chickens and dogfish, in which the two glands are separate and distinct. In the embryo, the thyroid arises from the thyroglossal duct in the anterior pharynx in the midline. The ultimobranchial gland (or body) arises from the ventral aspect of the last branchial pouch. It is present in all gnathostomes, and has hitherto been assigned no specific function.

In the Pacific Coast dogfish (*Squalus suckleyi*), a small shark weighing 2 to 4 kg, the ultimobranchial gland is located just above the pericardium on the left side in the triangle formed by the basibranchial and ceratobranchial cartilages and the coracobranchial muscle (*7*). The glands, which weighed 10 to 20 mg, were dissected out of fish caught 2 to 4 hours earlier, and were immediately frozen with dry ice. The chicken ultimobranchial gland is located in the chest near the bifurcation of the common carotid and axillary artery. The thyroid makes a good landmark, since the two parathyroids and the ultimobranchial glands are strung out in sequence below it. The glands were obtained from reject birds at a local poultry processing plant and were removed and frozen within 2 hours of the death of the bird. Extracts were prepared by the method of Hirsch *et al.* (*2*). The chicken glands were first defatted by extracting eight times with ten volumes of acetone. The dried material was then homogenized with ten volumes of 0.1*N* HCl and extracted at room temperature for 1 hour. It was then centrifuged for 10 minutes in a clinical centrifuge to remove cell debris. The dogfish glands contained little or no fat, and the glands were extracted directly. Thyroid tissue similarly treated served as control.

Extracts were brought to *p*H 4 by addition of 0.1*N* NaOH, and suitable dilutions were made with 0.9 percent NaCl so that the final volume injected was 0.5 ml. This was administered intraperitoneally to 44- to 49-day-old rats weighing 180 to 200 g. Samples of tail blood (0.2 ml) were collected at 0, 1, 3, and 6 hours after injection and were analyzed for calcium by the method of Copp (*8*). The response was assessed by the assay method of Copp and Kuczerpa (*9*) and is expressed as the area (in milligrams of calcium per 100 milliliters of plasma, per hour) between the plasma calcium curve after injecting extract and the plasma calcium level of the control. This evaluates both the intensity and the duration of the hormone effect. Five or six rats were used at each dose level.

The dose (per 100 g of the rat's body weight) is expressed in terms of the fresh weight of gland used to prepare the extract. The response was compared to that obtained in similar rats injected with a standard preparation of calcitonin from beef thyroid. This preparation contained 0.11 MRC units per milligram. These units