the endosymbionts that would become organelles (*3, 21*). This suggestion stems from the growing number of specific resemblances between archaebacteria and eukaryotes. For example, (i) the adenosine diphosphate ribosylation of archaebacterial elongation factor by diptheria toxin (*22*), (ii) the close resemblance between the archaebacterial and eukaryotic versions of the sequence of one or more of the (large subunit) ribosomal proteins (*6*), (iii) the findings that archaebacterial tRNA's may be more readily aminoacylated by eukaryotic than by eubacterial synthetases and that protein synthesis in archaebacteria starts with Met-tRNA, not F-Met–tRNA (*23*), and (iv) the finding that an immunological cross-reaction exists between archaebacterial and eukaryotic RNA polymerases (*24*). It is apparent that the matter of the earliest phylogenetic branchings is far from settled and that the origin of the eukaryotic cell is a deeper and therefore more interesting problem than it was once thought to be.

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5-Hydroxytryptophan Elevates Serum Melatonin

Abstract. *Daytime administration of 5-hydroxytryptophan to sheep elevated serum melatonin more than sevenfold within 2 hours. This suggests that administration of 5-hydroxytryptophan could be used as the basis of a clinical test of pineal function and that melatonin might mediate some clinical effects of 5-hydroxytryptophan.*

5-Hydroxytryptophan (5-HTP), which is used in the treatment of depression and myoclonus (*1*), is an intermediate in the synthesis of *N*-acetyl-5-methoxytryptamine (melatonin). The pathway is tryptophan → 5-HTP → 5-hydroxytryptamine (serotonin) → 5-hydroxy-*N*-acetyltryptamine (*N*-acetylserotonin) → melatonin (*2*). Melatonin synthesis by the pineal gland, which is thought to be the major determinant of the concentration of circulating melatonin (*3*), is low during the day and increases at night as a result of neural stimulation of the activity of serotonin *N*-acetyltransferase (*2*). Even though 5-HTP has been found to cause a small increase in rat pineal melatonin (*4*), the role of this precursor in the control of melatonin synthesis and serum melatonin has generally been ignored. We now report that administration of 5-HTP to sheep during the day increases the concentration of serum melatonin to nighttime levels within 2 hours.

Male sheep that had been housed for 1 week in a windowless stall with automatically regulated lighting were intraperitoneally injected with tryptophan (500 mg/kg), 5-HTP (20 or 200 mg/kg), or saline (*5, 6*). Blood samples were obtained at 1-hour intervals beginning immediately before treatment and ending 5 hours after treatment. Serum melatonin was measured by radioimmunoassay (*7, 8*) and serum tryptophan by a fluorometric technique (*9*). Statistical analysis was done with Duncan's multiple-range test (*10*).

A diurnal rhythm was seen in the level of serum melatonin in saline-treated sheep (Figs. 1 and 2) (*7, 11*). The injection of tryptophan elevated serum tryptophan 10- to 15-fold after 1 to 5 hours (*12*); this caused a very small increase in serum melatonin that was significant ($P < 0.05$) only at 2 hours after the injection. A tryptophan-induced increase in serum melatonin was not detected at night (Fig. 1).

In contrast to the weak effect of tryptophan on serum melatonin, 5-HTP had marked effects (Fig. 2). The 5-HTP injection (20 or 200 mg/kg) caused a statistically significant ($P < 0.05$) increase in serum melatonin after 2 to 5 hours. The peak of the increase induced by 5-HTP at 20 mg/kg occurred after 1 to 2 hours and was more than seven times greater than control values. The 200 mg/kg dose produced a larger and more lasting increase:

after 5 hours serum melatonin values were more than 20 times greater than control values. No significant effect of 5-HTP treatment was detectable at night, when serum melatonin was already elevated as a result of neural stimulation of the pineal gland. One animal died 4 hours after receiving the larger dose of 5-HTP.

The most reasonable explanation for the weak effect of tryptophan on serum melatonin is that, in sheep, pineal tryptophan hydroxylase is saturated by endogenous pineal tryptophan. This does not appear to be the case in the rat, because tryptophan administration increases pineal serotonin in this species (*13*).

We believe that the marked elevation of serum melatonin by 5-HTP is due to a mass-action effect on the 5-HTP → melatonin pathway in the pineal gland (*14*); the three enzymatic reactions—decarboxylation, *N*-acetylation, and *O*-methylation—do not seem to be saturated during daytime. This is supported by three lines of evidence: (i) during the day 5-HTP increases the level of pineal melatonin in rats in vivo (*4*); (ii) monoamine oxidase inhibitors, which elevate serotonin, also increase rat pineal melatonin (*15*); and (iii) treatment with 5-HTP (200 mg/kg) elevates sheep pineal serotonin, *N*-acetylserotonin, and melatonin (*16*). The finding that nighttime treatment with 5-HTP did not produce a marked increase in the already elevated levels of serum melatonin may indicate that at night at least one step in the 5-HTP → melatonin pathway is saturated.

If 5-HTP elevates serum melatonin in man, then 5-HTP administration might form the basis of a clinical test of nonneural pineal function. Such a test could indicate the ability of the pineal gland to convert 5-HTP to melatonin. With this approach it might be possible to identify and characterize pineal tumors. In addition, the 5-HTP–induced increase in melatonin might be useful in predicting whether patients will respond to 5-HTP therapy.

Some clinical effects of 5-HTP treatment might be mediated by melatonin. Patients receiving 5-HTP during the day may have unusually high, perhaps "nighttime," levels of serum melatonin that would modify or abolish the physiological melatonin rhythm. Also, individual differences in the effectiveness of 5-HTP treatment might be due to individual differences in the melatonin response to 5-HTP.

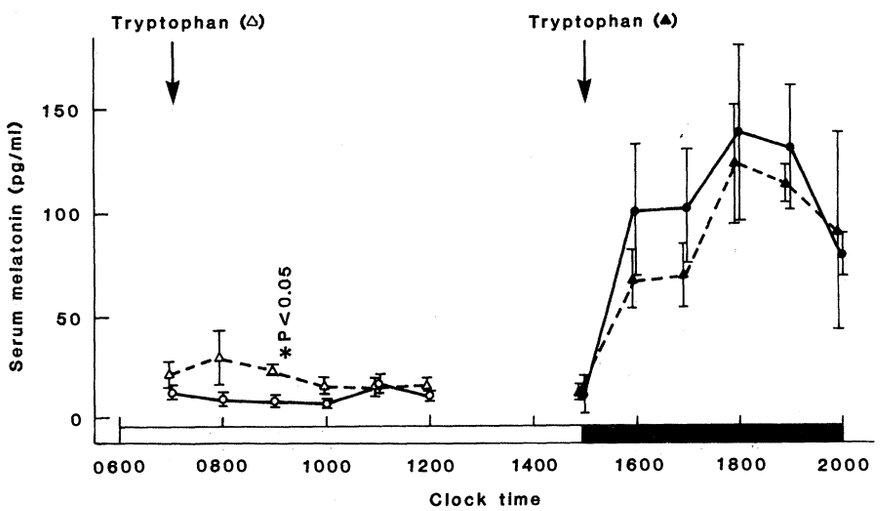

Fig. 1. Effect of tryptophan administration on serum melatonin. Each animal was injected intraperitoneally with 200 ml of a suspension of tryptophan (500 mg/kg) in saline or with saline at 0700. Blood was collected 10 minutes before the injection and every hour after the injection for 5 hours. The same protocol was followed at night; treatment was started just before lights out at 1500. The experiments were done during May and June. Serum melatonin was measured in duplicate (*7*). Data were collected over a 4-week period. Values are means ± standard errors for three sheep.

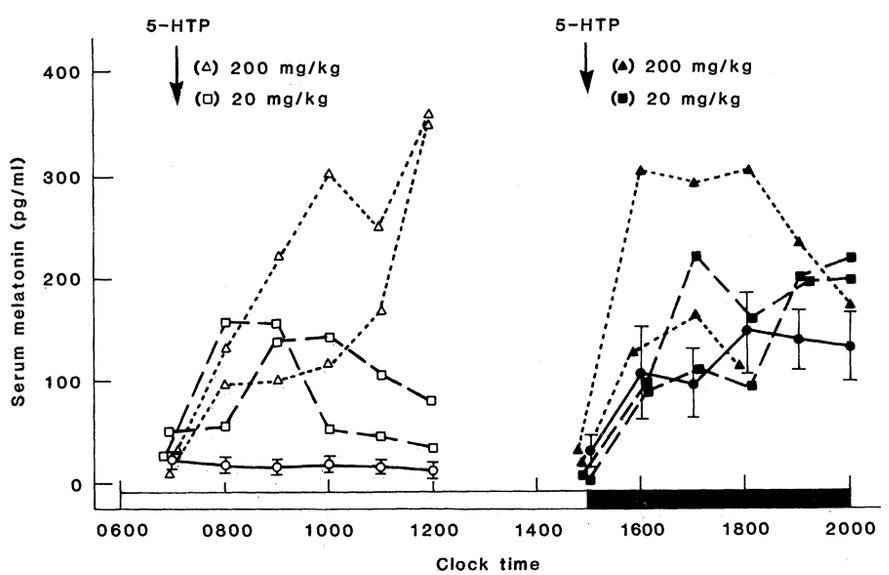

Fig. 2. Effect of 5-HTP administration on serum melatonin. The protocol described in the legend to Fig. 1 was used, except that 5-HTP was injected instead of tryptophan. These experiments were done during November and December. The control values are means ± standard errors for four animals; the experimental values are for individual animals.

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6. L-Tryptophan (Sigma) and L-5-hydroxytryptophan (ICN) were injected as suspensions in saline. No side effects of L-tryptophan or the smaller dose of 5-HTP were apparent. Animals

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Lignin-Degrading Enzyme from the Hymenomycete *Phanerochaete chrysosporium* Burds.

Abstract. *The extracellular fluid of ligninolytic cultures of the wood-decomposing basidiomycete* Phanerochaete chrysosporium *Burds. contains an enzyme that degrades lignin substructure model compounds as well as spruce and birch lignins. It has a molecular size of 42,000 daltons and requires hydrogen peroxide for activity.*

Lignin biodegradation plays a key role in the earth's carbon cycle. Not only is lignin the most abundant renewable organic material next to cellulose, but it also encrusts and, until degraded, prevents access of degradative enzymes to the cellulose and hemicelluloses in woody plant tissues (*1*). Lignin is decomposed preeminently by higher basidiomycetous fungi that cause the white-rot type of wood decay (*2*).

Past research has shown that oxidizing agents with low specificity are involved in the biodegradation (*3*), but has not revealed the nature of these agents. Recent indirect evidence indicates that nonenzyme-bound activated oxygen species derived from H_2O_2, rather than enzymes, are the actual degradative agents (*4*, *5*), and that H_2O_2 has a role in lignin degradation (*4*–*7*).

Essential to defining the biochemical mechanism is the identification of individual reactions of the lignin degradation process. Because of the complexity of the lignin polymer (Fig. 1), lignin substructure model compounds such as 1,2-bis-(3-methoxy-4-[^{14}C]methoxyphenyl)-propane-1,3-diol (**1**) and 1-(4-ethoxy-3-methoxy[U^{14}C]phenyl)-2-(*o*-methoxyphenoxy) propane-1,3-diol (**2**) have been used to define specific reactions. Compound **1** represents the 1,2-diarylpropane substructure (Fig. 1A), which accounts for ~ 7 percent of the linkages in lignins, and **2** represents the arylglycerol-β-aryl ether type of substructure (Fig. 1C), which is the dominant one in lignins, accounting for 50 to 60 percent of the interunit linkages (*8*). The degradative pathways of these and related model compounds in cultures of the white-rot fungus *Phanerochaete chrysosporium* Burds. have been partially elucidated (*9*–*12*). An oxidative C-C bond cleavage, which initiates the degradation of **1** (Fig. 1A) and related structures in cultures (*9*–*11*), has been described. We report the discovery of an extracellular enzyme from *Phanerochaete chrysosporium* which, in the presence of added H_2O_2, catalyzes that cleavage, not only in **1**, but also in compound **2** and in spruce and birch lignins.

The enzyme activity was detected by incubating **1** (*13*), in the presence of added H_2O_2, with the concentrated extracellular fluid from 6-day-old ligninolytic cultures (*14*); this compound (**1**) was cleaved between C-1 and C-2, with formation of vanillin methyl ether (**3**) from the C-1 moiety, and 1-(3′,4′-dimethoxyphenyl)ethane-1,2-diol (**4**) from the C-2 portion (Fig. 1A). The ^{14}C-labeled products were extracted and identified by coelution, after isolation by thin-layer chromatography (TLC), with unlabeled standards on TLC plates (*15*). They are the same products formed initially in intact cultures (*9*). Both intact cultures (*9*–*11*) and the reconstituted system (concentrated culture fluid + H_2O_2) further cleave the diol product (**4**) to form the aldehyde **3**, and both also oxidize diol **4** to ketol **5** as a minor reaction (Fig. 1A). Thus **3** is produced from both aromatic moieties.

The reconstituted system was active also against model compound **2** (*16*), which differs from **1** in having an aryl ether rather than an aryl substituent at C-2 (Fig. 1C). Like **1**, compound **2** is

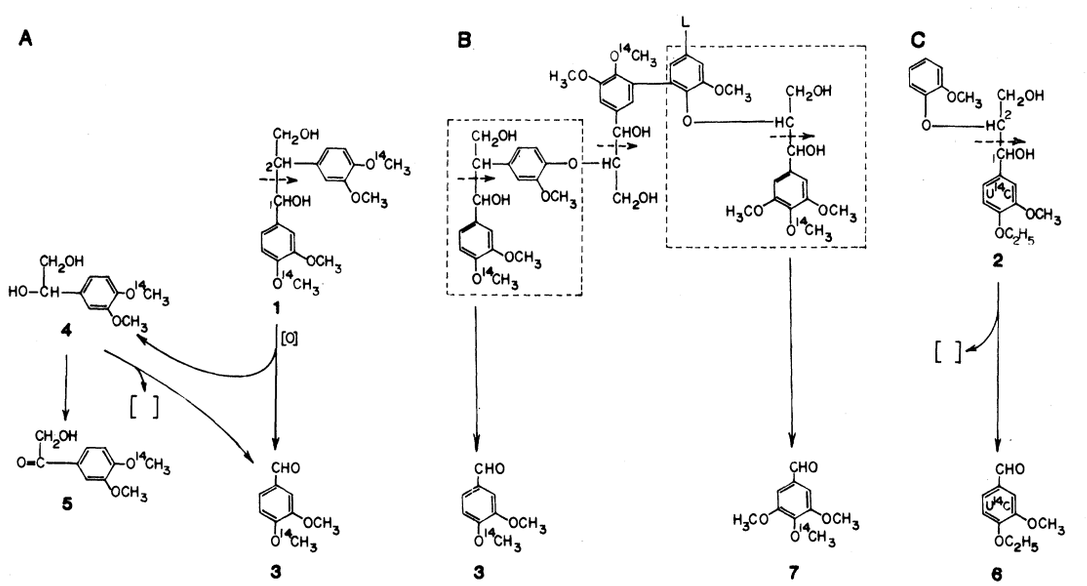

Fig. 1. A portion of a lignin macromolecule and substructure model compounds **1** and **2**. The fungal degradation products are shown. (A) Model compound **1**. (B) Lignin with phenolic hydroxyl groups ^{14}C-methylated. Dashed boxes enclose the 1,2-diarylpropane (left) and arylglycerol-β-aryl ether (right) substructures in the lignin corresponding to model compounds **1** and **2**. *L*, continuation of lignin polymer. (C) Model compound **2**. Dashed arrows indicate sites of C-C bond cleavages. Empty brackets in (A) and (C) indicate unidentified degradation products.