background of spontaneous afferent impulses reaching the cortex from the retina. This background activity may be inhibitory or excitatory, or both, and the changes in the cortical response might thus result from elimination of tonic inhibitory or occlusive influences, or both. It is also possible that the lesions alter cortical excitability secondarily, as a result of an alteration of excitability of the central structures (*6*) to which the slower-conducting optic nerve fibers project. These structures may, in part, mediate the prolonged effects of the conditioning volley on the test response.

Regardless of what mechanism is ultimately shown to operate, the present experiments indicate the large extent to which the recovery processes of the primary visual cortex depend upon impulses reaching the central nervous system from the peripheral visual apparatus.

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Utilization of Porphobilinogen Carbon-14 in Biosynthesis of Vitamin B_{12}

The structural formula of vitamin B_{12} proposed by Hodgkin (*1*) and by Bonnet (*2*) and their colleagues focused attention on the probable presence of a cobalt-binding porphyrin-like moiety in the molecule. Despite its unique character, the tetrapyrryl ring structure indicated the likelihood that common pyrrol precursors for this vitamin and for the various naturally occurring porphyrins were present. In the George R. Minot lecture (*3*) in June 1956, mention was made of our observation that porphobilinogen, the monopyrrolic precursor of uro-, copro-, and protoporphyrin, is readily utilized in the formation of vitamin B_{12} by a bacterial culture. At about the same time Shemin *et al.* (*4*) reported the incorporation of δ-amino-levulinic acid into vitamin B_{12} under similar conditions.

Table 1. Recovery of vitamin B_{12}-C^{14} from a bacterial culture incubated with porphobilinogen-C^{14}. Background count, 22.0 ± 0.6 count/min for experiment No. 1 and 26.4 ± 0.7 and 27.6 ± 0.7 count/min, respectively, for experiments No. 2 and 3 (60 min each). A correction factor was applied to correct for self-absorption of NaOH used as solvent and for differences in background counts.

	Expt. 1	Expt. 2	Expt. 3
Porphobilinogen added (mg)	12.6	22.5	22.2
Vitamin B_{12} produced (mg)	0.15	1.29	1.47
Carrier added (mg)	10.1	10.2	8.83
Carrier + B_{12} recovered (mg)	6.91	5.41	2.98
Amt. in planchet (mg)	0.33	0.216	0.119
Duration of count (min)	120.	300.	300.
Count/min above background	23.1 ± 0.6	20.4 ± 0.4	7.6 ± 0.4
Count/min corrected	38.4	28.9	10.2
Count/min mg of B_{12} produced	8000.	1200.	600.
Count/min μmole of B_{12} produced	10800.	1630.	810.
Count/min μmole of B_{12} per C^{14} carbon atom	1540.	233.	116.

Carbon-14 labeled porphobilinogen was prepared as follows: Two rabbits were treated with allyl-isopropyl-acetylcarbamide (Sedormid) for 10 days. Each rabbit then received a total of 160 μc of glycine-2-C^{14} subcutaneously in five divided doses over a 36-hour period. The urine was collected during this period and for the next 6 days. The pooled 975 ml of urine contained 310 mg of porphobilinogen as determined by quantitative analysis of the Ehrlich aldehyde reaction. The pooled urine was subjected to the method of Cookson and Rimington (*6*) for the isolation of porphobilinogen; 102 mg of crystalline porphobilinogen were obtained.

Some crystalline material was dissolved in H_2O and evaporated on a planchet for radioactivity measurement. A specific activity of 6300 count/min per milligram was observed. Solubility properties and the intensity of the Ehrlich aldehyde compound were similar to those found with repeatedly recrystallized porphobilinogen isolated from urine of patients with acute intermittent porphyria.

Ninety milligrams of the crystalline compound were placed in an evacuated and sealed ampule and sent to the Merck Laboratory for further study. Here the crystalline porphobilinogen was dissolved in 0.1*N* sodium hydroxide and sterilized by filtration before being added to the sterile nutrient medium (*4*). It was incubated with the bacterial culture in three separate experiments. In the first study, 12.6 mg (56 μmole) of porphobilinogen was added at time 0; in each of the other studies, a total of 22 mg (97 μmole) was added to three divided portions, at time 0 and again after 1 and 2 days' incubation, respectively. The broths were all harvested after 4 days. The vitamin B_{12} content of the broth was assayed microbiologically. Carrier B_{12} was then added to facilitate isolation. Radioactivity measurements were performed with a windowless gas-flow counter, with suitable correction made for self-absorption of the samples evaporated on a steel planchet.

The observed radioactivity of the porphobilinogen at the Merck Laboratories was 6900 count/min per milligram or 1560 count/min per micromole. This corresponds to an activity of 780 count/min per micromole for each of the two labeled carbon atoms in the molecule (*7*).

As is shown in Table 1, the calculated activities of the vitamin B_{12} produced ranged from 810 to 10,800 count/min per micromole of vitamin B_{12} produced. If one assumes labeling of seven carbon atoms in the vitamin corresponding to the two labeled carbon atoms in porphobilinogen (*7*) (one alpha carbon atom in each of the four pyrrole rings and three bridge carbon atoms), it would appear that approximately one-third and one-half of the porphyrin-like moieties were derived from the added porphobilinogen-C^{14} in experiments 2 and 3, respectively. In the first experiment, however, the radioactivity recovered is greater than can be accounted for on the basis of the observed production of only 0.15 mg of vitamin B_{12}. It is evident that the many possible additive errors inherent in such a calculation do not permit precise estimation of the dilution factor involved in the incorporation of the added porphobilinogen C^{14} into vitamin B_{12}.

The present result, coupled with that of Shemin and his coworkers with δ-aminolevulinic acid, clearly reveals that the biosynthesis of the porphyrin-like moiety of vitamin B_{12} is along the same primary pathway as that of other naturally occurring porphyrins. The

point of divergence in the pathway, beyond porphobilinogen, remains to be determined. It appears unlikely that uro- or coproporphyrin is involved, as this would require partial degradation—that is, removal of the δ-methene bridge to provide the curious C=C linkage in the porphyrin-like moiety of B_{12}. Nevertheless it would be of interest to determine whether uroporphyrinogen III might be utilized in B_{12} synthesis, as it is in that of the hemoglobin protoporphyrin (*8*).

While vitamin B_{12} is essential to normal erythropoiesis and resembles the heme compounds to the extent of having a porphyrin-like group in its molecule, there is no evidence that vitamin B_{12} deficiency is associated with diminished porphyrin or bile pigment formation. As discussed elsewhere (*9*), the available evidence indicates that in pernicious anemia there is plentiful formation of pyrrol pigment.

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30 October 1958

Ecological Significance of Red Light Sensitivity in Germination of Tobacco Seed

Abstract. The light transmission of different soil materials was measured in a Beckman spectrophotometer. The relative energy transmission was greatest at the red end of the spectrum. A seed sensitive to red light will have the capacity to germinate at nearly the maximum depth of penetration by visible light, where the risk of early desiccation is diminished.

It has been established that red light is much more effective than light of shorter wavelength in promoting germination of some light-requiring seeds, the blue end of the spectrum being least effective (*1*). Investigation of the light requirement of seeds of the tobacco, *Nicotiana trigonophylla* Dun., gave similar results in the present study.

If a deductive approach may be taken, the question is: What is the ecological significance of red light sensitivity for seed germination? We know that the amount of diffraction or scattering of light by small particles or openings varies inversely as the fourth power of the wavelength; therefore it seems likely that fine soil particles, and especially the finer interstices between them, might scatter blue light more than red. This means that red light would penetrate the soil to a greater depth than blue light, and that below the soil layer where the first scattering takes place (the surface), the penetrating light will be impoverished in the blue end of the spectrum compared with the incident light.

An investigation was made of the transmission spectrum of a medium-coarse quartz sand and of a silty clay, with the Beckman DU spectrophotometer. It was found that a 5-mm thickness of wet silty clay gave zero light transmission at all wavelengths measured, even when a blank was used which reduced the slit area, and hence the level of the blank signal relative to the sample signal. Therefore it was necessary to use a dilute suspension, and the blank for this sample was 5 mm of distilled water. The wet sand was more translucent than the silty clay, but it was necessary to use an arbitrary blank which reduced the slit area, since with the usual slit openings, the blank gave such a high signal relative to the sample signal that the latter registered zero transmission at all wavelengths. This blank was a series of holes in black tape arranged in linear fashion, so that all fell within the slit image.

The transmission spectra of these soils, in the wavelength range from 400 to 800 mμ, are presented in Figure 1; a comparison of the ratios of percentage transmission of longer to shorter wavelengths is provided in Table 1.

Table 1. Ratios of percentage transmission of longer to shorter wavelengths.

Soil	Percentage transmission ratios	
	655 mμ/450 mμ	735 mμ/655 mμ
Sand, 10 mm	6.0	1.6
Sand, 5 mm	2.5	1.2
Clay suspension	1.9	1.1

It is evident that the relative energy transmission in the shorter wavelengths is smaller than in the longer wavelengths when light is passed through these materials. The greater loss in the blue end of the spectrum may be due to specific absorption and refraction effects in addition to diffraction.

Radiation of wavelength 735 mμ is reported to inhibit the germination of many light-sensitive seeds (*1*); and since 735-mμ light has a higher percentage transmission in soil than 655-mμ light (see Table 1), it may be inferred that there is some level below the soil surface where the inhibiting effects of far-red radiation will prevail. But since this level would be just above the zone of perpetual darkness, the only effect is to extend that zone upward, as far as light-sensitive seeds are concerned.

With regard to the ecology of the genus *Nicotiana,* an outstanding characteristic is the adaptation to the rapid, early invasion of pioneer or disturbed sites, free of dense vegetation (*2*). This mobility is due primarily to the enormous production of very minute seeds with rough, reticulate coats, which provide great powers of dispersal, whether due to the agency of wind or animals. On the other hand, the small seed carries with it the disadvantage of a small food reserve, which limits the depth of successful germination to the magnitude of the relatively small dark-elongation of the hypocotyl. Hence much seed would be lost by dark germination following slight burial were it not for the fact that the seeds of many of the species are light

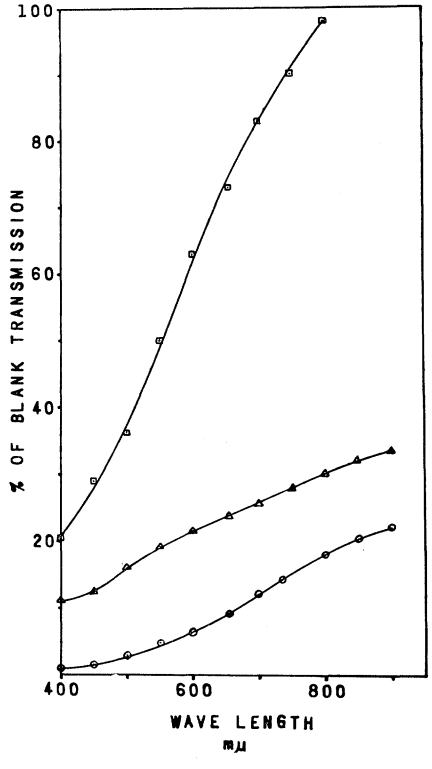

Fig. 1. Transmission spectra of quartz sand and a clay suspension. Top curve, quartz sand (5 mm); middle curve, quartz sand (10 mm); bottom curve, clay suspension (5 mm).