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 B₁₂. Nevertheless it would be of interest to determine whether uroporphyrinogen III might be utilized in B₁₂ synthesis, as it is in that of the hemoglobin protoporphyrin (8).

While vitamin B₁₂ 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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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,

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

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 mediumcoarse 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.

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-mu light has a higher percentage transmission in soil than 655-mu 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 lightsensitive 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).

requiring, which insures germination near the surface. However, the soil-air interface is a harsh environment. As the center of radiation exchange, it undergoes the most rapid and extreme temperature changes, and with regard to the moisture factor, it is alternately bombarded by splattering raindrops and subjected to severe droughts. Therefore, any depth below the surface, however slight, will be an improvement from the standpoint of an environment for germination of seeds. If then, a seed is lightrequiring, it is of some survival value for it to be red sensitive, since this property will facilitate germination at nearly the maximum depth of penetration by visible light. Of course, this will not prevent the seed from germinating on or near the surface, where the risk of early desiccation is greatest; it merely confers on the seed the capacity to germinate where this risk is diminished.

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Critical Period for Effects of Infantile Experience on Maturation of Stress Response

Abstract. Manipulated infant rats respond to cold with depletion of adrenal ascorbic acid (AAA) significantly earlier than nonmanipulated infants. The study discussed in this report examined the critical period for infantile manipulation on the depletion of AAA. It was found that infant rats manipulated immediately following birth exhibited significant AAA depletion, whereas infants manipulated later did not exhibit depletion.

Recently it has been reported (1) that infant rats which had been manipulated (handled) once daily from birth responded to cold stress with a significant depletion of adrenal ascorbic acid as early as 12 days of age, whereas nonmanipulated infant rats did not show significant AAA depletion until 16 days of age. One question which arose from this study was whether the age at which the experimental treatment of manipulation was initiated is a significant factor in the accelerated maturation of the systems which result in AAA depletion with stress.

The experiment discussed in this report (2) was directed, therefore, toward answering the question of whether there



Fig. 1. Comparison of depletion in AAA in the various groups of infant albino rats of the study. The bar represents the mean depletion; the lines, the range. The dotted bar and dotted line represent untreated animals that had previously been tested.

exists a critical period in the development of the organism during which manipulation has its greatest effect on the AAA depletion response to stress. The existence of such a period seemed likely, since critical periods have been documented for many other aspects of development (3).

Seventy-six infant Sprague-Dawley albino rats were used as subjects. The subjects were assigned at birth to one of four groups. For the infants in group I (N = 20), the treatment was initiated on the second day following birth and continued through day 5. The treatment was started on day 6 and was continued through day 9 for group II infants (N = 20). The treatment for the group III subjects (N = 20) was given from day 10 through day 13. The last group, group IV, received the treatment from day 2 through day 13. The experimental treatment was identical to that previously described (1) and consisted of removing the pup from the nest, placing it in a 2.5- by 3.5- by 6-in. compartment for 3 minutes, and then returning it to the nest. This procedure was followed once daily during the period assigned to the subject. At 14 days of age, approximately half the pups within each group were randomly assigned to either the stress or control condition to test for AAA depletion with stress.

The stress conditions and method of analysis for AAA are fully described in previous reports (1) and, therefore, will be only briefly described here. The nonstressed subjects within each group were killed by cervical spinal separation and weighed. The adrenals were removed, weighed, and assayed for AAA by the modified method of Glick *et al.* The stressed infants were subjected to a cold stress of 5°C for 90 minutes before removal of the adrenals and determination of AAA.

The results of this experiment are shown in Fig. 1 and are expressed in terms of milligrams percent change in AAA level. Change in AAA level was determined by subtracting the AAA present in the stressed animals from the mean for the nonstressed subjects.

The data clearly indicate that the age during which the infant rat is manipulated is a major variable in the effect described in this report. Only the animals in groups I and IV showed significant AAA depletion. In terms of percentage, the group I subjects showed a 25-percent depletion and the group IV subjects showed a 32-percent depletion. The depletion in AAA in the group II and group III animals (9 percent and 0 percent, respectively) did not differ significantly from that in the respective controls. Thus, in the groups (I and IV) which had been manipulated during the period directly following birth, a significant depletion in AAA is evidenced in response to cold stress at 14 days of age, whereas the groups manipulated later in infancy do not show significant AAA depletion.

Recent evidence has indicated that the early postnatal period is also critical for behavioral changes during adulthood.