the following year without further application of 2,4-D. The substitution of fluorine in 2,4-D would appear to make it equally stable in varieties that otherwise decarboxylate it.

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3 April 1961

Variation in Clones of Penstemon Growing in Natural Areas of Differing Radioactivity

Abstract. A study to determine whether or not the relatively high radioactivity of a natural area has produced detectable morphological effects on plants of the species *Penstemon virens*, a plant of rather plastic morphology, has been conducted in the Central City region of Colorado for over 2 years. While no differences in kinds of anomalies were found to exist between plants growing in the sites of highest and lowest radioactivity, the number of anomalies was greater in plants from sites of greatest radioactivity.

A study was made of 26 clones of *Penstemon virens* growing along the crest of a dike of nonporphyritic quartz bostonite. The ground-surface gamma radioactivity varied from 0.05 to 0.40 mr/hr. According to Phair (*1*), this dike may be the most radioactive igneous rock known on the North American continent. Except in radioactivity, the microenvironments of the clones appeared to be quite similar.

Plant organs that are commonly known to be radiosensitive were examined and measured. The total heights and internodal lengths were measured on 206 flowering spikes; stage of development was determined on more than 4000 floral elements; and the total numbers of nodes, peduncles, and anomalies such as those shown in Fig. 1 were counted for each spike. Simultaneously the gross radiation environment of each clone was determined with a portable Geiger counter, and by securing segments of flowering spikes and selected samples of the adjacent soil for radioassay.

Preliminary analysis of the field data indicate several differences between clones: The range of morphological variability was greater, the percentage of aborted flowers was larger, the numbers of nodes per flowering spike were less, and the number of gross anomalies was higher in clones from the most radioactive sites; however, the average height, numbers of floral elements (buds, flowers, and fruit), and numbers of peduncles of the flowering spikes were very nearly the same in all sites.

Several hypotheses to explain the differences can be suggested: The differences may result from (i) chance, (ii) errors in measurement, (iii) slight variation in microenvironment (other than radioactivity), and (iv) variations in radioactivity. The first two hypotheses can be disregarded because of the large number of comparative measurements made. The several thousand measurements are the aggregates of measurements made by eight to ten different people, and approximately 75 percent of the measurements were independently double checked. Data placed in questionable and arbitrary categories (such as "immature or aborted") were disregarded.

Although the clones all appeared to be growing in similar microenvironments, the possibility exists that very subtle differences in amounts of light, temperature, insect damage, and moisture during critical periods of plant growth and differentiation could account for the differences between clones. It is known that gene action, even in the uniform environment of a single clone, can vary in expression. However, one observation suggests that this is not the reason for the differences in numbers of anomalies between the clones in this case. A study, in 1959, of *Penstemon* growing in a wide range of habitats provided evidence that soil drought, excessively high temperatures, and other environmental factors can account for various "anomalies" but that one of the first general effects of such factors is that the plant is somewhat stunted. In the present study the clones growing in the more radioactive sites were as tall as those in the less radioactive sites. Furthermore, clones from the habitats examined in 1959, including those of the extremely dry and exposed rock outcrop, never showed as many anomalies as the clones from the most radioactive habitats.

There is a final consideration. The radioactivity of the substrate is high compared with that of other natural areas, but alone it would probably be regarded by most radiobiologists as too low to cause detectable morphological

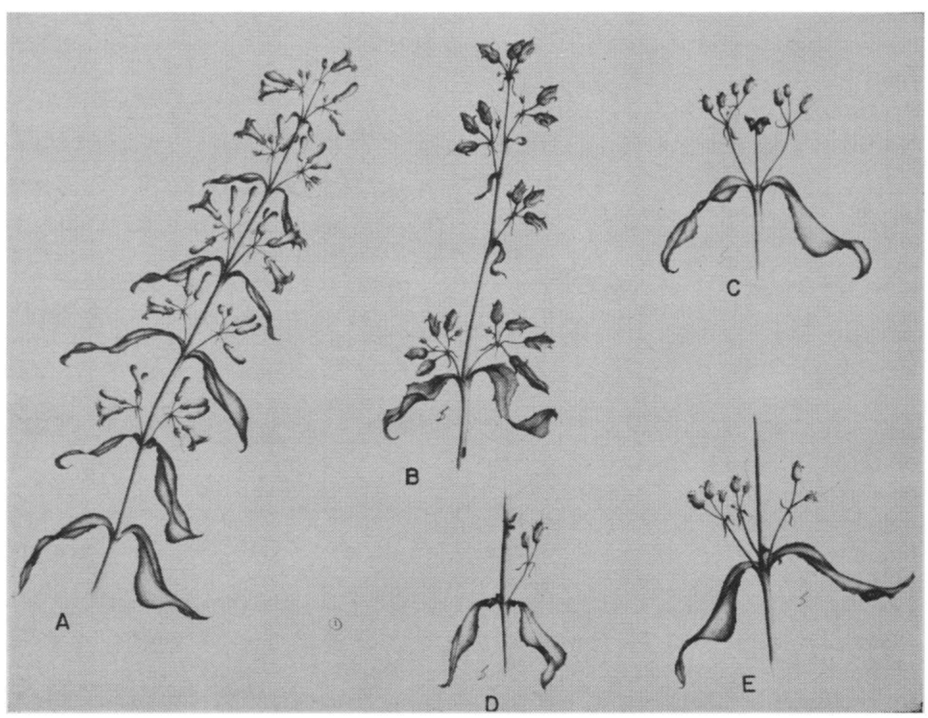

Fig. 1. (*A*) Typical flowering spike of *Penstemon virens*. Note opposite leaves, one axillary peduncle on each side of node, absence of aborted buds, and regularity of internodal lengths. (*B*) Spike has opposite and alternate arrangement of leaves and several aborted buds near the top. (*C*) Terminal development incomplete. (*D*) Blocked development of one peduncle and fragmentary nodal development. (*E*) Extra peduncle.

effects. In ecology there is a commonly accepted principle which is worthy of serious consideration in this situation: If conditions are less than optimum for an organism with respect to one ecological factor, the sensitivity of the organism to other factors may be increased. There is ample evidence that some environmental "stress" factors, especially suboptimum amounts of oxygen and abnormal temperatures, increase the sensitivity of plants to radioactivity (see *2*). Therefore, at the present time the most tenable hypothesis appears to be that background radioactivity, acting in conjunction with other suboptimum environmental factors, is responsible for the greater incidence of anomalous morphological forms (*3*).

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13 April 1961

Enzymatic Formation of Psychotomimetic Metabolites from Normally Occurring Compounds

Abstract. An enzyme has been found that N-methylates serotonin and tryptamine to psychotomimetic metabolites, bufotenine and N,N-dimethyltryptamine. This enzyme is highly localized in the rabbit lung and also N-methylates phenylethylamine derivatives such as tyramine, phenylethylamine, mescaline, and dopamine.

In recent years, there has been an active search for a biochemical cause of mental disease. Several compounds have been reported to be present in the body which produce abnormal behavior, but in rigorously controlled studies none of these findings have been confirmed (*1*). Cohoba, a snuff obtained from *Piptadenia peregrina,* has been used by Indian tribes of Haiti to enable them to communicate with unseen powers. Recently, bufotenine (N,N-dimethylserotonin) and N,N-dimethyltryptamine have been found to be present in *Piptadenia peregrina* (*2*), and both of these compounds have been shown to produce psychotomimetic effects in man (*3*). I wish to report that an enzyme has been found in the rabbit lung that can convert the normally occurring compounds, serotonin and tryptamine, to the psychotomimetic metabolites, bufotenine and N,N-dimethyltryptamine.

Table 1. Enzymatic N-methylation of indoleamines. Rabbit lung was homogenized with three volumes of isotonic potassium chloride and centrifuged at 80,000 *g* for 30 min. Soluble supernatant fraction obtained from 15 mg of rabbit lung was incubated at 37°C with 10 mμmole of S-adenosylmethionine-methyl-C^{14}, 100 μmole of phosphate buffer at *p*H 7.9, and 0.5 μmole of substrate. After 90 min of incubation, the methyl-C^{14} indoleamine was determined in the incubation mixture (*4*).

Substrate added	Product formed	
	Name	Amount (μg/g of tissue)
Serotonin	N-Methylserotonin	80
N-Methylserotonin	Bufotenine	56
Tryptamine	N-Methyltryptamine	71
N-Methyltryptamine	N,N-Dimethyltryptamine	20

When the methyl donor S-adenosylmethionine-methyl-C^{14} (C^{14}AMe) was incubated with the soluble supernatant fraction of rabbit lung, a normally occurring compound present in this tissue became radioactive. In trying to characterize this substance, it was found that, when serotonin was incubated with the soluble supernatant fraction of rabbit lung and C^{14}AMe, considerable amounts of a radioactive compound were formed. This derivative could be extracted into isoamyl alcohol at *p*H 10. After chromatography with three solvent systems—butanol, ethanol, and ammonia (8:2:1), *n*-propanol and ammonia (1*N*) (5:1); and isopropanol, ammonia, and water (16:1:3)—the radioactive compound formed from serotonin and C^{14} AMe had the same R_F values as authentic N-methylserotonin. When N-methylserotonin was incubated with C^{14}AMe and the rabbit lung enzyme preparation, a compound was formed having the same R_F values as bufotenine in several solvent systems. In addition, typtamine and N-methyltryptamine, when incubated with C^{14}AMe and the enzyme from rabbit lung, formed radioactive metabolites having the same R_F values as N-methyltryptamine and N,N-dimethyltryptamine, respectively, when chromatographed on Whatman No. 1 paper buffered at *p*H 8.0, with *n*-butanol or isoamyl alcohol saturated with water as the solvent systems. These compounds were isolated from the enzymatic reaction mixture by extraction into isoamyl alcohol at *p*H 10. The relative rates of enzymatic formation of the N-methylated derivatives are shown in Table 1. These observations were taken as evidence that serotonin and tryptamine are converted to their corresponding N-dimethyl derivatives by an enzyme present in the soluble supernatant fraction of the rabbit lung in two steps as follows:

$$\text{serotonin} \xrightarrow{\text{AMe}} \text{N-methylserotonin} \xrightarrow{\text{AMe}} \text{bufotenine}$$

$$\text{tryptamine} \xrightarrow{\text{AMe}} \text{N-methyltryptamine} \xrightarrow{\text{AMe}} \text{N,N-dimethyltryptamine.}$$

This enzyme was also found to N-methylate other phenylethylamine derivatives such as phenylethylamine, tryamine, mescaline, and dopamine (*4*); and it appeared to differ from other N- and O-methyltransferases with regard to substrate specificity and distribution (*5*).

Recently, Pollin *et al.* (*6*) found that the repeated oral administration of large amounts of *l*-methionine and *l*-tryptophan caused marked alterations of behavior in schizophrenic patients. Both of these amino acids can serve as precursors for the psychotomimetic compounds, bufotenine and N,N-dimethyltryptamine.

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6 April 1961