to the shoot. Factor I apparently can be converted to factor II by hydrolysis. The significance of these root factors to shoot metabolism is discussed, especially with regard to the influence of the root environment on the supply of these hormones.

The author is grateful to Dr. H. W. Johnson, USDA, Beltsville, for the supply of soybean seeds var. Acme, and to Dr. Y. Vaadia for the many helpful discussions.

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EVIDENCE FOR THE LOCALIZATION OF PLASTOCYANIN IN THE ELECTRON-TRANSPORT CHAIN OF PHOTOSYNTHESIS

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A reversible light-induced absorbance change near 600 mμ was observed recently in green plants and attributed to the functioning of the copper protein plastocyanin.[1] This light-induced increase of absorbance was linked to oxidation of plastocyanin. Since red and far-red actinic illumination had antagonistic effects on this change, it was suggested that plastocyanin served as a link between the two photochemical systems of photosynthesis. Far-red light (713 mμ) absorbed

largely by the long-wavelength forms of chlorophyll (system I) caused oxidation of plastocyanin while red light (651 mμ), absorbed by chlorophyll *b* and the short-wavelength absorbing forms of chlorophyll *a* (system II), brought about much less oxidation or even transitory reduction.

Trebst[2] has also suggested that plastocyanin functions as an intermediate in the electron-transport chain of photosynthesis. He found that salicylaldoxime, an inhibitor of copper-containing enzymes, stopped NADP reduction, oxygen evolution, and ATP formation. Photosynthetic NADP reduction could still take place in the presence of this inhibitor if dichlorophenol indophenol and ascorbate were supplied.

It was previously shown[1] that the complexing agent of copper, diethyldithiocarbamate, inhibited the plastocyanin absorption change measured at 591 mμ. We therefore continued this line of investigation to examine the effects of salicylaldoxime on the 591-mμ as well as on the 419-mμ (cytochrome *f*) absorbance changes. The 591-mμ absorbance change was inhibited completely after a short incubation in salicylaldoxime, while oxidation of cytochrome was not. However, the light-dependent reduction of cytochrome was inhibited by salicylaldoxime.

In a later investigation Trebst and Pistorius[3] showed that DCMU inhibition of NADP reduction could be relieved if certain N-substituted phenylenediamines, reduced by excess sodium ascorbate, were provided. With DAD plus ascorbate, ATP was formed along with reduced NADP. However, TMPD plus ascorbate allowed only NADP reduction. It therefore appears that these compounds feed electrons into the transport chain at different places.

We used the DAD + ascorbate donor system with DCMU-treated samples in an attempt to localize the site of plastocyanin function. With this system the absorbance change of plastocyanin at 591 mμ, inhibited with DCMU, could be restored. However, this system was ineffective for the restoration of the 591-mμ change inhibited by salicylaldoxime.

These results indicate that plastocyanin functions in the electron-transport chain of photosynthesis between the two light reactions and before cytochrome *f*.

Methods and Materials.—Light-induced changes of absorbance were measured as previously described.[1] The changes were recorded on a Massa oscillographic recorder, model BSA-250A.

Far-red actinic light (713 mμ), used to excite preferentially system I of photosynthesis, was isolated with a Bausch and Lomb interference filter and a Corning red cutoff filter (CS No. 2-58). This combination provided a monochromatic beam with a half-band width of about 9 mμ. The intensity of the 713 mμ used in all these experiments was 3.74×10^4 ergs cm^{-2} sec^{-1}. A broad-band actinic red beam, having wavelengths longer than about 625 mμ, was used to excite both systems I and II. This light was isolated by using the Corning filter CS no. 2-58 alone. The energy of this beam in the region from about 625 to 900 mμ was about 2.51×10^6 ergs cm^{-2} sec^{-1}. This intensity was used when Ulva was illuminated with "red" light. The actinic beams were provided by a 118-v, 625-w quartz-iodine lamp (Sylvania type DWY) and filtered through 27 mm of water.

The marine green alga *Ulva lobata* used in these experiments was collected at Half Moon Bay, California, and maintained in the laboratory as described before.[1] All experiments reported here were done using one disk of the algal thallus (2 cell

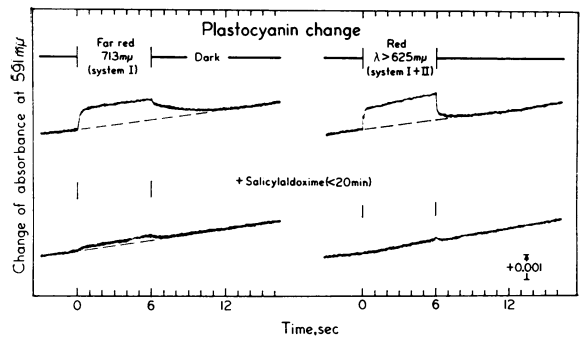

FIG. 1.—The effect on the 591-mμ absorbance change of a short incubation in 10^{-2} *M* salicylaldoxime in sea water in *Ulva lobata*. The far-red exposure (3.74×10^4 ergs cm^{-2} sec^{-1}) was made 18.7 min after adding salicylaldoxime, and the red (2.51×10^6 ergs cm^{-2} sec^{-1}) after 18.0 min. Temp., 20°C; gas phase, air.

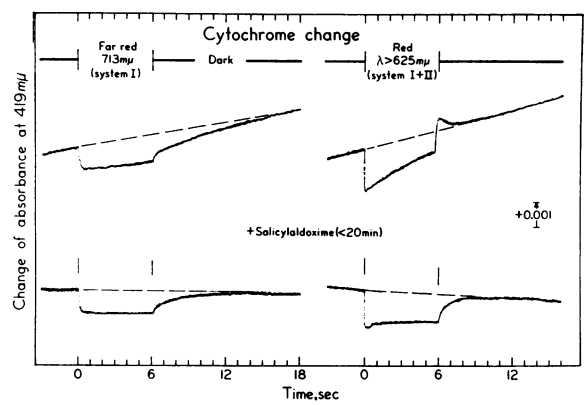

FIG. 2.—The effect on the 419-mμ absorbance change of a short incubation in 10^{-2} *M* salicylaldoxime in sea water in Ulva. The exposure to far-red light was made 15.0 min after adding salicylaldoxime and 12.0 min after for the red light. The same sample of Ulva produced the traces given in Figs. 1 and 2.

layers thick) in 10 ml of sea water equilibrated with air at about 20°C. Added inhibitors and dyes were each dissolved in 0.1 ml 95 per cent ethanol. Ethanol alone at the concentrations used was without effect on the absorbance changes.

Results.—Effect of salicyladoxime on 591- and 419-mμ absorbance changes: The upper part of Figure 1 shows the absorbance changes which occurred when Ulva was illuminated with red or far-red actinic light. A positive absorbance change at 591 mμ results from plastocyanin oxidation. The initial rate of rise, including a small spike of plastocyanin oxidation, was larger with red than with far-red actinic light. This may be attributed to the much higher intensity of light in the broad red beam absorbed by system I. As observed previously,[1] the decay of the 591-mμ absorbance change was much faster after red light than after far red ($t_{1/2}$ = 0.10 instead of 1.56 sec). Incubation for a short time in sea water with 10^{-2} *M* salicylaldoxime strongly inhibited plastocyanin absorbance changes as shown in the lower part of Figure 1.

We observed absorbance changes at 419 mμ before the addition of salicylaldoxime

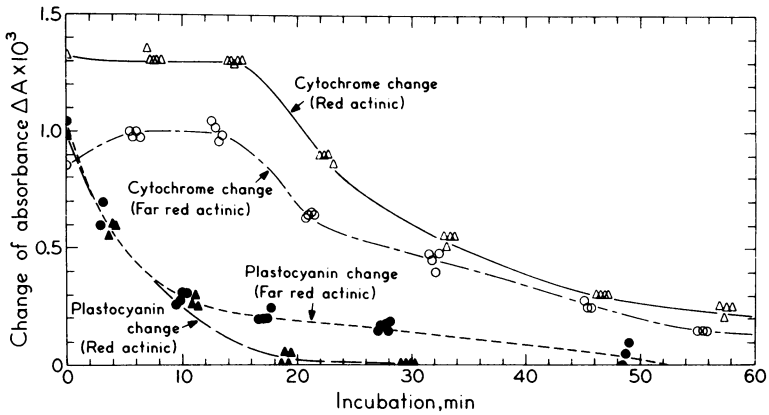

FIG. 3.—The inhibition of the 591- (plastocyanin) and the 419-mμ (cytochrome *f*) absorbance changes in Ulva as a function of the time of incubation in 10^{-2} *M* salicylaldoxime.

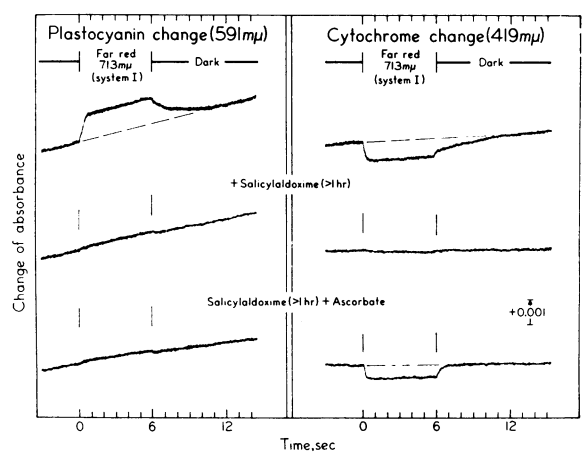

FIG. 4.—The inhibiting effect of a long incubation in salicylaldoxime on the 591- and the 419-mμ absorbance changes and the restoration of cytochrome oxidation by sodium ascorbate. The absorbance change at 591 mμ in Ulva was observed after 71.4 min and the 419-mμ change after 85.8 min incubation of the same sample of Ulva in 10^{-2} *M* salicylaldoxime. Sodium ascorbate was added to a concentration of 5.1×10^{-3} *M*.

in the same disk of Ulva which was used for Figure 1. As shown in the upper part of Figure 2, preferential excitation of system I with 713 mμ light caused a decrease in absorbance at 419 mμ which corresponds to cytochrome oxidation.[4, 5] In "darkness" (measuring beam still on) the oxidized cytochrome is slowly reduced to its former level. Excitation of both systems with the strong red light gave a more rapid and larger initial negative deflection probably for the same reason mentioned above that a higher intensity of light in the red beam is absorbed by system I. In contrast to the 713-mμ exposure, a slow reduction during the 6-sec red-light exposure was seen after the initial rapid oxidation. The decay after the red exposure was faster than after the far red ($t_{1/2} \cong 0.1$ instead of 2.50 sec). After a red exposure there was a temporary overshoot (reduction) followed by return (oxidation) to the former dark level.

Incubation in salicylaldoxime for a short time inhibited the fast decay and overshoot in the dark, as well as the reduction during a red-light exposure (Fig. 2, lower part). By contrast, cytochrome oxidation excited by 713-mμ light was little affected by salicylaldoxime for periods up to about 20 min after its addition.

Time course of salicylaldoxime inhibition for 591- and 419-mμ absorbance changes: Figure 3 shows the different sensitivities of the plastocyanin and the cytochrome changes to incubation with 10^{-2} M salicylaldoxime. Inhibition of the plastocyanin change was more rapid than inhibition of the cytochrome change. Figure 3 shows that about 13 min elapsed before there was an appreciable inhibiting effect of salicylaldoxime on the 419-mμ cytochrome change. Incubation for more than an hour inhibited both changes.

Restoration of salicylaldoxime-inhibited cytochrome change by sodium ascorbate: Figure 4 shows the complete inhibition of the plastocyanin and cytochrome changes after an incubation in salicylaldoxime longer than an hour. Addition of sodium ascorbate partially regenerated the cytochrome change but not the plastocyanin change. The steady-state cytochrome absorbance change regenerated by ascorbate was smaller ($\Delta A = 5.5 \times 10^{-4}$ instead of 8.8×10^{-4}), but the dark decay was faster ($t_{1/2} = 0.24$ instead of 1.76 sec) than without ascorbate.

Inhibition by DCMU of the 591-mμ absorbance change and its restoration by DAD and ascorbate: Figure 5 shows that DCMU at a concentration of 10^{-4} M inhibited the light-induced increase of absorbance at 591 mμ in both red and far-red actinic lights. The light-induced oxidation of plastocyanin could be restored by the addition of DAD plus ascorbate. A 72 per cent restoration of the original change of absorbance was seen with far red and 71 per cent with red light. About 95 per cent of the 591-mμ change has been restored in some experiments. After restoration by the DAD + ascorbate treatment, the dark decay times were about the same ($t_{1/2} = 0.24$ sec after far red and 0.35 sec after red actinic light).

Effects of DCMU and DAD + ascorbate on 419-mμ cytochrome change: The effects of 10^{-4} M DCMU on the cytochrome change are shown in the middle traces of Figure 6. The same disk of Ulva was used for Figures 5 and 6. Less oxidation of cytochrome was produced by far-red light after DCMU treatment ($\Delta A = 5.5$ instead of 9.2×10^{-4}). The fast decay ($t_{1/2} = 0.06$ sec) previously seen after a 6-sec red light exposure was dramatically inhibited by DCMU. The initial negative 419-mμ spike was also smaller ($\Delta A = 8.9$ instead of 12.9×10^{-4}). After DCMU inhibition a slow increase of absorbance during the light persisted. The reason for this slow increase is not clear. Addition of DAD and ascorbate resulted in smaller steady-state cytochrome oxidation in 713-mμ light, and accelerated dark decay. The fast dark decay after red light was seen again in DAD + ascorbate. The marked increase of absorbance during the red light exposure seems to be the result of an absorbance change of the dye itself. DAD has a faint yellow-green color, and when added to sea water alone at the concentration used in these experiments (6.6×10^{-5} M) caused no measurable absorbance change. Subsequent addition of excess sodium ascorbate to this solution of DAD also gave no detectable change. Nevertheless, when Ulva was incubated in these concentrations of DAD and ascorbate for at least 10 min and illuminated with bright red light, a dye change began to appear. This change was characterized by a slow increase of absorbance in the

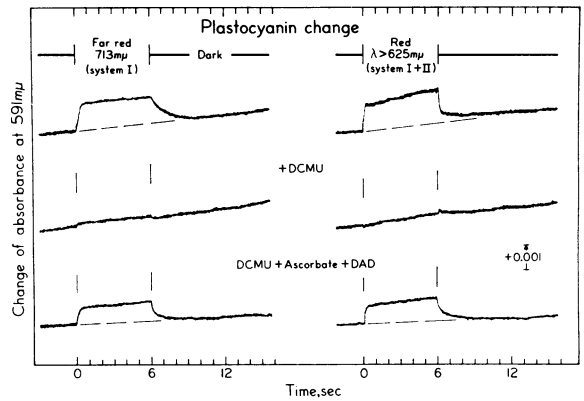

FIG. 5.—The inhibition of the 591-mμ absorbance change by DCMU in Ulva with red and far-red actinic lights and the restoration by DAD plus ascorbate. DCMU was added to a concentration of 10^{-4} M in the dark and allowed to incubate for 10 min. The concentration of DAD was 6.6×10^{-5} M and sodium ascorbate was 5.1×10^{-3} M. The DAD and ascorbate were incubated in the dark for about 5 min before the first actinic exposure was made (lower traces).

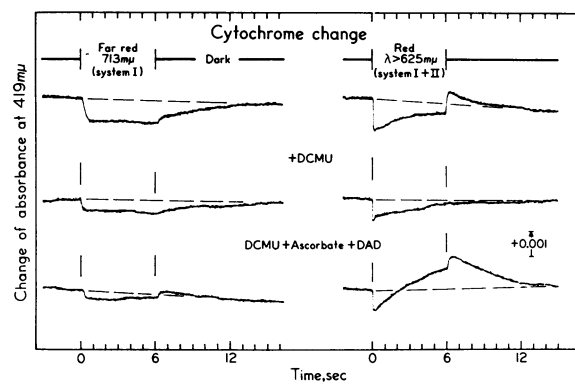

FIG. 6.—The effect of DCMU and DAD + ascorbate on the 419-mμ absorbance change in Ulva. DCMU was added in the dark to a concentration of 10^{-4} M. The middle traces were made after the sample had been incubated in DCMU for about 20 min. The lower traces were made after the sample was in DAD + ascorbate for about 9 min. The same sample of Ulva produced the traces given in Figs. 5 and 6.

light and a slow dark decay. The faster plastocyanin and cytochrome absorbance changes superimposed on the slower dye change could readily be distinguished.

The experiments showing the regeneration of the 591-mμ change by DAD + ascorbate (Fig. 5) were made soon after adding these substances and before a dye change could be detected. A further check that the plastocyanin change regenerated by DAD + ascorbate was real was made by looking for absorbance changes at 410, 440, and 448 mμ where, previously, no (or only very small) changes were detected with 713-mμ actinic light. If such a dye change occurred, we would have detected it at these wavelengths where the dye absorbs more strongly than at 591 mμ.

The fast dark decay of the cytochrome change which was seen after addition of the donor system cannot be attributed to a dye change because upon darkening, the dye change showed a decrease of absorbance. The reappearance of the fast dark

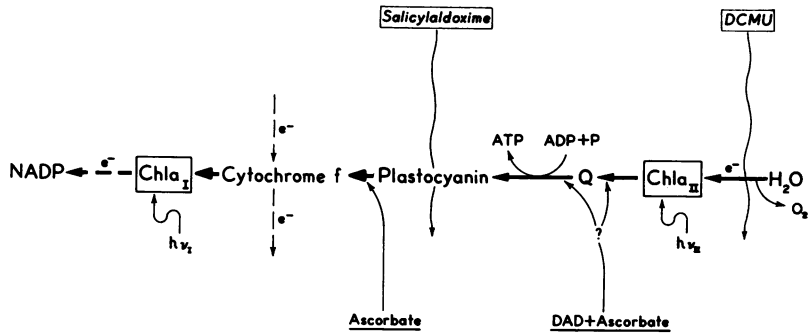

FIG. 7.—A diagram to show the presumed site of plastocyanin function in the electron-transport system of photosynthesis. *Q*, plastoquinone; other details in the text.

decay shows also that DAD + ascorbate regenerates the cytochrome as well as the plastocyanin change.

Discussion.—The copper-complexing reagent salicylaldoxime appears to inhibit specifically the 591-mμ absorbance change but not the negative 419-mμ change associated with cytochrome oxidation. The reduction of cytochrome seen during a red-light exposure (Fig. 2) and the subsequent, fast dark decay with a transient reduction overshoot above the former base line are almost completely inhibited by salicylaldoxime. Inhibition of plastocyanin functioning apparently also stops the flow of electrons to cytochrome coming from system II. These observations make it reasonable to place plastocyanin before cytochrome *f* in the flow of electrons coming from the second light reaction rather than in a side or parallel position (Fig. 7).

In the experiment shown in Figure 5, DCMU strongly inhibited the plastocyanin change. The loss of plastocyanin function was again accompanied by an impairment of cytochrome reduction during a red-light exposure and a loss of the rapid dark reduction afterwards (Fig. 6). DCMU had an effect similar to that of salicylaldoxime on the cytochrome change, which is another indication that plastocyanin functions in the electron flow from system II to system I.

DCMU apparently inhibits plastocyanin function indirectly. In order to be able to observe the absorbance change corresponding to plastocyanin oxidation, it must first be reduced. This reduction normally is mediated by electrons which are made available by the action of system II.[1] DCMU interrupts this reduction and in turn abolishes the plastocyanin change. However, the DCMU-inhibited plastocyanin change could be regenerated after the addition of the DAD + ascorbate donor system because DAD catalyzed the reduction of plastocyanin by ascorbate. Addition of ascorbate alone could not restore the plastocyanin change.

Regeneration of the plastocyanin change by the donor system also gave a parallel regeneration of the fast dark decay of cytochrome which was previously oxidized by red or far-red light. With the donor system the fast reduction reappeared after a red exposure and was similar to the trace observed before treatment.

The regeneration of both the plastocyanin and the cytochrome changes by the donor system after DCMU had blocked system II function indicates that electrons were coming from ascorbate via DAD. This can be seen from the similarity of the

dark decay times of the plastocyanin change after either red or far-red excitation (Fig. 5). The $t_{1/2}$ of the plastocyanin change after a red exposure was 0.14 sec (Fig. 5), but when chemical reduction by DAD + ascorbate substituted for reduction formerly mediated by system II, the $t_{1/2}$ was increased to 0.35 sec. By contrast, the dark decay time after a far-red exposure was accelerated from 0.94 to 0.24 sec.

It therefore appears that the DAD + ascorbate donor system is feeding electrons into the transport chain somewhere *after* system II and before plastocyanin (Fig. 7). Trebst and Pistorius[3] have suggested that the DAD + ascorbate system acts before a site of ATP formation because they could restore NADP reduction and ATP formation in DCMU-treated chloroplasts. When they used another substituted phenylenediamine, TMPD, plus ascorbate, Trebst and Pistorius could see no ATP formation but only NADP reduction. We also tried to determine the site of entry of electrons from the TMPD + ascorbate donor system in relation to the plastocyanin change. Unfortunately, we were not able to see the 591-mμ change itself because the oxidized form of the dye absorbs strongly in this region. We did observe, however, a reversible light-induced absorbance increase corresponding to TMPD photooxidation.

Incubation of the Ulva in salicylaldoxime for about an hour inhibited cytochrome oxidation. Salicylaldoxime appears to affect cytochrome indirectly because addition of ascorbate (which reduces cytochrome) served to regenerate light-induced oxidation. A similar regeneration of the salicylaldoxime-inhibited plastocyanin change by ascorbate was not seen (Fig. 4).

Apparently, electrons are available to reduce cytochrome from a source which is independent of system II. We always observed a dark reduction of cytochrome when the 591-mμ plastocyanin absorbance change was completely inhibited by salicylaldoxime or when the activity of system II was poisoned by DCMU (Fig. 2, lower traces; Fig. 6, middle traces). Goedheer[6] has suggested that the redox state of cytochrome can be adjusted by reduction or oxidation which is independent of the main photosynthetic electron flow. Long incubation in salicylaldoxime must inhibit this system II-independent cytochrome reduction in the dark because the cytochrome change disappeared after this treatment.

Summary.—A light-induced absorbance change near 600 mμ which is thought to represent the functioning of the copper protein plastocyanin was irreversibly inhibited by the copper reagent salicylaldoxime. This inhibition by salicylaldoxime was accompanied by a partial inhibition of the cytochrome *f* absorbance change. Light-induced cytochrome oxidation was unaffected while reduction of cytochrome mediated by system II was inhibited. Long incubation in salicylaldoxime abolished cytochrome oxidation, but this inhibition could be relieved by ascorbate.

DCMU had an effect parallel to salicylaldoxime on the cytochrome change because it did not affect cytochrome oxidation but only the light-dependent reduction of cytochrome caused by system II. DCMU stopped the plastocyanin absorbance change; however, this change could be restored with DAD plus ascorbate which served as a substitute source of electrons, normally supplied by system II.

These results are interpreted as indicating that plastocyanin functions in the main flow of electrons between systems I and II of photosynthesis and not in a side

or parallel position. Plastocyanin is thought to function after the second light reaction and before cytochrome *f*.

We thank Prof. A. Trebst for his many helpful comments, for providing us with his most recent results, and for supplying samples of DAD.

Abbreviations: NADP, nicotinamide adenine dinucleotide phosphate; ATP, adenosine triphosphate; DAD, 2,3,5,6-tetramethyl-p-phenylenediamine; TMPD, N,N,N′,N′-tetramethyl-p-phenylenediamine.

* Dr. Urbach's work was supported by a fellowship from the Charles F. Kettering Foundation.

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MICROCLIMATIC AND VEGETATIONAL CONTRASTS WITHIN A SUBALPINE VALLEY

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Most high mountain systems in the world are geologically young, precipitous, and topographically varied. As the mountains rise, glaciers and rejuvenated rivers sculpture them and provide habitats with contrasting exposures. The plant habitats of such mountains constitute a climatic mosaic with highly contrasting temperature zones in close juxtaposition.

An example of such a geologically active region is the Slate Creek Valley of the Harvey Monroe Hall Natural Area, Mono County, California, where the Timberline transplant station of our department is located. It is a recently glaciated, subalpine valley with predominantly north- and south-facing steep slopes that range in altitude between 10,000 and nearly 13,000 feet (3000–4000 m).

Location and Geologic History.—The Slate Creek Valley is located on the east side of the Yosemite National Park, the summits of three Sierran peaks forming the west boundary to the park. It is in a region where great uplifts have occurred during the later part of the Quaternary period. Its west end is within the granitic batholith of the Sierra Nevada, and its east end within ancient folded and highly metamorphosed sediments.

Slate Creek, which gives the valley its name, joins Lee Vining Creek that in crossing the Sierran uplift region drops 2500 feet within about 4 miles. This drainage is impounded in highly saline Mono Lake at 6400 feet of elevation on the western edge of the Great Basin.

Slate Creek Valley is approximately 2 miles long from west to east and is a typically U-shaped glaciated valley. As the glaciers retreated through the postglacial period, they left behind about 11 terminal moraines, now populated by sub-