We discussed previously (*1*) why a monolayer of silver halide grains will give optimum resolution, for a given emulsion. We find now, that with such a monolayer of L-4, the efficiency for a β^--emitter will range from 0.2 to 0.02 depending on the energy. Similar results might be expected for other nuclear emulsions with equally high sensitivity of the individual crystals. Since material suitable for electron microscopy must be thin, and therefore the amount of radioactive material included correspondingly reduced, sensitivity is one of the major points to be considered when planning an experiment.

We have calculated and demonstrated experimentally that the resolution obtained for a point source of H^3, using L-4 monolayers, is about 0.1 μ (*1*). Such calculations can be extended to grain-count distributions around a uniformly labeled circular area. The distribution calculated for H^3 is shown in Fig. 1, in which the expected grain counts are plotted against distance away from the circumference of a labeled circle 1 μ in diameter. Experimental points were obtained by measuring grain counts around cross sections of bacteria approximately 1 μ in diameter and labeled uniformly with tritiated uridine.

The various assumptions which had been made in the calculations of resolution for tritium are no longer valid with P^{32}. For all practical purposes the range of β^--particles from P^{32} can be considered infinite. There is probably very little change in the path of the particles near the origin due to the passage through silver bromide; the probability that a grain hit by a particle will be exposed is no longer 1, as we have seen before. If we make the simplifying assumptions that, with P^{32}, the particles are not absorbed or deflected and that the probability of exposure of a grain hit by a β^--particle remains constant over a fairly long range, then the probability of exposing a grain in a monolayer becomes exclusively dependent on the solid angle from which it is seen by the source.

The calculated distribution, while not as favorable as for H^3, would still give very good resolving power. We find, however, that, whereas the experimental points for H^3 fit the theoretical curve quite well, those for P^{32} do not. The actual distribution is much broader than expected. One possible explanation is that the probability that a grain, hit by a β^--particle, be exposed increases with the distance from the source. This is to be expected, but it does not seem that the energy loss in a path of 0.3 to 0.4 μ in tissue or emulsion is sufficient to affect the results significantly. The discrepancy is therefore unexplained.

The situation for P^{32}, while clearly worse than for H^3, is not hopeless (Fig. 2). For a total of 650 grains counted over circular cross sections of bacteria about 1 μ in diameter, 61 percent of the grains were over the cells, 75 percent within 0.1 μ of the cell, and 87 percent within 0.2 μ. It is therefore clear that structures of this size could be resolved fairly easily in tissue sections. By comparison with the results for tritium (Fig. 1), the resolution obtained can be set roughly at 0.3 μ.

Because of its short half-life, high specific activities can be obtained with P^{32}, overcoming, to some extent, the low sensitivity of the emulsion to it. It seems therefore that the inferior resolution and sensitivity obtained with P^{32} do not preclude its use in high-resolution autoradiography. Since practically all the isotopes used in biology emit β^--particles of lower energy than P^{32}, it can be safely predicted that they will give results falling somewhere between P^{32} and H^3, and that in high-resolution autoradiographs with L-4 emulsion they will give a sensitivity of 2.5 to 20 grains per 100 β^--particles and a resolution of 0.1 to 0.3 μ, depending on the energy spectrum of the β^--particle emitted.

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Reduction of Trimethylene Dipyridyl with Illuminated Chloroplasts

Abstract. *Chloroplasts photochemically reduce 1,1′-trimethylene-2,2′-dipyridylium dibromide and concurrently form adenosine triphosphate. Reduced trimethylene dipyridyl in darkness will reduce spinach ferredoxin,* Clostridium pasteurianum *ferredoxin, nicotinamide-adenine dinucleotide phosphate, and other viologen-type dyes.*

The present status of the terminal reaction sequence in photosynthetic electron transfer by plant chloroplasts can be summarized in the following scheme (*1*) in which reduced nicotinamide-adenine dinucleotide phosphate (NADPH) is the terminal product.

$$\text{Photochemical system} \cdots > \underset{\text{ferredoxin}}{\text{Spinach}} \cdots \blacktriangleright$$

$$\underset{\text{reductase}}{\text{NADP}} \longrightarrow \text{NADPH}$$

There has been considerable speculation regarding the nature of the components prior to the reduction of spinach ferredoxin (*1*, *2*). With the important demonstration that the standard electrode potential (E'_0) at *p*H 7.55 of spinach ferredoxin is near that of the hydrogen electrode (*3*) it was supposed that spinach ferredoxin is reduced either directly by the photochemical system or by way of an unknown component with an E'_0 below that of the hydrogen electrode. Prior to this report the compound with the lowest redox potential reduced by chloroplasts was methyl viologen, with an E'_0 of −446 mv (*4*). Data will be presented on the photochemical reduction with spinach chloroplasts of 1,1′-trimethylene-2,2′-dipyridylium dibromide with an E'_0 of −550 mv and the concurrent formation of adenosine triphosphate.

The standard reaction mixture con-

tained the following components: tris-HCl buffer, *p*H 7.8, $4.8 \times 10^{-2}M$; $MgCl_2$, $10^{-3}M$; inorganic phosphate (P_i), $10^{-3}M$ (containing P_i^{32}); adenosine diphosphate, $10^{-3}M$; and spinach chloroplast fragments. Chloroplast preparation, chlorophyll determination, and the adenosine triphosphate assay have been described (*5*). Anaerobic conditions were obtained by evacuation of the standard reaction mixture, flushing it with argon, and adding catalase and ethanol. All illuminations were at 2.6×10^4 erg cm^{-2} sec^{-1} and at 22°C. Spectra were determined with a Bausch and Lomb Spectronic 505.

Under anaerobic conditions the oxidized trimethylene dipyridyl, curve 1, Fig. 1, was photoreduced (Fig. 1, curves 2, 3, and 4). Admission of air after the reduction of the dye resulted in complete recovery of the spectrum shown in curve 1. This recovery was quicker than the recording time of the instrument employed. Thus, aerobically the reduced dye does not accumulate. In fact, one observes an oxygen uptake in a Mehler-type reaction in which hydrogen peroxide is formed, an indication that the dye is being reduced by way of the photochemical system and then reoxidized. Concurrent with these reactions, adenosine triphosphate is formed at the rate of 120 μmole per milligram of chlorophyll per hour.

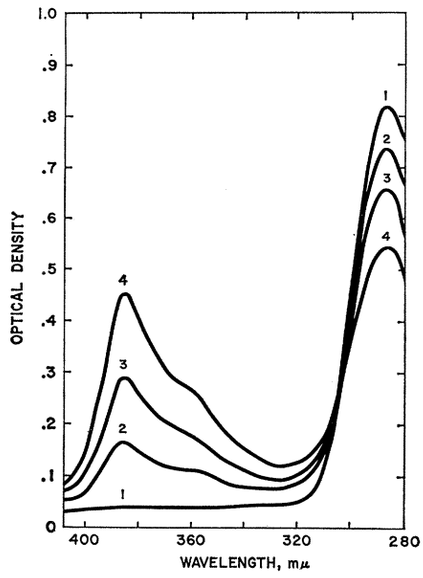

Fig. 1. Anaerobic photoreduction of 1,1′-trimethylene-2,2′-dipyridylium dibromide with spinach chloroplast fragments. Curve 1: zero illumination; curves 2, 3, and 4: after 5, 10, and 20 minutes of illumination, respectively. The standard reaction mixture contained 47 μg of chlorophyll per milliliter. The reduced peak is at 386 mμ and oxidized peak at 287 mμ.

Homer *et al.* (*6*) reported that the E'_0 of trimethylene dipyridyl is -549 ± 3 mv. The exact E'_0 was not determined in these experiments, but the following experiments indicate that the redox potential is lower than that of the hydrogen electrode near neutrality. Attempts to reduce the dye at *p*H 7 or 8 with hydrogen gas and palladium asbestos were not successful. At *p*H values above 10, reduced trimethylene dipyridyl accumulated with hydrogen gassing. Trimethylene dipyridyl was not reduced with hydrogen gas and *Clostridium pasteurianum* hydrogenase, although the hydrogenase readily reduced methyl viologen. After the reduction of trimethylene dipyridyl with the chloroplast photochemical system and addition of the following individual substances in the dark, one could observe their reduced forms spectrophotometrically: spinach ferredoxin; *Clostridium pasteurianum* ferredoxin; NADP; methyl viologen; benzyl viologen; and 1,1′-ethylene-2,2′-dipyridylium. The E'_0 of these compounds at *p*H 7.55 are -432, -417, -320, -446, -359, and -350 mv, respectively. If air was then admitted one observed the oxidized spectrum of each of the above-mentioned substances, with the exception of NADPH.

Homer *et al.* (*6*) have reported that the molar extinction coefficient (E_m) of oxidized trimethylene dipyridyl is 15,600 at 287 mμ. From the data in Fig. 1 the calculated E_m of the reduced trimethylene dipyridyl at 386 mμ is 25,000.

In other experiments under similar conditions no reduction of acridine dyes, E'_0 from -700 to -900 mv, was obtained; nor was oxygen evolved or taken up; nor was adenosine triphosphate formed. Indeed these dyes completely inhibited photophosphorylation catalyzed by methyl viologen in the range of 10^{-5} to $10^{-6}M$.

Thus plant chloroplasts can reduce substances with E'_0 at least 120 mv lower than that of spinach ferredoxin. The lower limit of the reducing capacity of illuminated chloroplast is unknown but appears to be above -900 to -700 mv since the acridine dyes were not reduced. It is relevant to note that in these studies we have only been successful in reducing trimethylene dipyridyl about 50 percent, whereas methyl viologen is completely reduced; this reduction indicates that -550 mv may be near the lower limit of chloroplast-reducing capacity at equilibrium. After the trimethylene dipyridyl is reduced with illuminated chloroplasts, oxidized ferredoxin and NADP may be added in the dark and the terminal reaction sequence (scheme 1) of photosynthetic electron transfer can be observed. There is also the possibility that some substance(s) in plant chloroplasts have an E'_0 lower than that of the hydrogen electrode.

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Psychrometric Measurement of Leaf Water Potential: Lack of Error Attributable to Leaf Permeability

Abstract. *A report that low permeability could cause gross errors in psychrometric determinations of water potential in leaves has not been confirmed. No measurable error from this source could be detected for either of two types of thermocouple psychrometer tested on four species, each at four levels of water potential. No source of error other than tissue respiration could be demonstrated.*

A fundamental requirement of psychrometric methods for determining leaf water potential (*1*, *2*) is that the leaf be brought to vapor pressure equilibrium with the small space in an equilibration chamber. A thermocouple psychrometer is used to measure the