(*9*) has confirmed these results and has shown in detail that the action spectrum for controlling the expansion of etiolated leaves on intact bean plants is identical to that for the other afore-mentioned morphological responses.

These results, then, add another morphological response to the ever-increasing list of responses that appear to be controlled through some primary photoreaction. However, one can only conjecture about the nature of the reactions that follow this primary light reaction and that eventually lead to the manifestation of the various responses.

References and Notes

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7 December 1954.

Communications

Prior Publication

This laboratory recently reported the production of cellotetraose during enzymatic hydrolysis of cellulose [*Science* **120**, 1033 (1954)]. Through *Chemical Abstracts* [**48**, 13746^h, (1954] we later became aware of related work by Kooiman *et al.* in a journal not available on this campus [*Enzymologia* **16**, 237 (1953)]. Since the abstract did not indicate that either the tetraose or other intermediate dextrins had been observed, we did not cite the Dutch paper.

When a reprint arrived from Kooiman, however, it became obvious that the tetraose and several other dextrins had been recognized. Our report, then, must be viewed as simply corroborating Kooiman's excellent initial observation. We have written to Kooiman apologizing for our error and would like to correct immediately the erroneous statement in our report that intermediate dextrins had not previously been observed in enzymatic cellulose hydrolysates.

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Spectral Absorption of Turbid Systems Using Diffuse Light

For a long time, absorption spectroscopy has found successful and even spectacular application to the measurement of the chemical composition and reaction kinetics of living cells. The effects of the turbidity of biological materials, which if ignored can lead to quantitative errors and even to qualitatively fallacious conclusions, have been widely realized in these researches, and artifices have usually been introduced in order to reduce turbidity, or to include much of the scattered light in the transmitted beam, or to insure that important changes of turbidity do not occur during the course of the reaction under investigation. Nevertheless, a method of general applicability has not emerged. In this paper we refer to preliminary experiments which suggest that such a general solution to the problem might be possible.

It is convenient to introduce the subject by referring to recent experiments by Burk (*1*) and Warburg and Krippahl (*2*), although our work was done without knowledge of theirs. In their experiments the vessel containing a turbid colored cell suspension was surrounded by a large spherical diffuse reflector. A measurement of the amount of light not absorbed by the cells when exposed to an incident monochromatic beam was obtained by measuring the light intensity at some point on the periphery of the globe, taking advantage of the fact (*3*) that the intensity at the wall of a diffusing sphere containing a source of radiation (the cell suspension in this case) is the same at all points even when the source does not emit equally in all directions.

In the other experiments (*1, 2*) the turbid absorber occupied only a very small fraction of the volume of the diffusing globe, so that the effect of double or multiple passage of diffusely reflected radiation through the absorber resulted in only a small correction term. In our own work the absorbers filled the globe, so that the process of diffuse reflection which gives the globe its essential "integrating" character for scattered radiation also has the effect of exaggerating the absorbing properties of the contents in a manner that may be expressed by comparing a globe of diameter d to a conventional absorption cell of thickness nd, where n is often much greater than unity.

The absorbing sphere with diffusely reflecting walls ("diffuse light absorption vessel," DLAV) was real-

ized very simply in the laboratory. A round-bottom fused quartz flask (about 60 cc) was imbedded in magnesium oxide powder; two small holes were left for admitting monochromatic light and for viewing the interior with a 1P21 photomultiplier tube.

A few very simple experiments served to establish the essential properties of the device. (i) When the flask contained a pure absorber (dilute aqueous $KMnO_4$), the response of the phototube as a function of wavelength I, compared with that obtained with pure water I_0, had the maxima and minima characteristics of the $KMnO_4$ absorption. The number n, representing the effective path length, was a function of the extinction, and was of the order of magnitude 10. (ii) With a colorless turbid material (Dow polystyrene latex), the relative phototube response I/I_0 was not very different from unity and decreased only threefold for a 10^{15}-fold decrease of transmission in a conventional absorption cell. (iii) Mixtures of Dow polystyrene latex and hemoglobin gave the same absorption curve as pure hemoglobin. (iv) Suspensions of intact red blood cells, that gave a very flat wavelength response in the Beckman spectrophotometer were qualitatively indistinguishable from the laked cells when they were observed in the diffuse-light absorption vessel; close quantitative agreement was obtained, except for relatively small differences, which require further investigation. (v) Measurements on a suspension of bacterial cells (*Escherichia coli*) in the presence and absence of free oxygen gave good spectra of the bacterial cytochrome system, particularly of the powerful Soret band at about 420 mμ; this band was completely masked in the Beckman absorption curve. (vi) A clear red colloidal gold gave the same absorption spectrum in the diffuse-light absorption vessel as in the Beckman cell, with a maximum at about 520 mμ. Increasing the average particle size by addition of sodium chloride made the sol turn blue, with a strong Tyndall sheen, and caused the apparent absorption observed in the Beckman cell to be displaced to some wavelength beyond 600 mμ. The maximum observed in the diffuse-light absorption vessel remained between 540 and 580 mμ, at wavelengths that appeared to shift somewhat with the sol concentration.

The foregoing experiments deal with several examples of spectral absorption associated with light scattering, including, at the one extreme, the case of an optically "clear" absorber associated with colorless scattering elements and, at the other extreme, a colloidal suspension of particles that themselves absorb strongly. The results of these experiments give some indication of the scope and limitations of the new method. It has proved reliable, at least as far as first-order effects are concerned, in determining the spectral absorption of a continuous phase that separates nonabsorbing scattering particles and the spectral absorption of a continuous phase (concentrated hemoglobin solution) enclosed within a scattering envelope.

The method has also made it possible to observe the same spectral absorption of bacterial cells and the same changes associated with removal of molecular oxygen as those already demonstrated in more elaborate researches (*4*). It shares with these researches the limitation that the absorbing material in the bacterial cell is not necessarily manifested in its totality; in the new method, as in others, the absorbing material either may be opaque or may be shielded by scattering components.

The nature of the association between absorbing and scattering structures in living cells is undoubtedly complex; even when all scattered light is collected, the contributions of different substances to the total absorption may depend not only on their quantity but also on their location within the cell. We presume that the observations on a polydisperse colloidal gold illustrate how complex the situation may become when scattering and absorption are interdependent and when the absorption index is so high that at least some of the particles in the system are opaque.

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Nutritional Changes in Diets Exposed to Ethylene Oxide

Ethylene oxide has been employed as an insecticide and bactericide for many years. It has been used in the food industry (*1*), and recent publicity (*2*) suggests that it may be a suitable sterilizing agent for a variety of products. This paper presents evidence that when diets are treated with ethylene oxide the nutritional properties are impaired.

The animals were female rats of the Osborne and Mendel strain. Except as otherwise noted, litter-mate controls were used and group mean starting weights were within the 38- to 40-g range. Each animal was housed individually on wide-mesh wire screen and given free access to food and water. The purified diet contained (in percentages) Labco casein 20, sucrose 69, Wesson oil 5 (*3*), Wesson salts 4 (*4*), and a vitamin mixture 2 (*5*). The stock diet was ground dog meal pellets (*6*).

For treatment with ethylene oxide, 500 g of diet was spread on large Petri dishes to a depth of 1 cm or less. The dishes were then stacked in a 10-lit desiccator (without desiccant). A small vent at the top prevented pressure accumulation and glass rods between the dishes assured free access of the gas to the diets. Ten milliliters (8.84 g) of liquid ethylene oxide