dialyzed, mounted, and shadowed for examination in the electron microscope. Most of the material from (i) dissolved in hot NaOH and showed only traces of amorphous substance (Fig. 1). Conversely, material from (ii) was fibrillar, resembling normal bacterial cellulose microfibrils (Fig. 2), and gave a positive anthrone reaction (*5*). Radioactivity was detected when the microfibrillar material was spread on a planchet under a counter and when it was exposed as a thin film to an x-ray emulsion. The combined ethanol and chloroform extract from (i) was radioactive, but no radioactivity was detectable in the combined extracts from (ii).

These results confirm the previous conclusion that the compound in the M4 fraction is an immediate precursor of bacterial cellulose. They also confirm the conclusion that a catalyst, which is probably an enzyme, is required for the formation of the cellulose microfibrils from the precursor, and that this enzyme is present in the extracellular medium of *A. xylinum*. This conclusion has been substantiated recently for *A. acetigenum* also (*6*). In the presence of the enzyme, the glucose is transferred mainly from soluble precursor into insoluble cellulose microfibrils by tip growth (*7*). No evidence for the necessity of a primer was observed, but these experiments do not exclude this possibility. These results thus support the postulation of a diffusible intermediate by Schramm *et al.* (*8*).

Presumably the precursor, containing lipid-bound glucose, is formed within the cells from glucose taken up during incubation. The bound activated glucose is then transported across the cell wall, perhaps aided by the presence of a substantial lipid moiety. After the extracellular enzymatic transfer of the glucose portion of the molecule to the growing microfibril, the lipid fraction is probably recycled since it does not accumulate in the medium (*9*).

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Nondestructive Method for Estimating Chlorophyll Content of Leaves

Abstract. A quantitative relationship is shown to exist between the chlorophyll content of soybean and Valencia orange leaves and their percentage reflectance of light of wavelength 625 mμ as measured by a colorimeter with reflectance attachment.

Incident to an investigation of the response of plants to iron supply, a method was needed whereby successive daily estimates of chlorophyll content could be made on the same leaf. Standard methods were not suitable because they require destruction of the leaf. Since chlorotic leaves become greener to the eye as the chlorophyll content increases, it seemed likely that reflectance of certain wavelengths from a leaf surface might be sufficiently well correlated with the chlorophyll content to serve as an estimate of that content. Reflectance measurements of leaves have been reported (*1*), and a general relationship has been recognized between the percentage of incident light reflected and the chlorophyll and carotenoid pigment concentrations (*2*). Apparently no attempt has been made to utilize this relationship to obtain quantitative indications of changes occurring in the chlorophyll content of leaves.

The basic instrument used was a colorimeter (Bausch and Lomb Spectronic 20) equipped with a reflectance attachment. It was soon established that the least difference in reflectance from the under surfaces of green and chlorotic soybean leaves occurred at wavelengths of about 665 and 465 mμ, while the greatest difference occurred at a wavelength of about 570 mμ. These wavelengths occur in the regions of maximum and minimum absorption of chlorophyll and agree with the results cited by Gabrielsen (*2*). Since the greatest difference in reflectance occurred at 570 mμ, this wavelength seemed to be the one to employ in developing a relationship between chlorophyll content and reflectance. It was found, however, that a still greater spread in reflectance from green and chlorotic leaves could be obtained by using a wavelength of 625 mμ and placing a filter with peak transmission at that wavelength between the leaf and the white magnesium carbonate reflecting surface.

To establish quantitative relationships between reflectance percentage and chlorophyll content, reflectance was measured for several hundred soybean leaves exhibiting different degrees of chlorosis induced by iron deficiency. These leaves were then sorted into groups. All leaves with reflectance readings between 33 and 37 percent (average 35) were placed in one group, those between 38 and 42 percent in the next group, and so on. Replicate determinations were made of the chlorophyll content of each group, by the spectrophotometric method described by the Association of Official Agricultural Chemists (*3*). The coefficient of variability of chlorophyll content within a group was found to be 8 percent. The results of these determinations (Fig. 1) show a high inverse relationship between the logarithm of the reflectance percentage and the chlorophyll content of the leaves within the range of concentrations studied.

To test the method on a different type of leaf, Valencia orange leaves were used. These leaves, exhibiting different degrees of chlorosis, regardless of the cause, were collected at random from local trees. The relationship between reflectance readings and chlorophyll content was found to hold about as well for these leaves (see Fig. 1) as for soybean leaves, except at the higher levels where the straight-line relationship no longer held.

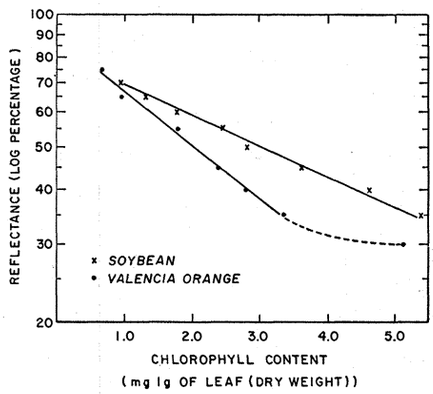

Fig. 1. Relationship between reflectance and chlorophyll content of leaves.

The close correlation between reflectance readings and chlorophyll content indicates that changes in reflectance from day to day may be used to estimate changes in the chlorophyll content of a leaf from day to day. This is especially true for chlorotic leaves; for greener leaves, when the chlorophyll content approaches the maximum, further increases may not be proportionally indicated by decreases in reflectance. Because of this and other limitations, the method can be used to the greatest advantage in detecting changes or relative differences in the chlorophyll content of leaves responding to different treatments.

Reflectance readings for a given chlorophyll content have been found to change with the age of the vacuum tubes and batteries of the instrument as well as with the species and variety of the plants being studied. The instrument should therefore be calibrated regularly against standard chlorophyll determinations.

Other precautions must also be taken. The readings must be made in the shade and not in direct light, and care must be taken that a major vein does not intercept the light beam to be reflected. Light must be prevented from leaking into the instrument around the edges of the leaf. A small cushion of black foam rubber around the opening for the light source is helpful for this purpose. Readings must be made consistently from the same surface and the same portion of the leaf. When checkered or mosaic types of chlorosis develop, such as in manganese deficiency, it may be necessary to calculate the mean of many readings on the same leaf.

The results shown in Table 1 indicate how the method has proved useful in indicating the rate of chlorophyll development in leaves receiving iron in different forms. One hundred such readings can easily be made in 1 day (*4*).

Table 1. Percentage reflectance and estimated chlorophyll content of soybean leaves in different iron treatments.

Days from start of treatment	Treatment 1*	Treatment 2†
	Percentage reflectance	
0	56	58
5	41	50
8	35	45
	Estimated chlorophyll content, dry weight basis (mg/g)	
0	2.3	2.1
5	4.4	3.0
8	5.3	3.8

* Iron added to Hoagland solution as 0.5 part of iron nitrilotriacetate per million. † Iron added to Hoagland solution as 0.5 part of iron ethylene bis-hydroxyphenylglycine per million.

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Action of Gamma-Irradiation on Dimethyl Uracil in Aqueous Solution in Absence of Oxygen

Abstract. The action of ionizing radiations on dimethyl uracil in aqueous solution, in the absence of oxygen, was found to lead to the formation of the 4-dihydro, 5-hydroxy dimethyl uracil (I), shown to be identical with that formed by the action of ultraviolet radiation. In addition, the corresponding 4,5-glycol (II) has also been identified as one of the reaction products.

It has been shown by Sinsheimer and Hastings (*1*) that the action of ultraviolet light on uracil in aqueous systems leads to a well-defined photoproduct, which was suggested to be 4-dihydro, 5-hydroxy uracil. This supposition was later confirmed by the synthesis of such compounds by various authors; in particular the dihydro-hydroxy compound from dimethyl uracil could be obtained in a crystalline form (*2*).

We have recently studied the action of ionizing radiation (Co^{60} γ-rays and 200-kv x-rays on aqueous solutions of uracil and dimethyl uracil. In the presence of oxygen, the pyrimidine bases give the more or less stable hydroxy-hydroperoxides and also the corresponding glycols (*3*). The radiation-induced formation of a glycol from cytosine has recently been reported by Eckert and Monier (*4*).

In solutions irradiated in the *absence* of oxygen, we have now been able to identify among the products the corresponding pyrimidine 4,5 glycol and, in the case of dimethyl uracil, the 4-dihydro, 5-hydroxy compound (Fig. 1). The mechanism of the formation of the dihydro-hydroxy compound and of the glycol could go by way of the successive addition of a hydrogen atom and of a hydroxyl radical or of two hydroxyl radicals at the 4 and 5 double bond or by dismutation between two pyrimidine radicals. It is, however, also possible that in this system the formation of the dihydro-hydroxy compound proceeds in a way somewhat similar to the photochemical process—that is, by excitation of the pyrimidine molecule—since it has been pointed out previously that the relatively low energy electrons, formed in the absorption of ionizing radiations, should be able to bring about excitation processes similar to those produced by ultraviolet radiation (*5*).

Dimethyl uracil, in particular, was chosen as a model compound as it had been previously investigated in detail.

In the presence of oxygen a product is formed which on treatment with acid gives an ultraviolet-absorbing compound having a peak at 283 mμ at *p*H 2 and at 310 mμ at *p*H13. This latter product was identified by chromatography as dimethyl isobarbituric acid, suggesting that the original radiation product was dimethyl uracil glycol (II).

In the absence of oxygen, irradiation gave rise to a product, which on treatment with acid also gave an increase in the ultraviolet absorption with an absorption peak at 269 mμ at *p*H 2; on subsequent treatment with alkali (to *p*H 13) this spectrum was modified to give two peaks, a major absorption at

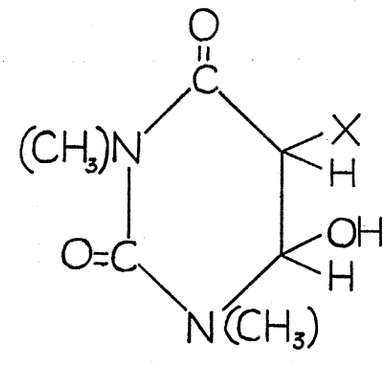

Fig. 1. Structure of the 4-dihydro, 5-hydroxy dimethyl uracil (I) and the corresponding 4,5 glycol (II). In I, *X* is H; in II, *X* is OH.