of Aspidistra has been purified by repeated salt precipitation or by differential centrifugation. Only about half this material is protein, as already found by Menke,[3] and now corroborated by us. To determine the ratio of chlorophyll to protein we estimated the protein chemically by the usual methods for N, and the chlorophyll spectrophotometrically by measuring the height of the absorption band in the red. Although the position of the absorption band in these preparations is different from that of chlorophyll in organic solvents, the same preparation has an identical extinction value in water as protein compound, or in ether or petroleum ether after precipitation of the protein with ten volumes of acetone. We have taken 5.4×10^4 as the value of the extinction coefficient estimated for three parts of chlorophyll *a* and one part of chlorophyll *b* from the values published by Zscheile.[4]

The measurements show a constant relation in the purified chloroplast material of about 16 parts of chlorophyll per 100 parts of protein. This indicates a little over three molecules of chlorophyll per Svedberg protein unit of 17,000 molecular weight. Because of the much smaller absorption at the standard wavelength of chlorophyll *b* compared to chlorophyll *a*, the fraction over 3 may represent an extra chlorophyll *b* molecule. This is in keeping with the observation of Willstätter and Stoll[5] that the leaves of most green plants show a ratio of about 3 to 1 of chlorophylls *a* and *b*. This constant stoichiometric combination of protein and chlorophyll strengthens the conclusion from other evidence that chlorophyll acts as the prosthetic group of a protein.

As stated earlier,[2] the chlorophyll-protein compound can be rendered water-soluble by the action of various detergents such as digitonin (= digitalin), bile salts or sodium desoxycholate. We have studied the action of an additional detergent, sodium dodecyl sulfate (SDS), in some detail. In addition to clarifying completely the green pigment, SDS also quantitatively converts the chlorophyll into phaeophytin, *i.e.*, removes magnesium from the molecule. This conversion, measured spectrophotometrically, proceeds at a rate which is directly proportional to the hydrogen-ion concentration, and takes place even in fairly alkaline solutions (pH 8 to 9). At constant pH, the rate is proportional to the SDS concentration until a maximum rate is achieved. Phaeophytin formation does not occur in 4 per cent. digitonin, or 10 per cent. bile salts at pH 4.5. At this pH, in 0.05 per cent. SDS the reaction is complete in a few minutes. It is therefore apparent that different detergents may have quite different actions on the same compound.

In the presence of SDS, the chlorophyll or phaeophytin (depending on the pH of the solution) remains attached to the protein, since the prosthetic group can not be separated by ultrafiltration, dialysis or by salt precipitations after removal of the SDS. This is confirmed by an ultracentrifugal study[6] of the solutions, which shows in addition that the protein is split into smaller units than in the other detergents. The action of SDS on the chlorophyll-protein compound of spinach differs from its action on the virus of tobacco mosaic disease; in the latter case, Sreenivasaya and Pirie[7] showed not only a splitting of the protein, but also a separation of the prosthetic group (nucleic acid) from the protein.

The fact that the phaeophytin remains attached to the protein indicates that in the smaller units magnesium plays no role in binding chlorophyll to the protein. The magnesium may, however, be concerned in the binding of the larger units, as indicated by the ease with which it is eliminated when the protein is split.

EMIL L. SMITH,
John Simon Guggenheim Memorial Fellow[8]
MOLTENO INSTITUTE,
CAMBRIDGE, ENGLAND, AND
LABORATORY OF BIOPHYSICS,
COLUMBIA UNIVERSITY

VITAMIN B_6 AS AN ACCESSORY GROWTH FACTOR FOR STAPHYLOCOCCUS ALBUS[1]

IT has been shown recently by Knight[2] that *Staphylococcus aureus* can be grown on a synthetic amino acid medium provided it contains nicotinic acid and thiamin. Koser, Finkle, Dorfman, Gordon and Saunders,[3] using *Staphylococcus albus*, have reported somewhat similar but less striking results. The present communication reports the result of a study to determine whether or not synthetic vitamin B_6[4] (2-methyl, 3-hydroxy, 4,5-di-[hydroxymethyl] pyridine) has any effect on *Staphylococcus albus*[5] grown in a synthetic amino acid medium.

[3] *Zeits. physiol. Chem.*, 257: 43, 1938.

[4] *Bot. Gaz.*, 95: 529, 1934. When the data of Winterstein and Stein (*Zeits. physiol. Chem.*, 220: 263, 1933) are converted from $\log_e$ to $\log_{10}$ by dividing by 2.303, they are in good agreement with those of Zscheile. The data of Rabinowitch and Weiss (*Proc. Roy. Soc. London*, A, 162: 251, 1937) have somewhat lower values.

[5] "Investigations on Chlorophyll," English translation by F. M. Schertz and A. R. Merz, Washington, D. C., 1928.

[6] E. L. Smith and E. G. Pickels, unpublished.

[7] *Biochem. Jour.*, 32: 1707, 1938.

[8] Present address: Connecticut Agricultural Experiment Station, New Haven, Conn.

[1] From the Department of Internal Medicine, University of Cincinnati College of Medicine and the Cincinnati General Hospital. This investigation was aided by a grant from the John and Mary R. Markle Foundation.

[2] B. C. J. G. Knight, *Biochem. Jour.*, 31: 731, 1937.

[3] S. A. Koser, R. D. Finkle, A. Dorfman, M. V. Gordon and F. Saunders, *Jour. Infect. Dis.*, 62: 209, 1938.

[4] Supplied through the courtesy of Merck and Company, Inc., Rahway, N. J.

[5] This culture was furnished through the kindness of Dr. Arnold G. Wedum, University of Cincinnati.

Medium IV of Koser and associates[3] was used first and was later modified by the addition of 0.2 gms NaCl and 0.2 gms asparagine per liter.

Repeated observations have shown that vitamin B_6, in amounts of 0.3 to 1.2 gamma per cc stimulates the growth and acid production of *Staphylococcus albus* when nicotinic acid and thiamin are present.

S. P. VILTER
T. D. SPIES

SCIENTIFIC APPARATUS AND LABORATORY METHODS

GRAPHICAL METHOD FOR DETERMINING WARBURG VESSEL CONSTANTS AT VARIOUS FLUID VOLUMES

IN the use of the Barcroft-Warburg manometer for studies on cell and tissue metabolism it is frequently necessary to know the value of the vessel constant, k, for a number of different values of V_F, the volume of liquid in the vessel, under otherwise constant experimental conditions. The vessel constant is ordinarily obtained by calculation from the well-known formula:

$$k = \frac{V_G \frac{273}{T} + V_F \alpha}{P_o}. \qquad (1)$$

where V_G is the volume of gas space in the vessel containing a volume of liquid V_F, both volumes being expressed in cubic millimeters; T is the absolute temperature of the thermostat; α is the absorption coefficient at the experimental temperature for the gas concerned; and P_o is the number of millimeters of manometer fluid equivalent to one atmosphere of pressure.

This calculation is somewhat tedious if there are a number of vessels concerned, and there is always the possibility of an unsuspected numerical error somewhere in the computation. We have found it much more convenient to use a graphical presentation of the relation between k and V_F, as shown in Fig. 1. In this graph the values of k_{O_2} and k_{CO_2} for a given vessel and experimental temperature are plotted on ordinary cross-section paper against various values of V_F. It can be seen that the relation between k and V_F is strictly linear, and that it is a simple matter to obtain the vessel constant for oxygen or carbon dioxide at any desired value of V_F, particularly when V_F is not a simple whole number.

The linear relationship between k and V_F is not readily evident from equation (1), since V_G is a function of V_F, but if it is recalled that $V_G = V_T - V_F$, where V_T is the total vessel volume as obtained in the usual calibration with mercury, equation (1) may be written:

$$k = \frac{(V_T - V_F)\frac{273}{T} + V_F \alpha}{P_o} \qquad (2)$$

from which the following equation may be obtained:

$$k = \frac{V_T}{P_o} \cdot \frac{273}{T} - \frac{V_F}{P_o}\left(\frac{273}{T} - \alpha\right) \qquad (3)$$

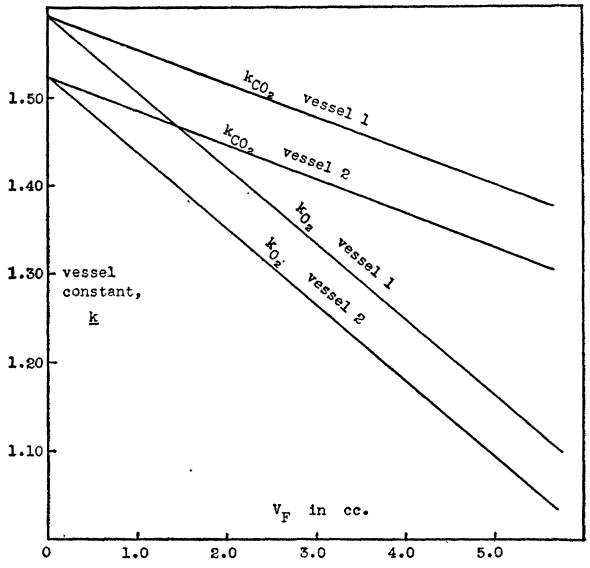

FIG. 1. Graphical presentation of the relation between the vessel constant, k, and the amount of liquid in the vessel, V_F, for two different vessels and for both oxygen and carbon dioxide.

Equation (3) is of the form $y = a - bx$ and expresses a linear relationship between k and V_F, since under ordinary conditions the other components of the equation are constants.

It will be noted that for a given temperature and manometer fluid the slope of the curve is determined by the solubility of the gas concerned, while the intercept on the y axis is determined by the vessel volume, V_T. Thus for a given vessel the curves for different gases will all start from the same point on the y axis, differing only in their slope, as shown in Fig. 1, while for a number of vessels the curves for a particular gas will all be parallel and will differ solely in their intercept on the y axis. It is thus a simple matter to plot the curves for a number of vessels, and for several different gases, on a single sheet of cross-section paper, and to read off at a moment's notice the value of the vessel constant for a particular vessel, gas, and V_F value.

To construct the curves for a number of vessels, the simplest procedure is to establish the intercept on the y axis for each vessel by dividing the value of V_T for the vessel (in cu. mm) by P_o, and multiplying the result by the value of 273/T. These points are laid