The concentration of the β form present in the solution at the time t being given by $x = x_\infty(1 - e^{-kt})$, it follows that

$$\ln(i_k - i_{k,\infty}) = \ln 0.00690x_\infty(k_1 - k_2)/\sqrt{k_1' + k_2'} - kt \quad (4)$$

where

i_k = total kinetic limiting current at time t
$i_{k,\infty}$ = total kinetic limiting current at time ∞
$= 0.00690\,[k_1a + (k_2 - k_1)x_\infty]/\sqrt{k_1' + k_2'}$ (5)
$= 1.43\,\mu$A.
a = total concentration of the glucose = 0.655 mole/liter
k = conventional first order rate constant of mutarotation[5]
$= 1/2[k_1 + k_1' + k_2 + k_2' - \sqrt{(k_1 + k_1' - k_2 - k_2')^2 + 4k_1'k_2'}]$[6]

or, if $k_1', k_2' \gg k_1, k_2$

$$k = (k_1k_2' + k_1'k_2)/(k_1' + k_2') \quad (6)$$

The over-all equilibrium constant is

$$x_\infty/(a - x)_\infty = k_1k_2'/k_1'k_2 = 1.740 \quad (7)[6]$$

A plot of ln $(i_k - i_{k,\infty})$ *vs.* t, derived from the current-time curve, could be fitted quite well by a straight line, as required by equation (4). The slope of this line is

$$k = 3.17 \times 10^{-3}\ \text{sec.}^{-1} \quad (8)$$

and the intercept is

$$\ln 0.00690x_\infty(k_1 - k_2)/\sqrt{k_1' + k_2'} = 0.115 \quad (9)$$

From the equations (5), (6), (7), (8) and (9), values for the constants in equation (1) are found: $k_1 = 5.80 \times 10^{-3}$ sec.$^{-1}$, $k_2 = 1.77 \times 10^{-3}$ sec.$^{-1}$, $k_1' = 69$ sec.$^{-1}$, $k_2' = 37$ sec.$^{-1}$, from which values the concentration of the free aldehyde form may be calculated. It was found to be 2.0×10^{-5} mole/liter, which is 0.0030% of the total glucose concentration.

A more complete report will be published later, together with work now in progress pertaining to a more detailed elucidation of the mechanism as given by equation (1).

(5) T. M. Lowry and W. T. John, *J. Chem. Soc.*, **97**, 2634 (1910).
(6) J. C. Kendrew and E. A. Moelwyn-Hughes, *Proc. Roy. Soc. (London)*, **A176**, 352 (1940).

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF NEW BRUNSWICK
FREDERICTON, N. B., CANADA
J. M. LOS
K. WIESNER

RECEIVED NOVEMBER 9, 1953

A NEW SOLUBLE CYTOCHROME

Sir:

Rhodospirillum rubrum, a photosynthetic heterotrophic bacterium, contains large amounts of a haemprotein which may be obtained by treatment with warm trichloroacetic acid, by extraction with phosphate buffer from cell residues after acetone treatment and by sonic disruption of cell suspensions. The protein so obtained can be purified in good yield by a modified Keilin–Hartree procedure.[1] The same protein is also obtained with similar yields from another photoheterotrophe, *Rhodopseudomonas spheroides*. Although this haem protein (I) possesses many properties usually attributed to mammalian cytochrome-c (II) it is, in fact, a new cytochrome. Thus, although I, like II, can be reduced with DPNH via DPNH-cytochrome-c reductase (prepared either from *R. rubrum* or pig heart), is not auto-oxidized, can be reversibly reduced or oxidized by reagents such as the ferro-ferricyanide couple, hydrosulfite and ascorbate, and exhibits an absorption spectrum in the visible identical with that of reduced II, it differs from II in the following important particulars: (a) I is not oxidized in air in the presence of the cytochrome oxidase system, whether the enzyme is prepared from pig heart, pig kidney or rat kidney. Preparations of I, purified electrophoretically (see below), do not inhibit the oxidase, as evidenced by unabated activity of the enzyme in catalyzing oxidation of II in the presence of I. (b) I is not absorbed on the NH_4^+ form of Amberlite IRC-50 ion exchange resin, prepared according to the directions of Margoliash.[2] (c) I

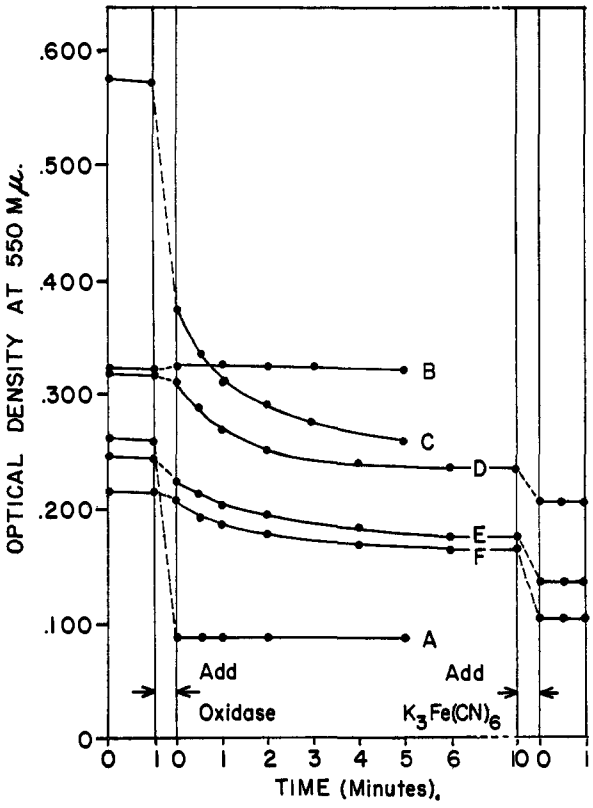

Fig. 1.—Action of cytochrome oxidase on bacterial cytochrome: tests were performed with a Beckman DU spectrophotometer, using absorption cells in which were placed 1.0-ml. volumes of solutions containing 50 μmoles of phosphate buffer *p*H 7.4, 0.4 μmole of $AlCl_3$, 1 mg. of protein of a pig heart cytochrome oxidase preparation and cytochrome preparations as indicated. Reduced cytochromes were prepared with stoichiometric amounts of ascorbic acid. Oxidase was added to the test systems after an initial one minute period of observation as well as in equal amounts to the water blank. Mixing time was about 15 seconds and all values for optical density prior to mixing were corrected for the dilution: A, 0.01 μmole of reduced cytochrome c; B, 0.012 μmole of reduced bacterial cytochrome; C, 0.01 μmole of reduced cytochrome c plus 0.012 μmole of reduced bacterial cytochrome. D, E and F all contain 0.008 μmole of reduced bacterial cytochrome with 0.01, 0.004 and 0.002 μmole of oxidized cytochrome c, respectively.

(1) L. P. Vernon, *Arch. Biochem. Biophys.*, **43**, 492 (1953).
(2) E. Margoliash, *Nature*, **170**, 1014 (1950).

can be only partially oxidized by a ferro–ferricyanide oxidation–reduction buffer (20:1) under conditions which permit complete oxidation of II. (d) The cathodic mobility of I on ionophoresis in 1% ammonium acetate on Whatman no. 5 paper is less than that of II by at least one order of magnitude.

Preparations of I, as obtained by the Keilin–Hartree procedure, usually exhibit a purity of 10–50%, based on spectral absorption properties and the assumption that the extinction coefficients at the characteristic absorption maxima are closely similar to those for II. Electrophoretic treatment of such preparations results in a 2- or 3-fold purification because of removal of colorless impurities which migrate rapidly toward the cathode.

The conclusion that I is not identical with II is further strengthened by the observation that I can be oxidized via mammalian cytochrome oxidase, if some II is added to the reaction mixture (see Fig. 1). Complete oxidation in this manner cannot be attained, indicating in agreement with (c) above, that the oxidative–reduction potential of I is slightly positive with respect to II.

It is tempting, but premature, to discuss the many intriguing possibilities raised by the presence of this new cytochrome in photosynthetic organisms. We may note, however, that the existence of the bacterial cytochrome may provide the basis for a mechanism of photochemical H-transport and an explanation for the absence of a light-stimulated respiration in the photoheterotrophes. It is suggested that I be called tentatively "cytochrome-c2".[3] Further researches on the properties of this and other haem-proteins in the photosynthetic bacteria are proceeding.

The investigations at Washington University have been made possible by grants from the C. F. Kettering Foundation and the U. S. Public Health Service. We are indebted to Dr. S. Velick, Dept. of Biochemistry, Washington University Medical School, for aid in the experiments on electrophoretic purification of the bacterial cytochrome.

(3) R. Hill and D. Keilin, private communication.

A.R.C. UNIT OF MICROBIOLOGY, THE UNIVERSITY
SHEFFIELD, ENGLAND S. R. ELSDEN
THE EDW. MALLINCKRODT INSTITUTE
OF RADIOLOGY, WASHINGTON
UNIVERSITY MEDICAL SCHOOL M. D. KAMEN
SAINT LOUIS, MISSOURI L. P. VERNON

RECEIVED DECEMBER 3, 1953

BEHAVIOR OF AN ION-EXCHANGE RESIN IN LIQUID AMMONIA

Sir:

In the course of a study of ion species present in liquid ammonia solution, use has been made of the cation exchange resin Dowex-50.[1] The ammonium form of the resin, dried 16 hours *in vacuo*, was washed copiously at −33° by repeatedly condensing fresh anhydrous ammonia upon it and then filtering. The resin was exposed, in a column operation, to a solution of liquid ammonia which contained the products of the reaction of potassium with monoammino boron trifluoride[2,3] (0.1901 g. K, 0.4380 g. $BF_3 \cdot NH_3$).

The total product from the 0.6281 g. of reactants dissolved in about 35 ml. of ammonia, was passed through a resin column, one cm.^{2} × 12 cm. at a rate that varied between 1 and 2 ml. per minute. The resin, which was largely in the ammonium form, quantitatively removed potassium from the solution. Boron and fluorine passed through the resin bed. The eluate solution was evaporated to dryness and the solid residue, which weighed 0.4457 g. after evacuation, was shown to be mainly $BF_3 \cdot NH_2$ by X-ray diffraction analysis.[4]

After the resin column had been washed until the washings gave negative tests for boron, a solution of ammonium chloride was passed through the column. The 11.7 g. of resin had a calculated capacity of 58.5 meq. cation, 10.9 meq. of potassium being on the resin after two exchange experiments. The passage of a total of 134 meq. ammonium ion (in approx. 1.5 *M* NH_4Cl solution) through the column resulted in the elution of only 0.4 meq. potassium.

The behavior of Dowex-50 in liquid ammonia parallels its behavior in water. A solution of ammonium chloride in ammonia behaves like a solution of hydrogen chloride in water: neither are efficient in stripping the resin of potassium ions. Potassium ion efficiently displaces ammonium ion from the resin in ammonia just as it displaces hydronium ion from the resin in water solution.

It appears that ion-exchange techniques of separations, purifications, and syntheses can be used successfully in the liquid ammonia medium.

(1) These studies were inspired by a paper which described the use of a cation exchange column in the study of KBF_3OH: see C. A. Wamser, THIS JOURNAL, **73**, 419 (1951).

(2) In a forthcoming publication it will be shown that $BF_3 \cdot NH_3$ reacts with *only one* equivalent of potassium in dilute ammonia solutions. When the resulting solution is evaporated to dryness and analyzed by X-ray diffraction the pattern of KF is found. Quantitative recovery of the total solid products and analysis of the gaseous and solid products support the following interpretation:

$$K + BF_3 \cdot NH_3 \longrightarrow KF + BF_2NH_2 + 1/2\ H_2$$

This equation accounts for all our observations, but it is emphasized that we have not as yet isolated the boron compound.

(3) For a description of the reaction of sodium with $BF_3 \cdot NH_3$ in liquid in ammonia see C. A. Kraus and E. H. Brown, *ibid.*, **51**, 2690 (1929).

(4) The X-ray powder diffraction of $BF_3 \cdot NH_3$ as obtained in this laboratory does not check with that reported by A. W. Laubengayer and G. F. Condike, *ibid.*, **70**, 2274 (1948). As prepared here in two ways, or as recrystallized from either water or liquid ammonia, the compound yields the following lines:

4.87 (m) 4.01 (s) 3.65 (s) 3.37 (s) **2.87** (m) 2.72 (s)
2.45 (m) 2.34 (m) 2.24 (s) 1.97 (w) **1.81** (m) 1.65 (w)

DEPARTMENT OF CHEMISTRY
THE UNIVERSITY OF TENNESSEE C. W. KEENAN
KNOXVILLE, TENNESSEE W. J. MCDOWELL

RECEIVED NOVEMBER 20, 1953

THE STRUCTURE OF PITHECOLOBINE

Sir:

Some time ago we described an alkaloid from the bark of *Pithecolobium saman* (Benth.)[1] $C_{22}H_{46}N_4O_2$ (one amide group, one hydroxy group, one ring, no double bond) which on reduction with $LiAlH_4$ gave desoxypithecolobine $C_{22}H_{48}N_4$ (one ring, no double bond). Analytical difficulties with even highly

(1) K. Wiesner, D. M. MacDonald, Z. Valenta and R. Armstrong, *Can. J. Chem.*, **30**, 761–772 (1952).