Anal. Calcd. for $C_6H_{11}N_5O_2$: C, 38.91; H, 5.99; N, 37.80; CH_3O, 33.53. Found: C, 38.92; H, 5.93; N, 37.72; CH_3O, 33.78.

An attempt to carry out this reaction in water solution was unsuccessful. Refluxing began around 80°, the solution turned progressively darker, and the temperature dropped to 60°. Apparently the methoxyl groups of the nitrile are easily hydrolyzed by aqueous alkali, but not by alcoholic alkali, before reaction with dicyandiamide takes place.

Diethoxyacetoguanamine.—A mixture of the diethoxyacetonitrile (25.8 g., 0.2 mole), dicyandiamide (18.6 g., 0.22 mole), potassium hydroxide (0.66 g., 0.01 mole) and 2-methoxyethanol (30 ml.) was heated as described above and worked up in the same manner, giving 40 g. (94%) of white crystalline product, m.p. 195–197°. It was recrystallized from 60% aqueous ethanol; m.p. 194–194.5°. It is thermally stable at least to 260°.

Anal. Calcd. for $C_8H_{15}N_5O_2$: C, 45.04; H, 7.09; N, 32.83; C_2H_5O, 42.25. Found: C, 45.26; H, 7.23; N, 32.72; C_2H_5O, 42.17.

When the quantities of reagents were tripled in this reaction, the exothermic reaction became so violent that half of the contents of the flask were expelled. Attempts to moderate the violence of this reaction were only partially successful. Portionwise addition of the dicyandiamide to the other reagents gave a 48% yield of the guanamine. Addition of the nitrile to the hot mixture of dicyandiamide and alkali gave an 84% yield on a 0.1-mole scale but only 38% on a 0.35-mole scale. When 2-methoxyethanol was replaced by 1-butanol, the reaction rate was decreased (probably because of lower boiling point and lower solubility of dicyandiamide) without impairing the yield; foaming, however, was troublesome. Weaker bases such as piperidine and potassium carbonate gave only dark, tarry products. Use of water as solvent caused hydrolysis of the nitrile.

Di-*n*-butoxyacetoguanamine.—A mixture of dibutoxyacetonitrile (13.0 g., 0.07 mole), dicyandiamide (6.7 g., 0.08 mole), sodium hydroxide (0.3 g., 0.007 mole) and 2-methoxyethanol (35 ml.) was heated to 110°. A mildly exothermic reaction caused the temperature to rise to 130°. The mixture was refluxed for 15 minutes, cooled and poured into water. The filtered and dried product was a white, crystalline material, 16.4 g. (87.2%), m.p. 165–168°. It was recrystallized from methanol; m.p. 166.5–167.5°.

Anal. Calcd. for $C_{12}H_{23}N_5O_2$: C, 53.50; H, 8.61; N, 25.97. Found: C, 53.62; H, 8.35; N, 25.91.

The reaction proceeded similarly when larger amounts of material were used.

Bis-(2-ethyl-1-hexyloxy)-acetoguanamine.—A mixture of bis-(2-ethyl-1-hexyloxy)-acetonitrile (38.2 g., 0.13 mole), dicyandiamide (16.0 g., 0.19 mole), potassium hydroxide (1.0 g., 0.015 mole) and 2-methoxyethanol (50 ml.) was heated as described above for the butyl analog. Ammonia was evolved and insoluble material (melamine[7] and similar materials) formed. The insoluble material was removed by filtration and the filtrate was poured into several volumes of water. The guanamine precipitated as an oil which rapidly hardened to a white, waxy solid. This was washed with water in a Waring blendor and dried. The weight of the crude product was 47.5 g. (96%), m.p. *ca.* 100°. It was recrystallized from hexane; m.p. 115–116°.

Anal. Calcd. for $C_{20}H_{39}N_5O_2$: C, 62.97; H, 10.31; N, 18.34. Found: C, 62.91; H, 10.26; N, 18.43.

Attempted Experiments.—α,α-Dimethoxypropionitrile did not give a guanamine under the conditions used in this study. It did not react exothermically with dicyandiamide and, after refluxing, a very dark solution was obtained which apparently contained none of the desired guanamine.

Heating dialkoxyacetoguanamines in the presence of an acidic condensing agent, such as zinc chloride, transformed them into brittle resins with the loss of the etherifying alcohol.

(7) Dicyandiamide, when heated under alkaline conditions, loses ammonia to form melamine and more complex triazine condensation products. This is especially true in the guanamine synthesis when relatively unreactive nitriles are used. See, for example, D. W. Kaiser, U. S. Patent 2,606,904 (Aug. 12,1952).

AMERICAN CYANAMID CO.
STAMFORD, CONNECTICUT

COMMUNICATIONS TO THE EDITOR

A POLAROGRAPHIC INVESTIGATION OF THE MECHANISM OF MUTAROTATION OF *d*-GLUCOSE

Sir:

It has been shown previously[1] that the polarographic reduction of equilibrium *d*-glucose is a completely rate-controlled process in a 10^{-2} molar solution of LiOH and in a phosphate buffer.

In the present case a solution which was 0.655 molar in *d*-glucose, 0.0183 molar in NaH_2PO_4, 0.0458 molar in Na_2HPO_4 and 0.0916 molar in LiCl gave a wave height of the limiting current of glucose of 57 mm. These current–voltage curves were recorded with a Sargent–Heyrovsky Model XII polarograph at 25°, some 40 minutes after the α-glucose was dissolved, and at $1/5$ of the maximum galvanometer sensitivity (0.0052 μa./mm.). Within the limits of the accuracy obtained this wave height was independent of the height of the mercury level. Immediately after dissolving the α-glucose a current–voltage wave was obtained with a limiting current considerably higher than 57 mm. This limiting current decreased with time, approaching the equilibrium value of 57 mm. The change in wave height was recorded as a function of time, yielding a continuous current–time curve of glucose in the phosphate buffer at 25°.

If the mechanism of mutarotation is considered to be essentially

$$\alpha \underset{k_1'}{\overset{k_1}{\rightleftarrows}} \mu \underset{k_2}{\overset{k_2'}{\rightleftarrows}} \beta \qquad (1)$$

where μ presumably is the aldehyde form, then the kinetic current due to α- and β-glucose separately is given by (*cf.*[2,3])

$$i_k(\alpha) = nF \times 10^{-3} \times 3/5 \times 0.85(mt)^{2/3}\sqrt{D}(k_1/\sqrt{k_1' + k_2'})C_\alpha \qquad (2)$$

and

$$i_k(\beta) = nF \times 10^{-3} \times 3/5 \times 0.85(mt)^{2/3}\sqrt{D}(k_2/\sqrt{k_1' + k_2'})C_\beta \qquad (3)$$

where all symbols have their conventional meaning (mt = 0.00475 g., $D = 6.16 \times 10^{-6}$ cm.^{2}/sec.)[4].

(1) K. Wiesner, *Collection Czechoslov. Chem. Commun.*, **12**, 64 (1947).

(2) K. Wiesner, *Chem. Listy*, **41**, 6 (1947).

(3) J. Koutecky and R. Brdicka, *Collection Czechoslov. Chem. Commun.*, **12**, 337 (1947).

(4) L. Friedman and P. G. Carpenter, THIS JOURNAL, **61**, 1745 (1939).

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).

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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

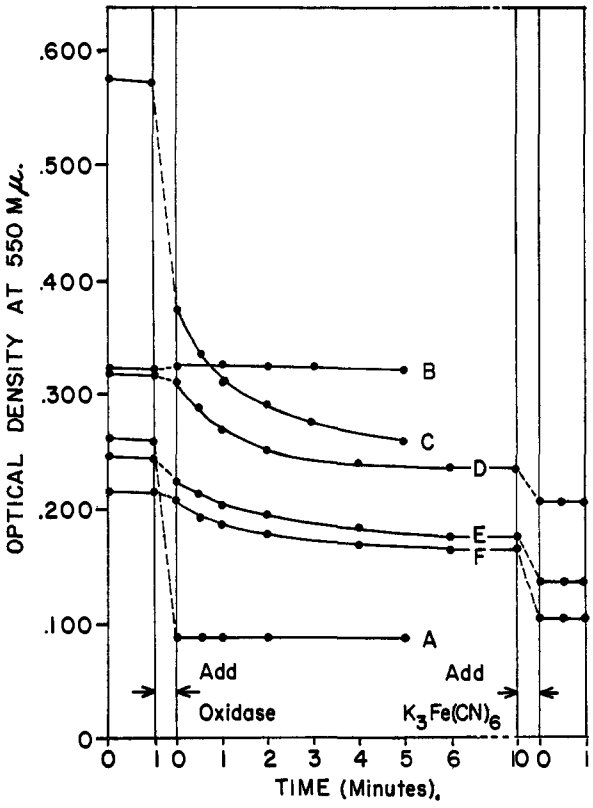

Fig. 1.—Action of cytochrome oxidase on bacterial cytochrome: tests were performed with a Beckman DU spectrophotometer, using absorption cells in which were placed 1.0-ml. volumes of solutions containing 50 μmoles of phosphate buffer *p*H 7.4, 0.4 μmole of $AlCl_3$, 1 mg. of protein of a pig heart cytochrome 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).