On the basis of these results we have tentatively identified the factor as ergothioneine or some closely related compound.

Details of the mechanism of the enzyme-catalyzed exchange reaction and the significance of ergothioneine will be discussed in subsequent papers.

(10) Postdoctoral fellow, National Institutes of Health, National Cancer Institute.

McCOLLUM–PRATT INSTITUTE LAWRENCE GROSSMAN[10]
THE JOHNS HOPKINS UNIVERSITY NATHAN O. KAPLAN
BALTIMORE 18, MARYLAND

RECEIVED JUNE 13, 1956

A NEW METHOD FOR THE PREPARATION OF BOROHYDRIDES

Sir:

The authors have succeeded in preparing a series of borohydrides by the general reaction of hydrolysis of magnesium diboride, MgB_2, with bases. The MgB_2 used in this study was prepared by direct combination of boron and magnesium at 950° in a closed system under an argon atmosphere. The MgB_2 (84%) dissolved in acid solutions, leaving only small amounts of acid insoluble borides (*e.g.*, MgB_4) as a residue; it reacted exothermically with water to give hydrogen, traces of boranes, a water soluble fraction and grey water insoluble solid. The latter consisted mostly of $Mg(OH)_2$ and magnesium borates; the dark brown water soluble fraction gave off large amounts of hydrogen when acidified.

Hydrolysis of MgB_2 in strong basic media gave similar results but in KOH and $(CH_3)_4NOH$ solutions, KBH_4 and $(CH_3)_4NBH_4$, respectively, were isolated from solution. For example, 23 grams of MgB_2, (82.4%) were digested for 8 to 12 hours in 250 ml. of 3*M* KOH. The reaction mixture was kept well stirred and cooled during the addition of the MgB_2 to the base and during the first few hours of the reaction. Thereafter the reaction ran smoothly at room temperature. The water soluble fraction was rapidly filtered and slowly evaporated under vacuum. Due to their relatively low solubility the first crystals were easily separated from the remainder of the solution by filtration. Analytical data showed that this product of the hydrolysis of MgB_2 and strong KOH was KBH_4 (B: Calcd. 20.06%; Found, 19.99%). Four moles of gas per mole of KBH_4 were evolved upon acidification, in agreement with the equation $KBH_4 + H^+ + 3H_2O \rightarrow H_3BO_3 + K^+ + 4H_2$. Our observed value of $a_0^{25°} = 6.7274 \pm 0.0003$ Å. is in complete agreement with the reported value for KBH_4 of $a_0^{25} = 6.7274$ Å.[1] A 13% conversion of boron to borohydride was obtained, as determined by the amount of hydrogen evolved upon acidification of the solution. Other crystals which formed in the solution were found by analysis to be a potassium borate of the formula $KBO_2 \cdot 1\ 1/4\ H_2O$. The powder diffraction pattern shows principal lines having "d" values of 5.5m, 3.78m, 2.97s, 2.73m, 2.48m, 2.25s, 1.85m and 1.60m.

The hydrolysis of MgB_2 was carried out with other bases with comparable results—*e.g.*, 7.7 g. of MgB_2 reacted in 85 ml. of 4M $(CH_3)_4NOH$, was filtered, and the filtrate evaporated slowly in vacuum. The first crystalline product to separate from the solution was $(CH_3)_4NBH_4$. A powder diffraction pattern of the crystals showed a tetragonal lattice with $a_0 = 7.29$, $c_0 = 5.696$ and $c/a = 0.719$.

The experimental results show that one can produce any borohydride from the general reaction of hydrolysis of MgB_2 in a strong basic medium. The borohydride can be isolated from solution if it is stable in the basic medium at room temperature and less soluble than its borate, also present in solution. In any event one has a simple means available for the preparation of laboratory quantities of a basic solution of most borohydrides.

(1) S. C. Abrahams and J. Kalnais, *J. Chem. Phys.*, **22**, 434 (1954).

DEPARTMENT OF CHEMISTRY A. J. KING
RESEARCH INSTITUTE F. A. KANDA
SYRACUSE UNIVERSITY V. A. RUSSELL
SYRACUSE, NEW YORK WALTER KATZ

RECEIVED JUNE 19, 1956

THE ENZYMATIC SYNTHESIS OF N^{10}-FORMYLTETRAHYDROFOLIC ACID AND ITS ROLE IN ATP FORMATION DURING FORMIMINOGLYCINE DEGRADATION

Sir:

Extracts of *Clostridium acidi-urici* and *Clostridium cylindrosporum* degrade purines to formiminoglycine ($NH{=}CH{-}NH{-}CH_2{-}COOH$)[1] in a series of hydrolytic reactions.[2] The further metabolism of FIG by a purified enzyme preparation requires ADP, P_i and a cofactor present in boiled extracts, and leads to the formation of ATP as shown by reaction (1).[3]

$$\text{FIG} + \text{ADP} + P_i \longrightarrow \text{glycine} + \text{HCOOH} + NH_3 + \text{ATP} \quad (1)$$

The activity of the boiled extract is completely replaced by 10-formyl-THF or THF, and evidence has now been adduced for the following steps in the over-all reaction (1)

$$\text{FIG} + \text{THF} \longrightarrow \text{glycine} + NH_3 + \text{10-formyl-THF} \quad (2)$$

$$\text{10-formyl-THF} + \text{ADP} + P_i \rightleftharpoons \text{HCOOH} + \text{THF} + \text{ATP} \quad (3)$$

With substrate amounts of 10-formyl-THF, FIG is not required for the formation of ATP (Table I) [equation (3)]. As in the over-all reaction (1), ATP cannot be demonstrated unless hexokinase, glucose and $MgCl_2$ are added as a trapping system. In the absence of the trapping system, the equilibrium lies far to the left and formic acid and THF are readily converted to 10-formyl-THF in the presence of ATP.[4]

(1) Abbreviations used are: FIG, formiminoglycine; THF, tetrahydrofolic acid; 10-formyl-THF, N^{10}-formyltetrahydrofolic acid; 5-formyl-THF, N^5-formyltetrahydrofolic acid (leucovorin or citrovorum factor); 5,10-formyll-THF, the cyclic N^5-N^{10}-imidazolinium derivative of 5-formyl-THF (anhydroleucovorin or anhydrocitrovorum factor).

(2) J. C. Rabinowitz and W. E. Pricer, Jr., *J. Biol. Chem.*, **218**, 189 (1956), and earlier references cited therein; J. C. Rabinowitz and W. E. Pricer, Jr., *ibid.*, in press.

(3) J. C. Rabinowitz and W. E. Pricer, Jr., THIS JOURNAL, **78**, 1513 (1956).

(4) The formation of 10-formyl-THF from HCOOH, ATP and THF with a purified pigeon liver extract has been described by Greenberg, *et al.*[7]