catalyzing the reversible interconversion of the two stereoisomers

$$l\text{-BOH-S-CoA} \rightleftarrows d\text{-BOH-S-CoA} \qquad (4)$$

Preparations of pig heart[8] or rat liver BOH-S-CoA dehydrogenase oxidize synthetic *d*-BOH-S-CoA with concomitant reduction of DPN as determined spectrophotometrically. *l*-BOH-S-CoA is not oxidized by the dehydrogenase, nor does it inhibit the oxidation of *d*-BOH-S-CoA (Fig. 1).

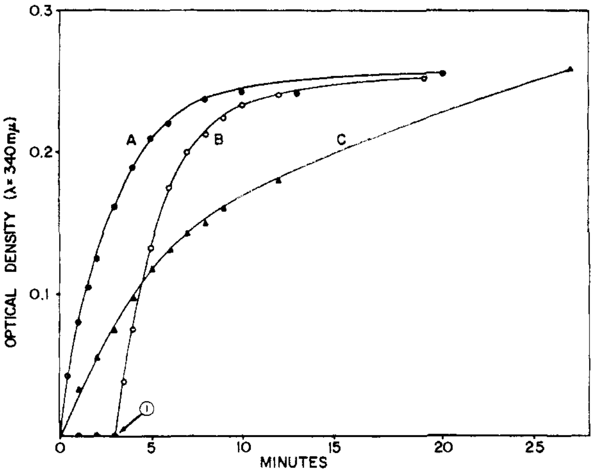

Fig. 1.—The experimental cell (d = 0.5 cm.) contained initially 100 μM. Diol buffer *p*H 9.5, 0.27 μM. DPN$^+$ and 0.14 μM. *d*-BOH–S–CoA (A), or 0.13 μM. *l*-BOH–S–CoA (B), or 0.21 μM. *l*-BOH–S–CoA (C). The reaction was started at zero time by addition of 20 γ of pig heart fraction (A and B) or 600 γ of ox liver fraction (C). At Arrow 1, 0.14 μM. *d*-BOH–S–CoA was added. A concomitant increase in O.D. at λ310 mμ (not shown) due to acetoacetyl-S–CoA formation was recorded in Experiments A and B, but was partly obscured in Experiment C by contamination of liver fraction with thiolase; volume, 1.5 ml.; temp., 25°.

Hence the dehydrogenase is stereospecific for the *d*-isomer. However, certain enzyme fractions from ox and rat liver which oxidize *d*-BOH-S-CoA were also found to oxidize *l*-BOH-S-CoA (*cf.* Figure 1), but at much slower rates. This suggested the presence either of a racemase or of a DPN$^+$-linked dehydrogenase specific for *l*-BOH-S-CoA which in concert with the *d*-BOH-S-CoA dehydrogenase could effect racemization. As shown in Table I, the dialyzed ox liver fraction catalyzes the conversion of *l*-BOH-S-CoA to *d*-BOH-S-CoA, measured with *d*-BOH-S-CoA dehydrogenase, and conversely the conversion of the *d*-isomer to a form which no longer reacts directly with DPN$^+$ in the presence of *d*-BOH-S-CoA dehydrogenase but which does react on addition of the ox liver fraction. Since (a) the liver fraction contains only 0.5–1 γ of DPN$^+$ per mg. protein[9] (final DPN$^+$ concentration $\leq 1.3 \times 10^{-6}$ M), and (b) the rate of interconversion of the isomers is not affected by addition of excess DPN$^+$ although the KM for DPN$^+$ of *d*-BOH-S-CoA dehydrogenase is 4.3×10^{-5} M,[6] the presence of a racemase rather than a specific *l*-BOH-S-CoA dehydrogenase is indicated. Preparations of ox and rat liver possessing racemase activity were found to be devoid of DPN$^+$-linked dehydrogenases acting on *d*- or *l*-BOH and of enzymes racemizing these isomers. BOH-S-CoA racemase activity, measured as DPN$^+$ reduction by *l*-BOH-S-CoA, has been found in extracts of mitochrondria from rat liver, kidney, heart and brain and in extracts of *R. rubum* and *Cl. acetobutylicum*, all of which contain the *d*-BOH-S-CoA dehydrogenase.

TABLE I

100 μM. diol buffer, *p*H 9.5, BOH-S-CoA (as indicated) and 1 mg. of ox liver fraction (final volume, 1.0 ml.) were incubated 30 minutes at 25°. Assays performed after acid heat deproteinization.

Addition	μM. recovered *d*-BOH-S-CoA[b]	μM. recovered *l*-BOH-S-CoA[c]
l-BOH-S-CoA[a] (0.21 μM.)	0.06	0.13
d-BOH-S-CoA[a] (0.20 μM.)	0.10	0.12

[a] Assayed by conversion to citrate in a multi-enzyme system.[2] [b] Assayed with heart *d*-BOH-S-CoA dehydrogenase fraction. [c] Measured by reduction of DPN on addition of ox liver fraction after oxidation of *d*-BOH-S-CoA by heart dehydrogenase fraction.

Crystalline crotonase[10] was found to dehydrate *d*-BOH-S-CoA but not *l*-BOH-S-CoA as determined by direct optical test.[11] Dehydration of *l*-BOH-S-CoA occurred only in the presence of the liver racemase system and crotonase. We find that crystalline crotonase hydrates *cis*-crotonyl-S-CoA as well as *trans*-crotonyl-S-CoA. With both isomers the product of hydration has been identified as *d*-BOH-S-CoA by enzymatic assay.

The enzymatic racemization of BOH-S-CoA may play a special role in linking ketone body metabolism to the fatty acid cycle. The properties of the racemase system and its significance are currently being investigated.

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[8] J. R. Stern, M. J. Coon and A. del Campillo, THIS JOURNAL, **75**, 1517 (1953).

[9] Assayed catalytically according to an unpublished procedure of Dr. S. Korkes.

[10] J. R. Stern, I. Raw and A. del Campillo, *Fed. Proc.*, **13**, 304 (1954).

[11] Compare F. Lynen and S. Ochoa, *Biochim. Biophys. Acta*, **12**, 299 (1953).

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A MECHANISM FOR THE PHOTO-OXIDATION OF WATER BY CERIC ION

Sir:

Chemical evidence has been obtained for the intermediate formation of OH radical in the photoreduction of ceric ion as proposed by Weiss and Porret.[1] The evidence is the effect of added solutes which have been demonstrated to react with OH radical in aqueous solution. Photoreduction of ceric ions by ultraviolet radiation, previously re-

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[1] J. Weiss and D. Porret, *Nature*, **139**, 1019 (1937).

ported[1] to occur only in perchloric acid solutions, also occurs in sulfuric acid solutions.

The following mechanism is proposed for the photoreduction of ceric ions in both perchloric and sulfuric acid solutions:

$$Ce^{++++}\cdot H_2O + h\nu \longrightarrow Ce^{+++} + H^+ + OH \quad (1)$$
$$OH + Ce^{+++} \longrightarrow Ce^{++++} + OH^- \quad (2)$$
$$OH + OH \longrightarrow H_2O_2 \quad (3)$$
$$2Ce^{++++} + H_2O_2 \longrightarrow 2Ce^{+++} + 2H^+ + O_2 \quad (4)$$

Reactions (1) and (4) express over-all chemical processes. Experimental evidence has been recently obtained[2,3] for reaction (3) through a study of the effects of Br^- and Cl^- upon the formation of "molecular" H_2O_2 in the decomposition of water by cobalt gamma radiation.

Br^- and Cl^- enhance the rate of photoreduction of ceric ions. This is interpreted as experimental evidence for the intermediate formation of OH radical postulated by Weiss and Porret.[1] Br^- and Cl^-, which readily react[4] with OH radical in acid solutions, prevent the occurrence of reaction (2). The quantum yield for cerous ion formation then becomes equal to the primary quantum efficiency of reaction (1). Bromine and chlorine atoms are formed which combine to yield molecular bromine and chlorine since, as reported[5] for the thermal reaction of ceric ion with Br^-, no back reaction occurs.

HCOOH also enhances the rate of photoreduction by prevention of reaction (2) through reaction with OH radical as postulated by Hart[6]

$$OH + HCOOH \longrightarrow H_2O + HCOO \quad (5)$$

The HCOO radical further enhances the rate of photoreduction by its behavior as a reducing agent according to reaction (6)

$$Ce^{++++} + HCOO \longrightarrow Ce^{+++} + H^+ + CO_2 \quad (6)$$

or through the sequence of reactions (7)[7] and (8).[8]

$$HCOO + O_2 \longrightarrow HO_2 + CO_2 \quad (7)$$
$$HO_2 + Ce^{++++} \longrightarrow Ce^{+++} + H^+ + O_2 \quad (8)$$

Hart[7] has concluded that HCOO is capable of reducing ferric ion which is a weaker oxidizing agent than ceric ion. The primary quantum efficiency for the formation of OH radicals, measured at 23°, is 0.10 in 0.8 N sulfuric acid solutions and 0.12 in 0.8 N perchloric acid solutions. These primary quantum efficiencies are equal to (a) the measured initial quantum yield (when Ce^{+++} concentration equals zero) for cerous ion formation in 0.8 N sulfuric acid when Br^- or Cl^- is present in 10^{-2} molar concentration and (b) one-half the measured initial quantum yields for cerous ion formation when HCOOH is present in 2×10^{-2} molar concentration both in 0.8 N sulfuric acid and in 0.8 N perchloric acid.

Since the primary quantum efficiency is about the same for sulfuric and perchloric acid solutions, the previous failure[1] to observe photoreduction in sulfuric acid solutions is attributed to the greater effectiveness of reaction (2) in sulfuric acid than in perchloric acid.

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[2] T. J. Sworski, THIS JOURNAL, **76**, 4687 (1954).

[3] T. J. Sworski, *Radiation Research*, **2**, in press (1955).

[4] H. Taube and W. C. Bray, THIS JOURNAL, **62**, 3357 (1940).

[5] E. L. King and M. L. Pandow, *ibid.*, **75**, 3063 (1953).

[6] E. J. Hart, *ibid.*, **73**, 68 (1951).

[7] E. J. Hart, *ibid.*, **74**, 4174 (1952).

[8] T. J. Hardwick, *Can. J. Chem.*, **30**, 23 (1952).

A NEW TYPE OF SUBSTITUTED BOROHYDRIDE

Sir:

The authors have shown[1] that two moles of carbon dioxide when present in excess react with one mole of solid sodium borohydride in a sealed tube at 125° to form a white solid from which it is possible to recover, by hydrolysis, one half of the carbon as methanol and the remaining half as formic acid. These observations suggest the equation[2]

$$NaBH_4 + 2CO_2 \longrightarrow NaBO(O_2CH)(OCH_3)$$

We wish to report the effect of reduced (room) temperatures and the use of liquid dimethyl ether as a solvent upon the course of the reaction. Under these conditions three, instead of two, moles of carbon dioxide are taken up per mole of sodium borohydride, and the product is a powdery white solid with a volume about 6 to 8 times as great as the sodium borohydride originally used. Treatment of this solid with hydrochloric acid produced 5.54 mmoles of hydrogen; when treated with dilute sulfuric acid, 5.94 mmoles of boric acid and 16.49 mmoles of formic acid were obtained (all values based on one gram of sample).

These data are consistent with a product formed according to the equation

$$NaBH_4 + 3CO_2 \longrightarrow NaBH(O_2CH)_3$$

The product thus represents the first reported isolation of a formatoborohydride. Although this material is fairly stable, there is some evidence that, on standing, it slowly liberates methyl formate. This latter substance is, indeed, obtained in appreciable quantity when sodium triformatoborohydride is heated to the temperature at which it appears to melt (about 125°). It may be noted that the material obtained in the high temperature reaction is *not* the same as that obtained by heating the product of the low temperature reaction.

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[1] In a paper presented to the Division of Physical and Inorganic Chemistry of the American Chemical Society at the New York Meetings in the Fall of 1954. This material will shortly be submitted for publication.

[2] Although the product has tentatively been assigned the formula shown above, it is possible that it is a mixture of $NaBO_2$ and the hitherto unreported $NaB(OCH_3)_2(O_2CH)_2$. This possibility will be discussed in a forthcoming publication.