tion of a dilute solution of dibutyl ethyleneboronate (I) and azobisisobutyronitrile (10 g. per mole of I) in carbon tetrachloride to refluxing carbon tetrachloride followed by distillation of the product (IIIa), yield 45%, b.p. 90–95° (0.07 mm.), $n^{29}D$ 1.4490. *Anal.*[5] Calcd. for $C_{11}H_{21}BCl_4O_2$: C, 39.10; H, 6.27; Cl, 41.97; B, 3.20. Found: C, 39.42; H, 6.44; Cl, 41.67; B, 3.46. The structure IIIa was proved by treatment with 2,4-dinitrophenylhydrazine and hydrogen peroxide, which yielded 73% of the 2,4-dinitrophenylhydrazone of 3,3,3-trichloropropionaldehyde, m.p. 109–112°, loses HCl (identified by tests for H^+ and Cl^-), solidifies, m.p. 161–163°.[6] *Anal.*[7] Calcd. for $C_9H_7Cl_3N_4O_4$: C, 31.65; H, 2.06; Cl, 31.14; N, 16.41. Found: C, 31.61; H, 2.16; Cl, 31.00; N, 16.25. Reaction of a fourfold excess of bromotrichloromethane with dibutyl ethyleneboronate (I) and a trace of initiator at 80° leads to an exothermic reaction and formation of the adduct IIIb in 94% yield, b.p. 95–100° (0.07 mm.), $n^{25}D$ 1.4720, structure proof the same as for IIIa but with a low yield, caused by decomposition in the presence of bromide ion. *Anal.*[5] Calcd. for $C_{11}H_{21}BBrCl_3O_2$: C, 34.55; H, 5.53; B, 2.83; g. silver halide/g. compound, 1.615. Found: C, 34.82; H, 5.67; B, 3.13; silver halide, 1.614. The adduct IIIc was prepared by irradiating 1.1 equivalent of *n*-hexyl mercaptan with the ester I at −70° under carbon dioxide with a mercury vapor lamp until the product solidified, then distilling, yield 93%, b.p. 115–118° (0.07 mm.), $n^{29.5}D$ 1.4501. *Anal.*[5] Calcd. for $C_{16}H_{35}BO_2S$: C, 63.56; H, 11.67; B, 3.58; S, 10.61. Found: C, 63.26; H, 11.70; B, 3.89; S, 10.81. The structure was proved by degradation to ethylene (confirmed by infrared) with solid potassium hydroxide at 110–127°.

(6) The 2,4-dinitrophenylhydrazone of 3,3-dichloropropenal has been reported, m.p. 164–165°, by M. S. Kharasch, O. Reinmuth and W. H. Urry, THIS JOURNAL, **69**, 1105 (1947). The report of the 2,4-dinitrophenylhydrazone of 3,3,3-trichloropropionaldehyde, m.p. 173°, J. Harmon, U. S. Patent 2,396,261, Mar. 12, 1946 [*Chem. Abstracts*, **40**, 3466 (1946)] is presumably inaccurate.

(7) Weiler and Strauss, Oxford, England.

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STIMULATION OF THE EXCHANGE OF FORMATE INTO PYRUVATE BY A DIALYZABLE COFACTOR[1]

Sir:

At *p*H 6.5, extracts of *Micrococcus lactilyticus* oxidatively decarboxylate pyruvate[2] to acetate, carbon dioxide and hydrogen. At *p*H 8.5, however, the products of pyruvate breakdown[3] are acetate and formate. Formate is not decomposed to carbon dioxide and hydrogen at either *p*H or under any conditions of testing[4] and at an alkaline *p*H formate is exchanged into the carboxyl of pyruvate.[3,5] The enzymes participating in this exchange may be separated from those catalyzing oxidative decarboxylation by fractionation with ammonium sulfate. This results not only in a separation of the two types of enzymatic activities, but also yields an increase in specific activity with regard to the exchange of formate.

TABLE I

EFFECT OF BES AND OTHER PREPARATIONS ON FORMATE EXCHANGE

For measurement of formate exchange, the reaction vessels contained: 1000 μM. of phosphate buffer at *p*H 8.0, 20 μM. of $MgCl_2$, 20 μM. of mercaptoethanol, 80 μg. of ferrous ammonium sulfate, 50 μM. of pyruvate, 0.5 μM. of C^{14}-formate (10^5 cpm.), 0.5 ml. of BES (obtained from a crude extract containing 30 mg. protein/ml.) or 0.5 ml. of charcoal eluate (equivalent to 2 ml. of original crude extract), and enzyme source equivalent to 10 mg. of protein in a total volume of 2.8 ml. The reaction mixture was incubated 90 minutes at 30° in a hydrogen atmosphere.

Preparation	Formate exchange activity[a] Expt. 1	Expt 2.
Crude extract	125	110
Treated crude extract[b]	3	1
+ BES	119	110
+ Charcoal eluate	..[c]	90
+ Eluate from chromatogram	71	..
$(NH_4)_2SO_4$ ppt., 0–45% satn.	1	1
+ BES	110	108
BES (alone)	1	1

[a] Cpm./μM. residual pyruvate/mg. protein, assayed by determining the radioactivity of the 2,4-dinitrophenylhydrazone of the residual pyruvate. [b] Prepared by treating with charcoal. [c] Not tested.

When crude extracts or fractions obtained by precipitation with ammonium sulfate are dialyzed for a short time, the ability to exchange formate into pyruvate is lost. Full activity may be restored by the addition of the supernatant fraction obtained by centrifugation of a heat-inactivated crude extract ("boiled extract supernatant," or "BES"). Treatment of crude extracts with charcoal (Norit A) also results in a complete loss of activity. Again, full activity may be restored either by addition of BES or of material eluted from the charcoal with 50% aqueous acetone containing 0.1 *N* NH_4OH (Table I). As seen in Table I, the active factor(s) can be recovered after chromatography on paper with butanol, pyridine, and water (1:1:1) as solvent.

BES cannot be replaced by yeast extract, mixtures of amino acids, or boiled extracts prepared from *Escherichia coli*, *Micrococcus aerogenes*, and *Clostridium kluyveri*. These compounds, too, when tested singly, also did not replace BES: nucleotide tri-, di-, and monophosphates, di-, and triphosphopyridine nucleotide, coenzyme A, lipoic acid, tetrahydrofolate, dihydrofolate, thiamine pyrophosphate, tocopherol derivatives, flavin adenine dinucleotide, biotin, or pyridoxal phosphate.

Further investigations are in progress into the nature of the component(s) of BES responsible for stimulating the exchange of formate into pyruvate.

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