free chlorine recovered from the liquid nitrogen trap agreed, within experimental error, with the diboron tetrachloride produced.

$$2BCl_3 \longrightarrow B_2Cl_4 + Cl_2$$

Only trace amounts, if any, of lower chlorides of boron were observed.

Various parameters which might increase the concentration of active species and at the same time inhibit recombination of chlorine with diboron tetrachloride are under investigation. The attractiveness of this procedure lies in its simplicity and probable adaptability to other systems.

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REQUIREMENT FOR BICARBONATE IN FATTY ACID SYNTHESIS[1]

Sir:

While studying a highly purified enzyme system[2] from avian liver which catalyzes the synthesis of palmitic acid from acetyl CoA in the presence of Mn^{++}, ATP[3] and TPNH we have observed an absolute requirement for bicarbonate ion (or its equilibrium forms). Table I shows the characteristics of this requirement. The half-maximal rate of fatty acid synthesis is attained at a HCO_3^- concentration of less than 2×10^{-3} *M*. HCO_3^- is not replaceable by phosphate, sulfate, chloride, formate, acetate, malonate, α-ketoglutarate, isocitrate or succinate. This absolute requirement for HCO_3^- applies to the system at all stages of purification[4] providing that (a) HCO_3^- is removed from enzyme and reagent solutions, and (b) systems which generate HCO_3^- are eliminated. Radio-labelled HCO_3^- is not incorporated into long-chain fatty acid. Hence HCO_3^- cannot be considered a substrate for fatty acid synthesis. We postulate a catalytic role for this component.

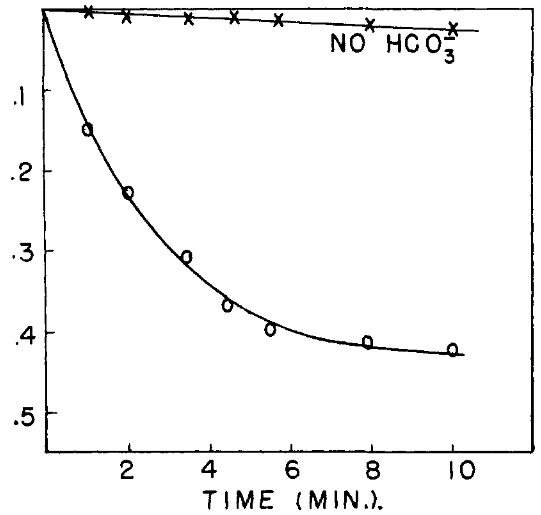

Fig. 1.—Oxidation of TPNH with and without added HCO_3^-: the conditions for these experiments are identical to those in Table I except that 32 mμmoles of acetyl-1-C^{14} CoA was added in each cuvette; 4.0 μmoles of $KHCO_3$ was added as indicated. In the complete system 17 mμmoles of acetyl CoA was incorporated into fatty acids, while there was no incorporation in the system without HCO_3^-.

TABLE I

BICARBONATE REQUIREMENT FOR FATTY ACID BIOSYNTHESIS

$KHCO_3$ added (μmoles)	0	1.0	2.0	4.0	5.0
Acetyl CoA incorporated into fatty acids (mμmoles)	1.0	8.0	19.0	25.0	24.0

Each experimental tube contained the following reagents (in a final volume of 0.50 ml.): 50 μmoles of potassium phosphate buffer (*p*H 6.5); 1.0 μmole of ATP; 0.3 μmole of $MnCl_2$; 0.05 μmole of TPNH; and 50 mmoles of acetyl-1-C^{14} CoA. The reaction was started by addition of the purified liver enzymes[2,4]: 0.7 mg. R_{1g} and 0.4 mg. R_{2g}. All samples were incubated five minutes at 38°.

The oxidation of TPNH in the complete system provides an alternative measure of biosynthesis.[2] The absolute requirement for HCO_3^- is also readily demonstrable in this spectrophotometric assay (*cf.* Fig. 1).

The requirement for HCO_3^-, as well as ATP,[2] have suggested to us the possible participation of still another cofactor. In this regard it is significant that one of the purified enzyme fractions contains a considerable concentration of protein-bound biotin.[2]

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[2] S. J. Wakil, E. B. Titchener and D. M. Gibson, *Biochim. Biophys. Acta*, in press.

[3] The following abbreviations are used: ATP, adenosine triphosphate; and TPNH, reduced triphosphopyridine nucleotide.

[4] S. J. Wakil, J. W. Porter and D. M. Gibson, *Biochim. Biophys. Acta*, **24**, 453 (1957); J. W. Porter, S. J. Wakil, A. Tietz, M. I. Jacob and D. M. Gibson, *ibid.*, **25**, 35 (1957).

[5] This work was carried out during the tenure of an Established Investigatorship of the American Heart Association, Inc.

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THE PRESENCE OF N_3^+ AND N_4^+ IN THE MASS SPECTRA OF MOLECULAR NITROGEN

Sir:

We have used two 60° sector instruments equipped with viscous and molecular leaks to study the mass spectra of purified tank nitrogen[1] at high source pressures. A 42^+ and a 56^+ ion current were observed in addition to those currents normally produced by the ionization and fragmentation of nitrogen. At first, it was suspected that the two unusual currents resulted from an increase in the background due to the high operating pressures. High purity argon was used to check this suspicion. It was found that the small 42^+ and 56^+ background ion currents remained constant when argon was run at the same pressures as those used to determine the nitrogen spectra. We also found that nitrogen prepared by the hypobromite oxidation of reagent grade $(NH_4)_2SO_4$ gave the same 42^+

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[1] Prepurified grade obtained from the Matheson Co., Joliet, Illinois.