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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RECEIVED APRIL 19, 1958

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.

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°.

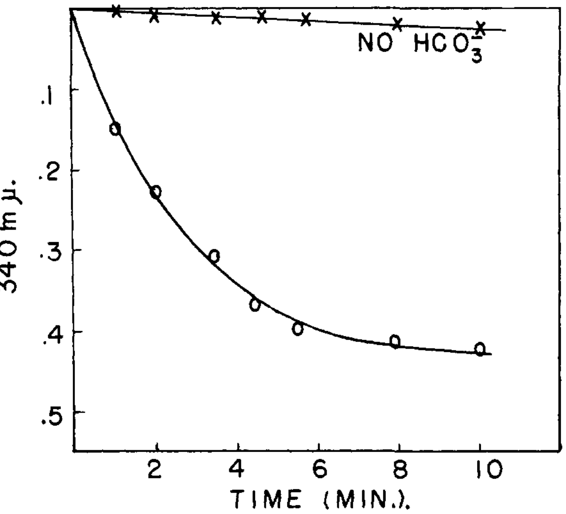

Fig. 1.—Oxidation of TPNH with and without added HCO_3^-: the conditions for these experiments are identical to those in Table I except that 32 mμmoles of acetyl-1-C^{14} CoA was added in each cuvette; 4.0 μmoles of $KHCO_3$ was added as indicated. In the complete system 17 mμmoles of acetyl CoA was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

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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[1] This investigation has been supported by a research grant, MN-3, from the American Cancer Society; by a research grant H-2236(C2) and postdoctoral training grant, HTS-5006(C8), from the National Heart Institute of the National Institutes of Health, Public Health Service; and Contract Nonr-1202 from the Office of Naval Research.

[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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RECEIVED FEBRUARY 14, 1958

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.

as was observed with the tank nitrogen. This observation eliminated the possibility that the 42$^+$ might be due to ^{84}Kr^{++} resulting from a krypton contamination in the tank nitrogen, since the commercial production involves the fractional distillation of liquid air.

Extensive study of the increase in the 42$^+$ and 56$^+$ as a function of source pressure was undertaken. The source pressure was monitored by observing the 29$^+$ ion current. It was found that the 42$^+$ varied linearly with the pressure and that the 56$^+$ varied linearly with the square of the pressure. Neither the change in charge mechanism[2,3] nor the ion-molecule collision mechanism[4,5] explains both of the observations, although the ion-molecule collision mechanism fully accounts for the presence and behavior of the 56$^+$.

Since we were using 70-volt electrons, which is below the appearance potential of $^{28}N_2^{+++}$, the change in charge mechanism cannot be used to explain the presence of the ion current at mass 42. The present ion-molecule mechanism is inadequate since this current varied linearly with the first power of the pressure. We suggest that this ion current was caused by $^{42}N_3^+$ produced by a reaction which occurs on the surfaces of the source somewhere in the region of the electron gun. The reaction is zero order with respect to the adsorbed species and first order with respect to the species in the gas phase.

The following observations on a 1:1 mixture of pure $^{28}N_2$ and pure $^{30}N_2$ are consistent with our postulates. The 42$^+$, 43$^+$, 44$^+$ (uncertain due to high background) and 45$^+$ ion currents were observed to increase linearly with the pressure which corresponds to the formation of N_3^+ by some surface mechanism. The 56$^+$, 58$^+$ and 60$^+$ increased linearly with the square of the pressure, which corresponds to a collision of gas phase molecules and/or ions.

These observations of N_3^+ and N_4^+ are not forbidden by the generally accepted hypothesis which proposes a low probability of endothermic reactions[4,6] occurring in the mass spectrometer.

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(2) J. Mattauch and H. Lichtblau, *Physik. Z.*, **40**, 16 (1939).

(3) F. J. Norton, Natl. Bur. Standards Circ. No. 522, pp. 201–204 (1953).

(4) F. H. Field and J. L. Franklin, "Electron Impact Phenomena and the Properties of Gaseous Ions," Academic Press, New York, N. Y., 1957, pp. 217–219.

(5) J. A. Hornbeck and J. P. Molnar, *Phys. Rev.*, **84**, 621 (1951).

(6) F. H. Field and F. W. Lampe, THIS JOURNAL, **79**, 4244 (1957).

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RECEIVED APRIL 3, 1958

THE SEPARATION OF ORTHOHYDROGEN AND PARAHYDROGEN

Sir:

We have found that orthohydrogen and parahydrogen can be separated[1] by gas-adsorption chromatography. Activated alumina columns (swept with nitrogen for 35 min. at 190–200°) operated at 77°K. employing purified helium as the carrier gas give sharp separation of these nuclear spin isomers (Fig. 1). The gas stream from the column was passed through a short combustion tube (35–48 mesh copper oxide, 750°) before entering a thermal conductivity cell held at 100°. Signal amplification resulted from the oxidation of hydrogen to water.

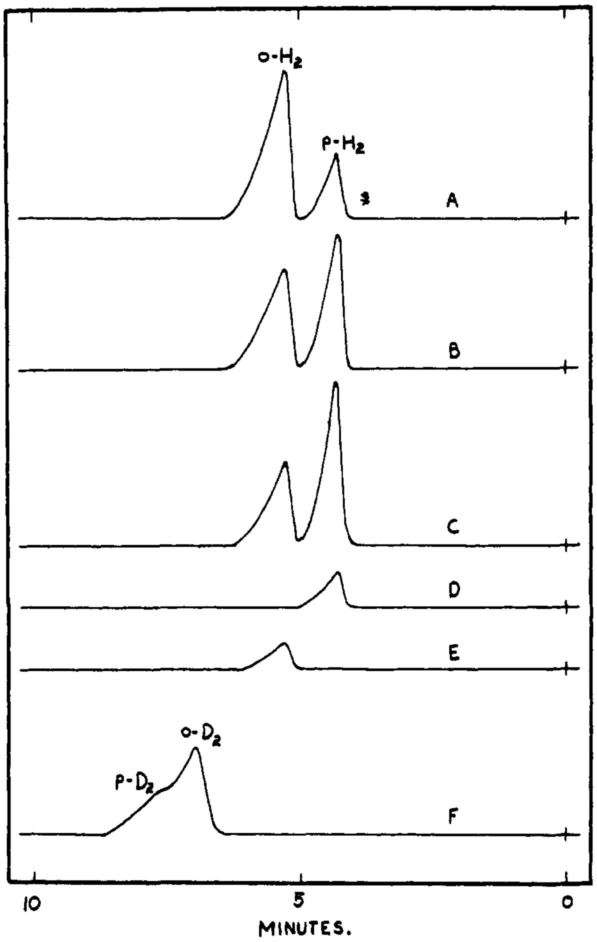

Fig. 1.—Chromatograms of samples of hydrogen and deuterium on an alumina column (150–200 mesh, 120 × 0.5 cm.; 77.4°K.; helium flow, 240 cc./min.): (A) 1.5 cc. of normal hydrogen; (B) 1.5 cc. of hydrogen equilibrated at 77.4°K.; (C) 1.5 cc. of hydrogen equilibrated at 64°K.; (D) parahydrogen and (E) orthohydrogen obtained by chromatographing 28-cc. samples of the eluent gas withdrawn from each of the two peaks produced by 6 cc. of hydrogen; (F) 1.0 cc. of deuterium.

Since the peak area ratios represent mole ratios, the positive identification of each isomer is demonstrated clearly by the results of analyses of samples of hydrogen equilibrated[2] at different temperatures (Table I).

The homogeneity of the separated components was shown by sampling the gas stream from the column (ahead of the combustion tube) at the peak maximum for each isomer. Each component,

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(1) E. W. Becker, H. Henkes and U. Seifert, *Z. physik. Chem.* (*Frankfurt*), **5**, 90 (1955), have reported a very slight enrichment in orthohydrogen by thermal diffusion and demonstrated that K. Schäfer and H. Corte, *Naturwissenschaften*, **33**, 92 (1946), who claimed a greater degree of separation by the same method were in error.

(2) A. Farkas, "Orthohydrogen, Parahydrogen and Heavy Hydrogen," Cambridge University Press, London, 1935, p. 30.