cently when Lappert[2] prepared pyridinium tetrachloroborate. Lappert suggested that the existence of pyridinium chloroborate and the non-existence of the alkali metal chloroborates may be due to a required stabilization of the complex anion by large cations. We have now found that potassium, rubidium and cesium chloroborates can be formed.

The alkali metal chloroborates were prepared by heating 10 to 30 g. samples of the metal chlorides with 60 g. of boron trichloride in a 145-ml., "Hastelloy C" lined pressure vessel to temperatures of 400–500° for about $^1/_2$ hour, and then cooling the vessel over a period of eight hours. Complete reaction at 400° was not effected; typical empirical compositions of the products were $3.3KCl \cdot BCl_3$, $2RbCl \cdot BCl_3$ and $1.7CsCl \cdot BCl_3$. The order of reactivity of the chlorides appeared to be Cs > Rb > K. Cesium and rubidium chlorides underwent essentially complete reaction at 500°; the solid products showed weight increases that indicated BCl_3/MCl ratios of 0.95 to 1.12. These products were usually contaminated with 3–8% extraneous material derived from the reactor.

Anal. Calcd. for $CsBCl_4$: Cs, 46.4; B, 3.78; Cl, 49.7. Found: Cs, 44.07; B, 3.13; Cl, 45.51; atomic ratios: Cs, 1.0; B, 0.88; Cl, 3.9.

X-Ray analysis of the cesium chloroborate showed the lines of a new component; the pattern could be indexed as rhombohedral[3] with a_0 = 10.0 ± 0.5 Å. and α = 24° ± 30′. Potassium chloride at 500° consistently yielded a mixture of the chloroborate (~65%) and unreacted chloride. Reaction of boron trichloride (250% excess) with potassium fluoborate at 400° and ~100 atm. also gave potassium chloroborate, along with boron trifluoride, in about a 30% conversion. In contrast to the above halides, sodium chloride showed no evidence of reaction with boron trichloride.

All of the chloroborates reacted exothermally with water. The resulting clear solutions gave an immediate precipitate when aqueous silver nitrate was added. The precipitate, however, continued to form over a short period of time. This behavior suggested that the hydrolysis of the chloroborate anion might be sufficiently slow to permit purification of the salts by rapid solution and crystallization. However, this procedure yielded only gross mixtures of the metal chloride, boric acid and possibly a metal borate. The ease of hydrolysis of the chloroborates seems surprising in view of the stability of boron trichloride–trimethylamine[4] toward attack by water and by alcohols; however, proton attack of the negatively charged chloroborate anion should occur more readily than proton attack of the neutral amine adducts.[5] The relatively higher order of stability of the amine adduct was indicated in partial displacement of Cl^- in BCl_4^- (as $KBCl_4$) by trimethylamine at 150° to give boron trichloride-trimethylamine.

The formation of chloroborates only by alkali metal ions of low polarizing power and the extreme hydrolytic instability suggest that the stability of the chloroborate lattice is marginal. Crude measurements of the dissociation pressures of $KBCl_4$, $RbBCl_4$ and $CsBCl_4$ showed average values of 3 atm. at 225, 275 and 370°, respectively, and approximate heats of dissociation in the range 8–15 kcal. The corresponding fluoborates have dissociation pressures of <1 mm. at these temperatures and heats of dissociation of about 27–29 kcal.[6] Thus the chloroborates are thermodynamically more prone toward dissociation than the fluoborates.

(2) M. F. Lappert, *Proc. Chem. Soc. (London)*, 121 (1957).

(3) $CsBF_4$ appears to be dimorphic and one form is reportedly rhombohedral. H. S. Booth and D. R. Martin, "Boron Trifluoride and its Derivatives," John Wiley and Sons, Inc., New York, N. Y., 1949, p. 108.

(4) E. Wiberg and W. Sütterlin, *Z. anorg. Chem.*, **202**, 35 (1931).

(5) The fluoroborate anion also is attacked more readily by water than is boron trifluoride–trimethylamine. However, in contrast to the facile hydrolysis of the chloroborate anion, hydrolysis of the fluoborate anion is difficult to force to completion.

(6) J. H. de Boer and J. A. M. van Liempt, *Rec. trav. chim.*, **46**, 124 (1927).

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RECEIVED OCTOBER 16, 1957

PHOSPHORYLATION OF SERINE IN RAT LIVER[1]

Sir:

We wish to report that serine is converted to O-phosphorylserine in rat liver both *in vivo* and *in vitro*.

Eight rats were injected intravenously with L-serine-3-C^{14} and killed 30 minutes later. Serine and phosphorylserine were isolated from a picric acid extract of the pooled livers by a combination of ion exchange and paper chromatography.[2]

From Table I it may be seen that the specific activity of the isolated phosphorylserine is about one-

TABLE I

ISOTOPE CONCENTRATION OF RAT LIVER CONSTITUENTS AFTER INJECTION OF L-SERINE-3-C^{14}[a]

Compd. isolated	μM. per g. liver (wet wt.)	C.p.m. per μM.[b]
Free serine	1.1	351
Phosphorylserine	0.04	100
Protein serine	...	15.1

[a] 10.8 μM. per 100 g. body wt. of L-serine-3-C^{14} containing 4.67×10^4 c.p.m. per μM. were injected in the tail vein. [b] Counted in a proportional flow counter on stainless steel planchets of 1.54 cm.² area, corrected to 5 mg. per planchet (M. L. Karnovsky, *et al.*, *Anal. Chem.*, **27**, 852 (1955)).

fourth that of the free liver serine and much higher than that of protein serine or any other liver constituent isolated, *e.g.*, phosphorylethanolamine, choline, etc. In contrast the concentration of phosphorylserine is quite low, only one-thirtieth that of free serine.

In further experiments, rat liver homogenates or cellular fractions have been incubated one hour with L-serine-3-C^{14}. Phosphorylserine was isolated with

(1) This work was supported by grants from the Nutrition Foundation, Inc., and the William Milton Fund of Harvard University.

(2) Details of these procedures will be reported elsewhere.

the aid of carrier and counted. The amount of radioactivity has been expressed as μmoles of added serine.

The results (Table II) show that homogenates also effect a rapid conversion of serine to phosphorylserine and that this activity resides almost exclusively in the supernatant fraction. The extent of the reaction, about 4–6 μmoles per g. liver per hour, is not significantly altered by dialysis or subsequent re-addition of ATP. When carrier phosphorylserine was added at the end of the incubation (Homogenate I) less than 0.02 μmole of radioserine was recovered as phosphorylserine.[3] Hence there is an apparent need for phosphorylserine in the incubation medium to serve either as a trap or to participate in an exchange of phosphate groups.

TABLE II

FORMATION OF PHOSPHORYLSERINE FROM L-SERINE-3-C^{14} IN LIVER HOMOGENATES AND CELLULAR FRACTIONS

Liver fraction	Added serine phosphorylated, μM./hr./g. liver
Homogenates	
I[a]	0.016
II[b]	4.2
Cell fractions[c]	
Homogenate	4.4
Mitochondria	0.03
Microsomes	0.2
Supernatant	6.8
Supernatant fraction[d]	
Undialyzed	4.0
Dialyzed—ATP added	3.0
Dialyzed—no ATP added	3.6

[a] 1 g. of liver homogenate incubated 1 hour with 10–13 μM. L-serine-3-C^{14}, containing 4.67×10^4 c.p.m. per μM., in phosphate buffer, *p*H 7.0. Final volume 9.3 ml. 50 μM. DL-phosphorylserine carrier added at end of incubation. Average of 3 experiments. [b] Conditions same as above, except that carrier phosphorylserine added at start of incubation. Average of 2 experiments. [c] Cell fractions prepared according to G. Hogeboom in N. Kaplan and S. Colowick, "Methods in Enzymology," Vol. I, Academic Press, New York, N. Y., 1955, p. 16. Each fraction incubated 1 hour in final volume of 10 ml. with 25 μM. L-serine-3-C^{14} containing 1.4×10^3 c.p.m. per μM. 2 μM. ATP and 50 μM. DL-phosphorylserine added at start of incubation. A similar experiment gave nearly identical results. [d] Dialyzed 6 hr., with stirring, against 20 l. of 0.05 *M* phosphate buffer. Conditions as above except that final incubation volume was 16 ml. and 5 μ*M* ATP was added except where absence is indicated.

Phosphorylserine has been shown to be an intermediate in the synthesis of serine from carbohydrate precursors.[4] The present study shows that phosphorylserine is normally present in liver and is, in part, derived from serine. From the results of the *in vitro* experiments the question arises as to what extent this conversion of serine to phosphorylserine represents a net phosphorylation or an exchange reaction.

Phosphorylserine may be involved in the conversion of serine to carbohydrates and in the synthesis of phosphatidylserine, in analogy to the synthesis of lecithin from phosphorylcholine.[5]

These and other possible pathways of phosphorylserine metabolism are currently under study.

(3) This value is high owing to isotopic contamination in the isolation procedure.

(4) A. Ichihara and D. M. Greenberg, *J. Biol. Chem.*, **224**, 331 (1957).

(5) E. P. Kennedy and S. B. Weiss, *ibid.*, **222**, 193 (1956).

(6) Predoctoral Fellow of the National Paraplegia Foundation for 1957–1958.

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THE ENZYMATIC SYNTHESIS OF HYDROXYMETHYLTETRAHYDROFOLIC ACID (ACTIVE HYDROXYMETHYL)[1,2]

Sir:

The chemical synthesis of "active hydroxymethyl" (hFH_4)[3] according to equation 1 has been reported by several investigators,[4,5,6] but the multiplicity of binding sites (*e.g.*, N^5, N^5, N^8 and N^{10})

$$HCHO + FH_4 \rightleftharpoons hFH_4 \qquad (1)$$

on FH_4 makes it difficult to prepare hFH_4 uniquely by the simple admixing of HCHO and FH_4. An unambiguous synthesis of hFH_4 may be achieved, however, with the *formaldehyde-activating* enzyme, which has been purified from extracts of pigeon liver acetone powder by adsorption (at *p*H 6.2) and elution (*p*H 7.5) from calcium phosphate gel, and by ammonium sulfate fractionation at *p*H 8 (55–75% fraction).

When FH_4 at a concentration of 7×10^{-5} *M* (λ_{max} at 298 mμ, $\epsilon = 22 \times 10^6$ cm.^{2} mole^{-1})[7] in phosphate buffer, *p*H 7.5, containing 10^{-2} *M* mercaptoethanol[8] is mixed with a 4- to 5-fold excess of HCHO in the presence of purified enzyme (50 γ), the spectrum of FH_4 is shifted rapidly to that of hFH_4 (λ_{max} at 290 mμ, $\epsilon = 26 \times 10^6$ cm.^{2} mole^{-1}). At this level of HCHO, no appreciable chemical reaction can be detected spectrophotometrically during the same time period (5–10 minutes) in the absence of enzyme.

hFH_4 can be separated from FH_4 by chromatography on Solka-Floc columns, or on Whatman No. 1 paper using 0.1 *M* phosphate buffer, *p*H 8, as the solvent system[5] (hFH_4, $R_f = 0.25$; FH_4, $R_f = 0.13$).

The authenticity of hFH_4 after isolation has been established by enzymatic assays. In the presence

(1) Paper V in the series "Folic Acid Coenzymes and Active One-Carbon Units"; for Paper IV see M. J. Osborn and F. M. Huennekens, *Biochim. Biophys. Acta*, in press.

(2) Supported by research grants from the Life Insurance Medical Research Fund and the United States Public Health Service (CY-3310).

(3) FH_4 represents 5,6,7,8-tetrahydrofolic acid; hFH_4 and fFH_4, hydroxymethyl and formyl FH_4 (position of the C_1 group not specified); h^5FH_4, $h^{10}FH_4$, N^5- and N^{10}-hydroxymethyl FH_4; $h^{5-10}FH_4$, N^5,N^{10}-hydroxymethyl FH_4; f^5FH_4, $f^{10}FH_4$, and $f^{5-10}FH_4$, the corresponding formyl derivatives of FH_4.

(4) R. L. Kisliuk, *J. Biol. Chem.*, **227**, 805 (1957).

(5) G. R. Greenberg and L. Jaenicke, "The Chemistry and Biology of Purines," ed. by G. E. W. Wolstenholme and C. M. O'Connor, Little, Brown and Company, Boston, 1957, pp. 204–232.

(6) R. L. Blakley, *Biochim. Biophys. Acta*, **23**, 654 (1957).

(7) Y. Hatefi, P. T. Talbert, M. J. Osborn and F. M. Huennekens, submitted to *Biochemical Preparations*.

(8) In the presence of mercaptoethanol FH_4 is stabilized considerably against oxidation to dihydrofolic acid (λ_{max} at 282 mμ at *p*H 7.5).