Table I gives the amounts of vitamin B_{12} required for half maximum growth at various salt concentrations. *The logarithm of the vitamin B_{12} requirement appears to be a linear function of the ionic strength of the salt solutions*

$$\log (B_{12})_{1/2\ max.} = a + b/\mu$$

This equation seems to apply well within the "reversible range" and the values of the constants a and b, respectively, are not too far apart for most of the salts examined.

It is possible to arrive theoretically to a similar functional relationship between $(B_{12})_{1/2\ max.}$ and μ from simple kinetic equations, if we make two basic assumptions: first, vitamin B_{12} combines with a protein apoenzyme (Ea) to give the enzyme $B_{12}Ea$: $B_{12} + Ea \rightleftarrows B_{12}Ea$; second, the available concentration of Ea is controlled by the ionic strength of the salt solutions in accordance with Cohn's "salting-out" equation for proteins.[5,6] These two assumptions allow the derivation of a theoretical equation which has the same form as the experimental formula. The derivation itself, together with a critical appraisal of such interpretation of our data, will be presented elsewhere.

(5) E. J. Cohn, *Physiol. Rev.*, **5**, 349 (1935); *Ann. Rev. Biochem.*, **4**, 93 (1935).

(6) M. Ingram, *Proc. Roy. Soc., Ser. B*, **134**, 181 (1951).

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RECEIVED MARCH 18, 1953

ASCORBIC ACID DEFICIENCY AND CHOLESTEROL SYNTHESIS[1]

Sir:

In continuing studies of chemical changes characteristic of or regulated by ascorbic acid[2,3] and related metabolites,[4] we have recently observed a relationship to steroid metabolism that is of considerable interest. Although 1-C^{14}-labeled ascorbic acid is not appreciably incorporated into cholesterol, the vitamin does exert a marked effect upon the conversion of acetate-1-C^{14} to cholesterol and other steroids in guinea pigs. Preliminary findings showed that severely scorbutic guinea pigs, compared with normal animals fed *ad lib.*, incorporated 6 times as much C^{14} from acetate-1-C^{14} into cholesterol isolated from adrenals.

Guinea pigs of comparable age (10–12 weeks) and size (350–400 g.), on a vitamin C- and cholesterol-free chow diet showed the following values (3 animals per group) for specific activities in purified adrenal and liver cholesterol, respectively, four hours after receiving the last of three intraperitoneal injections of labeled sodium acetate (1 mg., 2.68×10^7 c.p.m./mg. each at 9 hour intervals): normal, fed *ad lib.*, 100 and 80; mild scurvy (15–20 days depletion), 170 and 75 (pair-fed controls, 150 and 80); severe scurvy (21–28 days depletion), 600 and 145 (pair-fed controls, 195 and 90).

(1) This work was supported in part by grants from the Nutrition Foundation, Inc., and the Division of Research Grants, U. S. Public Health Service.

(2) L. L. Salomon, J. J. Burns and C. G. King, THIS JOURNAL, **74**, 5161 (1952).

(3) J. J. Burns, H. B. Burch and C. G. King, *J. Biol. Chem.*, **191**, 501 (1951).

(4) Hugh H. Horowitz and C. G. King, *ibid.*, **200**, 125 (1953).

Initial cholesterol fractions showed the presence of small quantities of similar C^{14}-labeled components but there was only a slight change in activity of the cholesterol after purification by precipitation of the digitonide, dibromination[5] and recrystallization. The observed changes in C^{14} content were not accompanied by comparable changes in total cholesterol present in the tissues[6] but they were sufficient to indicate changes in the C^{14}-content of other sterols.

(5) E. Schwenk and N. T. Werthessen, *Arch. Biochem. Biophys.*, **40**, 334 (1952).

(6) K. Guggenheim and R. E. Olson, *J. Nutrition*, **48**, 345 (1952).

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ENZYMATIC PHOSPHORYLATION OF NUCLEOSIDES BY PHOSPHATE TRANSFER

Sir:

We have found a phosphatase preparation, obtained by the fractionation with ammonium sulfate of Merck malt diastase, which is able to phosphorylate ribose and desoxyribose nucleosides in the presence of sodium phenylphosphate. The reaction is dependent on the concentrations of both nucleoside and phenylphosphate. The *p*H activity curves for transphosphorylation and dephosphorylation have the same shape, with an optimum around *p*H 5.2. Both reactions are partially inhibited by inorganic phosphate to the same extent.

The organic phosphates formed were separated by paper chromatography with aqueous isobutyric acid buffered with ammonium isobutyrate as the solvent.[1] Their R_F values were identical with those of the corresponding nucleotides.

In a large-scale experiment, 166 μ moles of ribocytidine was incubated, in a total volume of 4 ml., with 800 μ moles of phenylphosphate and 8 mg. of enzyme in 0.1 *M* acetate buffer of *p*H 5 for 87 hours at 30°. At this stage, 80% of the phosphate donor were split and 17 μ moles of cytidylic acid (10.2% of the nucleoside) were formed. The cytidylic acid fraction, isolated by ion-exchange chromatography,[2] contained equimolar quantities of organic phosphorus and of nucleoside (determined spectrophotometrically) and was completely dephosphorylated by the 5-nucleotidase of rattlesnake venom which, under the conditions used, failed to attack commercial cytidylic acid consisting, presumably, of a mixture of the 2′- and 3′-nucleotides. This evidence tends to indicate that the 5′-nucleotide had been produced.

All nucleosides tested could thus be phosphorylated. Preliminary results, listed in Table I, apparently show that, under identical conditions, desoxyribonucleosides[3] are phosphorylated with greater ease than the corresponding ribosides.

(1) B. Magasanik, E. Vischer, R. Doniger, D. Elson and E. Chargaff, *J. Biol. Chem.*, **186**, 37 (1950).

(2) W. E. Cohn and E. Volkin, *Nature*, **167**, 483 (1951).

(3) Uracil desoxyriboside was obtained through the courtesy of Prof. A. R. Todd.

TABLE I

PHOSPHORYLATION OF NUCLEOSIDES BY MALT ENZYME

The solutions containing, per ml. of 0.1 *M* acetate buffer of *p*H 5, 40 μ moles of nucleoside, 200 μ moles of phenylphosphate and 2 mg. of enzyme were incubated at 30° for 30 hours, when about 55% of the phosphorus has become inorganic

Nitrogenous constituent	Extent of phosphorylation, as % of nucleoside: Riboside	Desoxyriboside
Hypoxanthine	5.0	8.9
Uracil	7.7	9.9
Cytosine	7.6	13
Thymine		9.4

The transphosphorylation system described here seems to be the first instance of a general enzymatic reaction leading to the formation of nucleotides from nucleosides. In some of its characteristics it resembles the transfer reactions studied by Axelrod[4] and Green and Meyerhof.[5]

This work was supported by research grants from the National Institutes of Health, U. S. Public Health Service, and the Rockefeller Foundation. One of us (G.B.) was aided by a Predoctorate Research Fellowship from the U. S. Public Health Service.

(4) B. Axelrod, *J. Biol. Chem.*, **172**, 1 (1948).
(5) H. Green and O. Meyerhof, *ibid.*, **197**, 347 (1952).

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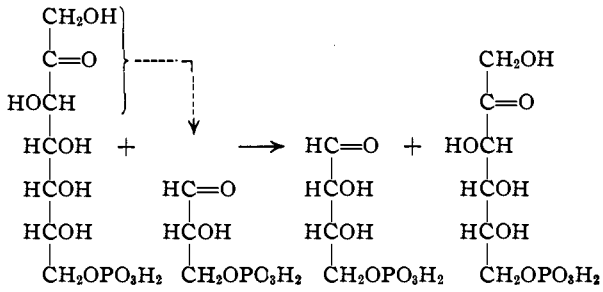
TRANSALDOLASE: THE FORMATION OF FRUCTOSE-6-PHOSPHATE FROM SEDOHEPTULOSE-7-PHOSPHATE

Sir:

Previous reports[1-3] have described the formation of ribulose-5-phosphate and sedoheptulose-7-phosphate as intermediates in the oxidation of hexosemonophosphate. This pathway is now established as a cyclic process by the finding that sedoheptulose-7-phosphate is converted to fructose-6-phosphate. Preparations from liver and yeast have been obtained which catalyze this reaction, but only in the presence of a source of triose phosphate (Table I). With a purified preparation from brewer's yeast the reaction is stoichiometric with respect to either sedoheptulose-7-phosphate or triose phosphate. The reaction mechanism has been clarified by the use of C^{14}-triose phosphate. Hexosemonophosphate produced from unlabeled sedoheptulose-7-phosphate and uniformly labeled triose phosphate (derived from fructose diphosphate) had a specific activity of 53,000 c.p.m. per micromole compared with 58,000 c.p.m. per micromole for the triose phosphate, indicating that three carbon atoms of the hexosemonophosphate were derived from triose phosphate. Degradation of the glucose obtained by hydrolysis of the hexosemonophosphate showed the label to be present only in carbon atoms 4, 5 and 6; carbon atoms 1, 2 and 3 which contained no radioactivity must have come from sedoheptulose-7-phosphate.[4] It is concluded from these results that the reaction mechanism involves a transfer of the dihydroxyacetone group from sedoheptulose-7-phosphate to glyceraldehyde-3-phosphate to form fructose-6-phosphate and erythrose-4-phosphate.



Since this enzyme catalyzes a transfer of aldol linkages, rather than their hydrolytic cleavage, it will be referred to as transaldolase.

The formation of fructose-6-phosphate as the initial reaction product is confirmed by the results in Table I, since little glucose-6-phosphate is formed in the absence of hexose phosphate isomerase.

TABLE I

THE FORMATION OF GLUCOSE-6-PHOSPHATE FROM SEDOHEPTULOSE-7-PHOSPHATE

The complete system contained 3.1×10^{-4} *M* sedoheptulose-7-phosphate, 3.3×10^{-4} *M* fructose diphosphate, 2.5×10^{-5} *M* triphosphopyridine nucleotide (TPN), 5.6 μg. of purified yeast transaldolase, 83 μg. of aldolase, 110 μg. of glucose-6-phosphate dehydrogenase and 340 micrograms of hexose phosphate isomerase, in 0.04 *M* triethanolamine buffer, *p*H 7.6. The total volume was 1.10 ml. and the temperature was 25°. The production of glucose-6-phosphate was followed spectrophotometrically by measuring the reduction of TPN in the presence of glucose-6-phosphate dehydrogenase. Hexose phosphate isomerase was a crude rabbit muscle ammonium sulfate fraction; all other enzymes were purified preparations.

System	Glucose-6-phosphate produced[a]
Complete system	4.7
Sedoheptulose-7-phosphate omitted	.0
Hexosediphosphate omitted	.0
Hexose phosphate isomerase omitted	.4
Transaldolase omitted	.4

[a] Millimicromoles per minute.

Preliminary evidence for a tetrose ester in the reaction mixture has been obtained with paper chromatography following hydrolysis with potato phosphatase. The chromatogram was developed with a 70–30 acetone–water mixture and sprayed with aniline phthalate. A fluorescent spot was observed with R_f = 0.65, identical with authentic erythrose.[5] However, since this solvent does not separate the aldo- and keto-tetroses, the nature of the tetrose remains to be determined.

The transfer of aldol linkages from one sugar to another, like the transfer of the ketol groups previ-

(1) B. L. Horecker and P. Z. Smyrniotis, *J. Biol. Chem.*, **193**, 383 (1951).
(2) J. E. Seegmiller and B. L. Horecker, *ibid.*, **194**, 261 (1952).
(3) B. L. Horecker and P. Z. Smyrniotis, THIS JOURNAL, **74**, 2123 (1952).

(4) We are indebted to Dr. M. Gibbs of the Brookhaven National Laboratory for the degradation of the labeled glucose.
(5) Kindly furnished by Dr. H. S. Isbell of the National Bureau of Standards.