

Table I gives the amounts of vitamin B₁₂ required for half maximum growth at various salt concentrations. *The logarithm of the vitamin B₁₂ requirement appears to be a linear function of the ionic strength of the salt solutions*

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

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 $(B_{12})_{1/2 \text{ max.}}$ and μ from simple kinetic equations, if we make two basic assumptions: first, vitamin B₁₂ combines with a protein apoenzyme (Ea) to give the enzyme B₁₂Ea: $B_{12} + Ea \rightleftharpoons B_{12}Ea$; 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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ASCORBIC ACID DEFICIENCY AND CHOLESTEROL SYNTHESIS¹

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-C¹⁴-labeled ascorbic acid is not appreciably incorporated into cholesterol, the vitamin does exert a marked effect upon the conversion of acetate-1-C¹⁴ 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 C¹⁴ from acetate-1-C¹⁴ 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 C¹⁴-labeled components but there was only a slight change in activity of the cholesterol after purification by precipitation of the digitonide, dibromination⁵ and recrystallization. The observed changes in C¹⁴ content were not accompanied by comparable changes in total cholesterol present in the tissues⁶ but they were sufficient to indicate changes in the C¹⁴-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 *pH* activity curves for transphosphorylation and dephosphorylation have the same shape, with an optimum around *pH* 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 *pH* 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, desoxyribonucleosides³ 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*, **187**, 483 (1951).

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