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A NEW ENZYMATIC SYNTHESIS OF HEXOSE PHOSPHATES[1]

Sir:

An enzyme which catalyses the formation of hexose-6-phosphate and ammonia from potassium phosphoramidate (PNH_2) and hexose (reaction 1) has been obtained from extracts of succinate grown *E. coli*. The enzyme, presently named phosphoramidic hexose transphosphorylase (PHT), has been purified about 60-fold by fractionation with protamine sulfate, ammonium sulfate and diethylaminoethyl cellulose. The formation of P^{32} labeled organic phosphate esters from the free sugar and labeled PNH_2 was used as an enzyme rate assay. The results shown in Table I indicate that PHT catalyzed a phosphoryl transfer from PNH_2 to several hexoses, although the rate differed considerably with different hexoses. Neither pentoses nor nucleosides were phosphorylated by partially purified PHT.

TABLE I

FORMATION OF HEXOSE PHOSPHATES FROM PHOSPHORAMIDATE

The reaction mixture contained in 1 ml. 2-amino-2-methyl-1,3-propanediol buffer, *p*H 8.0, 100 μmoles; PNH_2, 9 μmoles (2160 c.p.m. per μmole); carbohydrate, as shown, 10 μmoles; enzyme *ca.* 0.9 mg. protein (obtained from a protamine treated extract of *E. coli* by precipitation with $(NH_4)_2SO_4$ at 68 to 78% saturation). Reaction was incubated at 37°, and stopped by addition of 0.5 ml. of 12% trichloroacetic acid and boiled for two minutes to hydrolyze remaining PNH_2.

Organic substrate	Total incorporation[a] c.p.m./15 min.	PNH_2 utilization[b] μmoles/15 min.
D-Fructose	3810	1.85
D-Fructose[c]	3030	1.40
L-Sorbose	2480	1.15
D-Glucose	1800	0.80
D-Glucosamine	980	0.45
D-Galactose	370	0.20

[a] Organic and inorganic phosphates separated by method of S. O. Nielson and A. L. Lehninger, *J. Biol. Chem.*, **215**, 555 (1955). [b] Measured as inorganic phosphate by method of C. H. Fiske and Y. SubbarRow, *ibid.*, **66**, 375 (1925). [c] Enzyme pretreated with charcoal at *p*H 5.5, 0.7 mg. of protein in assay.

The PHT reaction could be coupled to the reaction catalyzed by glucose-6-phosphate dehydrogenase, when glucose was used as the phosphate acceptor. Thus, the rate of the over-all reaction could be followed by reduced triphosphopyridine nucleotide (TPNH) formation and furthermore glucose-6-phosphate could be assumed as the product of transphosphorylation reaction. When individual hexoses were used as phosphate acceptors, with PHT and PNH_2, the corresponding hexose-6-phosphate was isolated and identified chromatographically using the solvent systems of Mortimer.[2]

PHT showed neither a divalent metal requirement nor a dependence on added nucleoside diphosphates and treatment of the partially purified enzyme with charcoal (*p*H 5.5), or with Dowex-I or with Versene (*p*H 7.5) failed to remove any cofactors participating in the reaction. Furthermore, when PHT was coupled with glucose-6-phosphate dehydrogenase no TPNH formation was observed if PNH_2 was replaced by adenosine triphosphate (ATP). When crystalline yeast hexokinase replaced PHT in the coupled system TPNH formation was observed only if ATP was added.

The enzyme preparation used in these studies was contaminated with PNH_2 hydrolase activity,[3] making stoichiometric measurements of NH_3 in reaction 1 unreliable. Further work on the purification and characterization of PHT is in progress.

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(1) This investigation was supported in part by grants from the Williams–Waterman Fund and the United States Public Health Service.

(2) D. C. Mortimer, *Can. J. Chem.*, **30**, 653 (1952).

(3) R. A. Smith and D. J. Burrow, *Biochim. et Biophys. Acta*, **34**, 274 (1959).

DEPARTMENT OF CHEMISTRY
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THE EFFECT OF PRESSURE ON SEDIMENTATION RATE

Sir:

Recently, Fujita[1] has derived relationships relating the boundary position to time for a monodisperse species in a sector-shaped cell, when the sedimentation coefficient depends on both pressure and concentration.

The boundary position $y_* = (r/r_0)^2$, the dilution (or concentration) factor $\theta_* = C/C_0$ and the reduced time, $\tau = 2\omega^2S_0t$, are related as shown in eq. (1).

$$\frac{dy_*}{d\tau} = \frac{y_*}{1 + \alpha\theta_*}(1 - m(y_* - 1)) \qquad (1)$$

where m is a pressure dependence parameter[1] and α is a concentration dependence parameter.[1]

We have solved Fujita's system of Equations (69) through (72), p. 3603 of reference (1), by the use of Runge-Kutta integration combined with trial and error iteration, using a Bendix G-15D digital computer programmed in pseudocode. A range of α from 0.1 to 1.0 was covered, and of m from 0.1 to 0.9. For flotation, negative values of τ were used. In the case of flotation, the reference pressure is the pressure at the cell bottom (see reference (1)) for definition of symbols.

It is the purpose of this communication to show that a simple relation between boundary position and reduced time can be developed, which fits the exact solution of this system of equations to a very good approximation. Oth and Desreux[2] developed a relationship which is essentially equivalent to letting $\theta_* = 1/y_*$ in Equation (1).

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(1) H. Fujita, THIS JOURNAL, **78**, 3598 (1956).

(2) J. Oth and V. Desreux, *Bull. soc. Chim. Belges*, **63**, 133 (1954).

Instead of this, Fujita's expression for θ_* at $\alpha = 0$, was used: namely

$$\theta_* = \frac{1}{y_*(1 + m - my_*)} \tag{2}$$

On substitution of (2) in (1) and integration, the result is

$$\tau = \frac{1}{1+m}\left\{1 + \frac{2m\alpha}{(1+m)^2}\right\} \ln\left(\frac{y_*}{1 + m - my_*}\right) + \frac{\alpha}{(1+m)^2}\left\{1 - m - \frac{1 + m - 2my_*}{(1 + m - my_*)y_*}\right\} \tag{3}$$

The agreement for $\alpha = 1.0$, $m = 0.5$ is shown in the table.

	τ by		
y^*	Computer solution	Equation (3)	Oth-Desreux
1.0406	0.08000	0.08002	0.07959
1.0823	.16000	.15996	.15835
1.1250	.24000	.23977	.23622
1.1687	.32000	.31960	.31318
1.2132	.40000	.39927	.38918
1.2584	.48000	.47878	.46420
1.3042	.56000	.55816	.53819
1.3503	.64000	.63711	.61111
1.3968	.72000	.71602	.68294
1.4433	.80000	.79443	.75363

For lower values of α and m agreement is even better, for example, at $m = 0.1$, $\alpha = 1.0$, $\tau = 0.79889$ *vs.* 0.8000 at $y = 1.5298$; at $m = 0.1$, $\alpha = 0.2$, the two values of τ differ by 2.5 parts in 10,000 at $y = 1.4938$. As would be expected, a very good approximation always is obtained for y close to unity.

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RECEIVED JUNE 25, 1959

BOOK REVIEWS

The Chemical Behavior of Zirconium. By WARREN B. BLUMENTHAL, Chief of Chemical Research, Titanium Alloy Manufacturing Division, National Lead Company, Niagara Falls, New York. D. Van Nostrand Company, Inc., 120 Alexander Street, Princeton, N. J. 1958. vi + 398 pp. 15.5 × 23.5 cm. Price, $11.00.

This volume is offered "with the intent of providing students and research workers with a broad and detailed account of the chemistry of zirconium as an element and as a component of compounds, interstitial solutions, and alloys." To this end, the author has summarized the available information on zirconium chemistry in a monograph which far exceeds in comprehensiveness and scope any previously published treatise on this element. In doing this, he has avoided the purely encyclopedic approach by the liberal interpolation of interpretative comments and semi-theoretical concepts.

The book treats in order background chemistry, the element, the important interstitial and intermetallic phases, the simple compounds, and the complex compounds. Each section is liberally documented with original literature citations. Illustrations, except for phase diagrams, are at a minimum, but the text is replete with graphic formulations of various compounds and ionic species.

As a source of information about zirconium chemistry, the volume is highly recommended. As an interpretative treatment, however, the book cannot be as highly recommended. The theoretical and semi-theoretical portions are definitely weaker than the descriptive and are commonly more speculative than definitive. Thus, much of what is given as structural material has no support in experimental fact; many explanations are circuitously worded and couched in such general terms as to be without real meaning; and more attention is sometimes paid to the "rules" of zirconium chemistry than the available evidence would warrant. Typical is the comment on p. 37: "Although zirconium occurs both in cations and anions, it is incorrect to associate the positive charge of zirconium-containing cations with the zirconium atom. It is rather to be associated with the oxygen atom."

Mechanically, the book is well done and attractive. Errors in printing and proof reading are at a minimum. An excellent author index is provided. The subject index, however, is much too brief and non-inclusive to do the volume justice.

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THERALD MOELLER

Vitamins and Hormones. Advances in Research and Applications. Volume XVI. Edited by ROBERT S. HARRIS, Professor of Biochemistry and Nutrition, Massachusetts Institute of Technology, Cambridge, Massachusetts, G. F. MARRIAN, Professor of Medical Chemistry, University of Edinburgh, Edinburgh, Scotland, and KENNETH V. THIMANN, Professor of Biology, Harvard University, Cambridge, Massachusetts. Academic Press Inc., 111 Fifth Avenue, New York 3, N. Y. 1958. xi + 437 pp. 16 × 23.5 cm. Price, $11.60.

Volume XVI of "Vitamins and Hormones" is a book which covers nine separate subjects of great current interest. Each chapter is an authoritative account of the present stage of development of the particular subject and written so that it is of interest to the uninformed as well as to the expert in the field. The literature references are particularly valuable in most of the chapters because they have been gathered from such a wide variety of sources.

Three chapters, "Nutritional Effects of Parasitic Infections and Disease," "The Dependence of Gonadal Function upon Vitamins and Other Nutritional Factors" and "Vitamins and Other Nutrients in Cardiovascular Disease," deal principally with nutrition and physiology. Three of the chapters, "Chemical Structure in Relation to Biological Activities of Vitamin B_6," "Glucagon" and "Synthetic Derivatives of Cortical Hormones" are largely chemical in nature. The remaining three, "Hormonal Aspects of Coronary Artery Disease," "The Physiology of Secretin" and "Chemistry and Physiology of the Thyroid-Stimulating Hormone," deal mostly with physiological and biological aspects.

For many years it has been realized that diet had a considerable influence on parasitism and disease, but the interdependence of host and parasite nutrition has been too complex and variable to permit any generalization. The chapter by Geiman indicates that progress has been made because of a better understanding of the metabolism of both host and parasite. No simplification of the problem has yet resulted from this progress.

A similar evaluation could be made of the relation of nutrition and gonadal function covered by Lutwak-Mann. A number of pertinent questions are asked at the beginning of the chapter. It presents many data bearing on the questions, but in most cases these are not yet adequate for definite answers.

The same is true for the relation of nutrients and cardiovascular disease. In the opinion of the authors of this chapter, Felch, Sinisterra, Itallie and Stare, the theory of hyper-