been highly purified by Hilmoe.[7] The degradation of compounds of the type T_nP_{n-1} by this preparation was studied. It was found that, again, all the lower homologs were formed successively and that thymidine appeared only toward the end. However, the mononucleotide which accumulated was thymidine 3'-phosphate, as in the ribonucleotide series.[8] These results show that *the action of this enzyme as well is stepwise but begins from the end bearing the 5'-hydroxyl group.*

TABLE I

The reaction mixture consisted of *M* trihydroxymethylaminomethane, *p*H 8.9 (10 μl.), T_5P_4 (14 optical density units measured at 267 mμ), venom diesterase (31 μg. in 7 μl.) and water (20 μl.). Aliquots (6 μl.) were mixed with glacial acetic acid (1 μl.) and chromatographed in isopropyl alcohol–ammonia–water (7–1–2). The figures are the % of the total optical density in each aliquot.

Compound R_f	T_5P_4 0.05	T_4P_3 0.065	T_3P_2 0.24	T_2P 0.48	T 0.76	TP 0.18
			Time, min.			
2	36	34	5	0	0	25
5	10	21	19	8	0	42
15	9	6	11	14	2	58
30	7	4	3	10	6	70
60	4	3	1	3	13	76

It should be noted that both the snake venom and spleen phosphodiesterase preparations hydrolyzed, even if slowly, cyclic oligonucleotides[1] in which the 3'-hydroxyl group is involved in an ester linkage with the 5'-phosphate group at the other end of the chain. It is therefore possible for these preparations to attack at a point within a polynucleotide chain (*cf.* ref. 3).

The stepwise and complementary action of the two phosphodiesterases is strikingly reminiscent of the two proteolytic enzymes, carboxypeptidase and (leucine) aminopeptidase, which degrade polypeptide chains from the opposite ends, and is promising for the structural and sequential analysis of polynucleotides.

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(7) R. J. Hilmoe, unpublished work. We are grateful for a gift of this preparation.

(8) L. A. Heppel and R. J. Hilmoe in "Methods in Enzymology," Vol. II, Academic Press Inc., New York, N. Y., 1955, p. 565.

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ENZYME STUDIES ON THE BIOSYNTHESIS OF VALINE IN YEAST

Sir:

In previous reports[1-3] based on isotope tracer data, a mechanism for the biosynthesis of valine was proposed, which involved α-acetolactic acid as an intermediate. This hypothesis has recently received support from Umbarger, *et al.*[4] In the present communication we wish to describe the properties of a cell-free preparation from *Saccharomyces cerevisiae* which converts acetolactic acid to α-ketoisovaleric acid, the keto analog of valine.

Fresh baker's yeast (Fleischmann) was disrupted in 0.02 *M* phosphate buffer, *p*H 7.2, by means of a high-speed refrigerated centrifuge shaker described by Shockman, *et al.*[5] The crude, cell-free supernatant obtained after centrifugation at 80,000 *g* was either used as such or was dialyzed against cold distilled water for 18–20 hours. The enzyme preparations were incubated at 37° with acetolactic acid, synthesized as described by Krampitz.[6] After 4 hours, the solution was deproteinized with tungstic acid, and the 2,4-dinitrophenylhydrazones of the keto acids were prepared, extracted and chromatographed on paper, essentially according to the procedure of Cavalini, *et al.*[7]

Results of a single, typical experiment, illustrative of the properties of this system, are shown in Table I. Mixtures of undialyzed extract and acetolactic acid produced a strong hydrazone spot, not observed in the absence of either enzyme or substrate. The identity of this material with the hydrazone of α-ketoisovaleric acid was established by identical R_f values in four different solvents, by identical absorption spectra over the wave length range of 350 to 700 mμ, and by reduction of material isolated from a strip chromatogram, according to Towers, *et al.*,[8] to a substance identical chromatographically with valine in three different solvent systems.

TABLE I

ENZYMATIC CONVERSION OF α-ACETOLACTATE TO α-KETOISOVALERATE

Each tube contained 80 μmoles potassium phosphate buffer, *p*H 8.0, and where indicated in the table, 0.05 mg. TPN, 0.5 mg. DPN, 1.0 mg. ATP, 27 μmoles neutralized acetolactate, *p*H 8.0, 27 μmoles K acetate, 27 μmoles ethanol, 15 μmoles magnesium sulfate, and 0.3 ml. of dialyzed or undialyzed enzyme, containing approximately 4 mg. protein (by ultraviolet absorption) and representing 0.15 g. of fresh yeast. Total volume was 1.6 ml. and incubation was conducted 4 hours in air at 37°.

Enzyme preparation	Acetolactate	Cofactor(s)	α-Ketoisovalerate micromoles
Crude	+	—	1.28
Crude	+	TPN	2.10
Dialyzed	+	—	0.06
Dialyzed	+	Crude heated	1.83
Dialyzed	+	DPN	0.14
Dialyzed	+	DPN, ATP, Mg^{++}	2.19
Dialyzed	+	TPN	2.97
Dialyzed[a]	Acetate + ethanol	TPN	0.02

[a] This was run to check the effects of acetate and ethanol present as a result of hydrolysis of the ethyl acetoxyacetolactate.

The values given in Table I were obtained by elution of the α-ketoisovaleric acid hydrazone spots

(1) M. Strassman, A. J. Thomas and S. Weinhouse, THIS JOURNAL **75**, 5135 (1953); **77**, 1261 (1955).

(2) E. A. Adelberg, *ibid.*, **76**, 4241 (1954).

(3) M. Strassman, A. J. Thomas, L. A. Locke and S. Weinhouse, *ibid.*, **76**, 4241 (1954).

(4) H. E. Umbarger, B. Brown and E. J. Eyring, *ibid.*, **79**, 2980 (1957).

(5) G. D. Shockman, J. J. Kolb and G. Toennies, *Biochim. Biophys. Acta*, **24**, 203 (1957).

(6) L. O. Krampitz, *Arch. Biochem.*, **17**, 81 (1948).

(7) D. Cavalini and N. Frontali, *Biochim. Biophys. Acta*, **13**, 439 (1954).

(8) G. H. N. Towers, J. F. Thompson and F. C. Steward, THIS JOURNAL, **76**, 2392 (1956).

and measurement of their absorption at 510 mμ.[7] A mixture of undialyzed enzyme and acetolactic acid produced 1.3 μmoles of α-ketoisovaleric acid, and the yield was almost doubled by TPN addition. Crude extract or substrate alone, or dialyzed extract with substrate yielded only negligible amounts. Addition of boiled crude extract, TPN, or DPN and ATP restored activity of the dialyzed extract.

These data demonstrate that acetolactate is a precursor of valine in *S. cerevisiae*, and suggest that TPN (probably TPNH) is involved with this transformation. Further efforts are proceeding for purification and fractionation of this enzyme system and for identification of several other keto acids which appeared in the chromatograms.[9]

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THE EFFECT OF OXYGEN ON FERRIC ION YIELDS IN AQUEOUS SOLUTIONS CONTAINING POLONIUM

Sir:

A revised $G(Fe^{+++})$ yield of 5.2 ± 0.15 has been obtained for α-particles from dissolved Po^{210} in the aerated Fricke ferrous sulfate dosimeter. The previous value of about 6.0 reported by various workers[1,2,3] is probably too high. Other lower values[4,5,6] have been reported recently. $G(Fe^{+++})$ has been found to vary from 3.7 ± 0.2 in air-free solutions to values approaching 10 at oxygen concentrations above 0.1 *M*.

Solutions studied were 3 m*M* in $FeSO_4$, 1 m*M* in NaCl, 0.8 *N* in H_2SO_4 and contained on the average 0.4 mc./ml. of Po^{210}. $G(Fe^{+++})$ was independent of dose rate between 0.01 and 0.90 mc./ml. Oxygen pressures below 1 atmosphere were obtained by evacuating the samples and reintroducing known partial pressures of oxygen. Oxygen, at a known pressure, was introduced into a stainless steel bomb containing cells with polonium solutions for high pressure studies. Oxygen concentrations were calculated from the data of Zoss, Suciu and Sibbitt.[7] Suitable corrections were made for the dark reaction and oxidation at atmospheric pressures. The change in ferric ion concentration was followed spectrophotometrically and the rate of energy absorption in the solutions was obtained from the rate of Po^{210} disintegration. This rate was measured by absolute counting in a calibrated pulse ionization chamber. The full energy of the 5.3 Mev. alpha particle was assumed to have been expended within the solution.

$G(Fe^{+++})$ increases rapidly from its air-free value of 3.7 to 5.2 for air-saturated solutions (2.2×10^{-4} *M* O_2). Above oxygen concentrations of 10^{-3} *M*, however, $G(Fe^{+++})$ is nearly a linear function of the logarithm of the oxygen concentration. The highest experimental $G(Fe^{+++})$ value obtained in this work was 9.1 ± 0.4 at 1905 p.s.i. oxygen.

The increase in $G(Fe^{+++})$ with oxygen concentration is attributed to a competition of reaction (1) with reactions (2), (3), and (4) in the mechanism of the Fricke ferrous sulfate dosimeter.[5]

$$H + O_2 = HO_2 \quad (1)$$

$$H + H = H_2 \quad (2)$$

$$H + OH = H_2O \quad (3)$$

$$H + H^+ + Fe^{++} = H_2 + Fe^{+++} \quad (4)$$

Work with higher than 3 m*M* initial ferrous ion concentration $[(Fe^{++})_0]$ indicates a marked dependence of $G(Fe^{+++})$ on $(Fe^{++})_0$ for oxygen concentrations in the regions of 10^{-2} to 10^{-1} *M*. In contrast, at atmospheric pressures the ferric ion yield is much less sensitive to $(Fe^{++})_0$. Further work is being carried out on the effect of ferrous ion concentration and on the effect of pressures above 2000 p.s.i.

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(1) N. Miller and J. Wilkinson, *Trans. Faraday Soc.*, **50**, 690 (1954).
(2) W. R. McDonell and E. J. Hart, THIS JOURNAL, **76**, 2121 (1954).
(3) M. Haissinsky and M. C. Anta, *Compt. rend.*, **236**, 1161 (1953).
(4) W. R. Henke, P. K. Kruys and P. P. Lifland, Project Report for the School of Nuclear Science and Engineering, (Argonne National Laboratory) 2nd Session, June 1, 1956.
(5) A. O. Allen, *Int. Conf. on Peaceful Uses of Atomic Energy*, Vol. 7, 513, United Nations, N. Y., 1956.
(6) M. Lefort, *Compt. rend.*, **245**, 1623 (1957).
(7) A. Zoss, S. N. Suciu, W. L. Sibbitt, *Trans. Am. Soc. Mech. Eng.*, **76**, 69 (1954).