this latter glass, a much smaller number, ca. 25 phosphorus atoms per chain, is calculated for the dividing line between precipitation and no precipitation.

The data given in the last line of Table I show that a polyphosphate with a chain length of 16 appears to be quantitatively separated from a polyphosphate having a chain length of 1600 by precipitation with albumen in the presence of trichloroacetic acid. The fact that a mixture which is approximately 50–50 in the long- and short-chain phosphates can be separated into two equal-sized fractions is in accord with the conclusion that the phosphate glass having the shortest average chain length should give only a trace of precipitate instead of the ca. 25% observed. Presumably, a large part of this 25% is attributed to coprecipitation or some mechanism other than the well-known "metaphosphate-protein reaction"[8].

The data presented suggest that the differential separation into two polyphosphate fractions, which occurs when yeast cells are partitioned with trichloroacetic acid, may be due to the difference in protein complexing properties between polyphosphates which differ in chain length. The observations do not appear to be an artifact, as has been suggested by SCHMIDT[9]. Naturally, the nature of the protein-polyphosphate interaction is dependent upon many variables, and a more comprehensive investigation of this phenomenon is under way. In addition, an attempt is being made to determine the chain length (molecular weight) of the polyphosphate fractions in yeast, using physical-chemical methods. The exact role of molecular size in determining the different metabolic activities of the polyphosphates in yeast is not clear at the present time.

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ENZYMIC DECOMPOSITION OF THE ACTIVE INTERMEDIATE IN CITRULLINE SYNTHESIS

by

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In 1949 one of the authors (S.G.) made the observation that skeletal and cardiac muscle homogenates when added to mitochondrial preparations of rat liver inhibited the reaction ornithine $\longrightarrow$ citrulline. Recent studies on the reaction ornithine $\longrightarrow$ citrulline[1,2,3] have shown this reaction to be composed of two main enzymic steps, reactions 1 and 2.

1. $CG^{**} + CO_2 + NH_3 + ATP \xrightarrow[Mg^{++}]{\text{Enzyme I}}$ Compound X + ADP

2. Compound X + Ornithine $\xrightarrow{\text{Enzyme II}}$ Citrulline + CG + $PO_4^{\equiv}$

* This work was done during the tenure by the senior author of an Established Investigatorship of the American Heart Association.

** The following abbreviations are used throughout this paper: CG, carbamyl-L-glutamate; Compound X, the active intermediate for citrulline synthesis[2] which contains one mole each of CG, CO_2, NH_3, and $PO_4^{\equiv}$. ATP and ADP, adenosine tri- and di-phosphates respectively; Enzymes I and II, enzyme systems as previously described[2]. All analytical methods and procedures have been described in preceding papers[1,2,3].

These advances in the understanding of the mechanism of the reaction ornithine → citrulline have permitted us to reinvestigate the action of tissue extracts upon Compound X. It is shown in this paper that soluble preparations obtained from a variety of tissues enzymically decompose Compound X.

The enzymic decomposition of Compound X with time is clearly seen in Fig. 1. The muscle extract increases the rate of decomposition by a factor of approximately 2.5. That the decomposition is of an enzymic nature is also indicated in the figure since heated muscle preparations show no effect whatsoever on the non-enzymic decomposition rate. Enzymic decomposition of Compound X is also achieved by similar extracts from acetone dried preparations of other tissues. Referring the activity to brain on the basis of activity per milligram of protein, the following comparative values were obtained with rabbit tissue preparations; brain 100, cardiac muscle 17, skeletal muscle 12, liver 7, and kidney 7.

It is also possible that the enzyme described in this paper may possess high activity when tested with intermediates formed from other glutamyl derivatives active in citrulline synthesis[3]. Work to this effect is in progress.

Although the intimate mechanism of the enzymic decomposition of Compound X is unknown, it is reasonable to assume that it is due to a dephosphorylation since the phosphate of Compound X is extremely labile[1]. If this is the case, it is perhaps most likely due to the action of a phosphatase

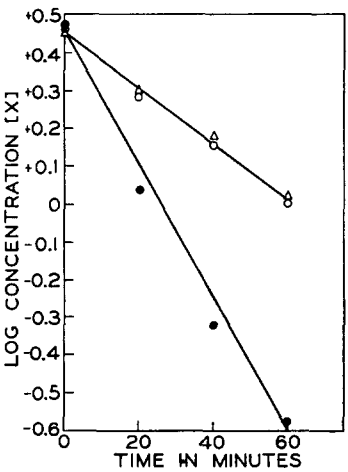

Fig. 1. The enzymic decomposition of Compound X. Each tube contained the following, expressed in micromoles per 2.5 ml; tris(hydroxymethyl)aminomethane buffer, pH 7.4, 75; Compound X, potassium salt 2.98; 2000 × *g* supernate of a water extract of acetone dried rabbit skeletal muscle, 26 mg protein per tube. Temperature 38° C. Experimental points marked with open triangles refer to spontaneous decomposition of Compound X. Experimental points marked with open circles refer to values obtained in the presence of heated (5 minutes at 100° C) muscle preparation. Experimental points marked with solid circles represent the values obtained in the presence of the unheated muscle preparation. Compound X was estimated in all cases as previously described[2].

and not to a transfer reaction. The energy level of the phosphate bond in Compound X is unknown and it has not been possible so far to achieve phosphate transfer from Compound X to ADP even in the presence of hexokinase and glucose with liver preparations (R. O. MARSHALL AND S. GRISOLIA, unpublished experiments) or to citrulline (S. RATNER AND S. GRISOLIA, R. O. MARSHALL AND S. GRISOLIA, unpublished experiments). Regardless of the mechanism of the enzymic decomposition of Compound X, the experiments reported here appear to be the first clue to a direct interference of sufficient magnitude to alter the efficiency of the urea cycle; thus it is conceivable that this phenomenon may be related to the specific dynamic action of proteins and related compounds. The synthesis of Compound X is an endergonic reaction; therefore if, for example, the cell were unable to cope with the utilization of Compound X or related compounds[3] due to the presence of active enzyme systems which will decompose the compound, a considerable leakage of high energy phosphate might result.

The early observations with mitochondrial preparations may indicate that under certain conditions these cell units may be somewhat permeable to Compound X. At any rate, these experiments should be extended in the direction of the intimate mechanism of the reaction with soluble preparations and also with mitochondria or at even higher levels of organization.

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