then submitted to paper chromatography using xylol as the developing solvent on Whatman No. 1 paper treated with formamide-acetone (1:3 by volume)[8]. The spots were marked through the ultraviolet absorption with the aid of fluorescence screen. Valine which has been shown to be N-terminal amino acid of trypsinogen both by dinitrofluorobenzene method[2] and Edman's method[9] was detected throughout the whole stage of the activation with practically constant strength. *Iso*leucine which has been shown to be N-terminal amino acid of di*iso*propylphosphofluoridate-treated trypsin (DIP-trypsin)[2] and of trypsin formed by autoactivation[9] was detected with increasing strength accompanying the appearance of tryptic activity. Other spots localized at the original points or of low R_F values were considered to be of minor interest in this connection. The spots corresponding to valine and *iso*leucine were cut off and the phenylthiohydantoin derivatives were eluted by 1 ml of 95% ethanol for one hour at room temperature. The eluates were measured on ultraviolet absorption at 270 mμ. The amounts of *iso*leucine were calculated against those of valine at each run. The agreement between the percentages of the activation and those of *iso*leucine referred to valine is shown in Fig. 1. The elution techniques have been worked out at least for valine, *iso*leucine and alanine[10] and are being applied to other amino acids[11].

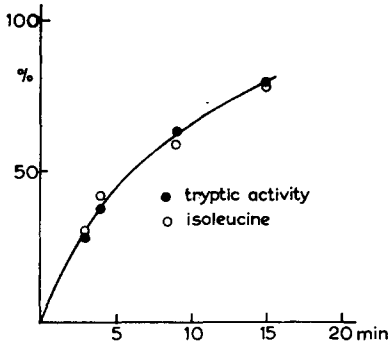

Fig. 1. Relationship between tryptic activity and N-terminal *iso*leucine.

Judging from these experimental results, it is likely that enterokinase acts as a peptidase-splitting valylpeptide[3] from trypsinogen. The details will be published in *Acta Chem. Scand.* in 1956. This work was made possible through a research grant to Professor ERIK JORPES from the Swedish State Medical Research Council. The author's thanks are also due to Dr. PEHR EDMAN for his help during the performance of the work.

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Mitochondrial oxidative phosphorylation in a magnesium-free medium

The assumption, that magnesium is a cofactor in the reactions involved in oxidative phosphorylation[1,2,3], is based on the fact that maximum P/O values for mitochondrial preparations could be obtained only in media containing added Mg^{++}. An assembly of respiratory enzymes, capable of oxidative phosphorylation without added Mg^{++}, has recently been obtained by LEHNINGER'S group[4]. Its endogenous magnesium content is unknown.

With intact rat liver mitochondria, using the rapid platinum electrode technique for simultaneous measurement of respiratory and phosphorylative effects[5], we have obtained respiratory control and maximum P/O values for the oxidation of β-hydroxybutyrate or succinate in a medium containing neither magnesium ions nor other divalent cations. The mitochondria were prepared as in ref. [5]. Table I shows P/O values for β-hydroxybutyrate oxidation in a magnesium-containing (I) and a magnesium-free (II) medium.

TABLE I

P/O VALUES FOR THE OXIDATION OF β-HYDROXYBUTYRATE

Experiment	*Medium*	*P/O*
1	I*	2.8
	II**	2.9
2	I	2.8
	II	2.8

* 16 m*M* phosphate buffer, 12 m*M* sodium fluoride, 6 m*M* magnesium chloride and 84 m*M* potassium chloride.

** 16 m*M* phosphate buffer, 12 m*M* sodium fluoride and 93 m*M* potassium chloride. The pH of I and II was 7.2.

An uncoupling event of a kind not previously reported occurs some minutes after incubation in medium II at room temperature with β-hydroxybutyrate as substrate. Uncoupling is here defined as loss of respiratory control. The event is manifested by a temporary, two- to fourfold increase of the respiration rate. During this interval the ability of adenosine diphosphate and 2,4-dinitrophenol to stimulate the respiration disappears. The uncoupling event can be correlated with a rapid fall in optical density at 510 mμ, due to swelling and structural disorganization of the mitochondria[6]. The controls in medium I had a constant respiration rate and optical density at 510 mμ. As the fall occurs somewhat before the uncoupling event (see Fig. 1) one can postulate that the morphological change causes the respiratory stimulation and not *vice versa*. With succinate the changes appear later and adenosine phosphates have a stabilizing effect: the changes occur after a period several times longer and are more gradual. Thus, especially for succinate oxidation, in a magnesium-free medium containing an adenosine phosphate the stability should be great enough to permit maximum P/O values even with the Warburg technique. This was indeed recently observed by ERNSTER AND LÖW[7].

Our results show that the lack of Mg^{++} in the medium causes a great and significant morphological change in the particles and indicate that a role of magnesium in oxidative phosphorylation of isolated mitochondria is to maintain the proper mitochondrial structure. The question whether magnesium also takes part in the actual oxidative phosphorylation reactions remains open.

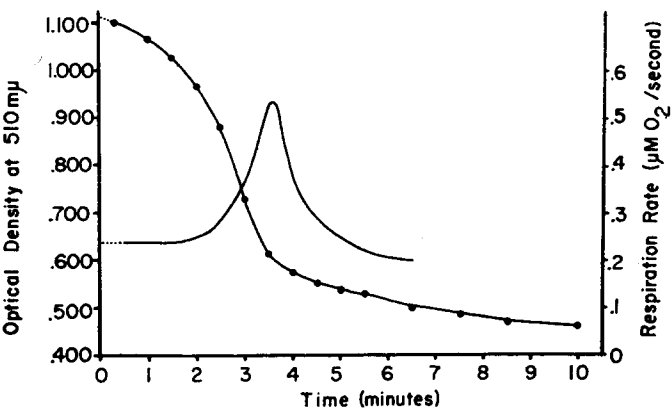

Fig. 1. Change of respiration rate and optical density at 510 mμ with time for mitochondari suspended in medium II. Zero time: addition of 10 m*M* (final concentration) of β-hydroxybutyrate 30 seconds after the mitochondria were added. Temperature: 25° C. In the measurement of the respiration rate the mitochondria were twice as concentrated as in the measurement of the optical density. ● = optical density, — = respiration rate.

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