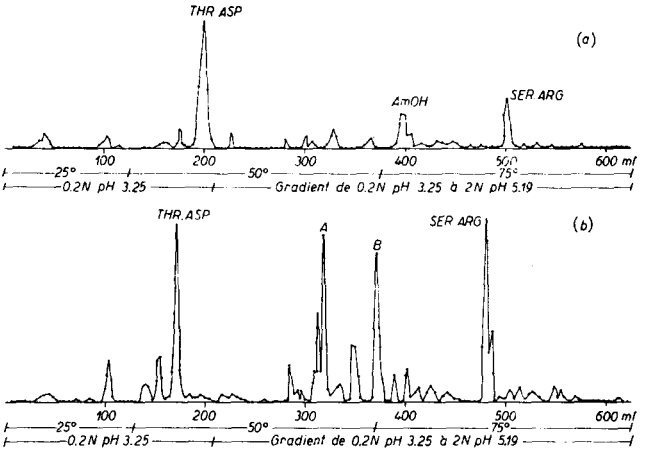

Fig. 1. Peptides formés pendant l'attaque chymotrypsique du chymotrypsinogène (a) et pendant l'activation "lente" de ce zymogène (b). (a): ChTg: $4.4 \cdot 10^{-3}$ *M*; ChT-α: $0.45 \cdot 10^{-3}$ *M*; Temp.: 0°; pH = 7.6; Durée du traitement: 46 h. (b): ChTg: $4.4 \cdot 10^{-3}$ *M*; Trypsine: $4.8 \cdot 10^{-7}$ *M*; SO_4Am_2: 0.3 *M*; Temp.: 6°; pH: 7.6; Durée du traitement: 46 h. Après traitement des hydrolysats par le di*iso*-propylfluorophosphate, les peptides qu'ils renferment sont débarrassés des protéines et éventuellement des ions SO_4 par adsorption sur Dowex-50 × 12 forme H^+ et élution avec NH_35N. Colonne de 87 cm. Chambre de mélange: 330 ml. Débit: 8 ml/h. Fractionnement par ml. Dessalage des pics (en vue de l'étude ultérieure sur papier de leurs hydrolysats): par échange de leurs ions avec ceux de l'acétate d'ammonium.

Nos expériences ne sont pas encore assez avancées pour permettre d'interpréter de façon définitive le phénomène de l'activation "lente". Nous ignorons en particulier si certains peptides ne sont pas perdus pendant le traitement préalable des hydrolysats par le Dowex-50 × 12 et si une élution plus complète des colonnes de Dowex-50 × 4 ne ferait pas sortir d'autres pics. Mais il est utile de signaler dès maintenant que cette activation engendre au moins trois peptides. Deux d'entre eux sont formés par des processus d'ores et déjà connus. Nous cherchons actuellement l'origine du troisième.

Les résultats du Tableau I suggèrent enfin que la thréonine N-terminale apparue pendant l'activation appartient bien à une protéine active, et non à une impureté peptidique comme on le pense quelquefois[8-6]. Si tel est le cas, il faudrait prévoir l'existence d'un nouvel enzyme de l'activation "lente", la chymotrypsine-α_1 possédant tous les résidus terminaux de l'enzyme-α, sauf l'alanine qui serait remplacée par la thréonine.

M. ROVERY
M. POILROUX
A. CURNIER
P. DESNUELLE

Laboratoire de Chimie Biologique, Faculté des Sciences, Marseille (France)

[1] M. ROVERY, M. POILROUX, A. CURNIER ET P. DESNUELLE, *Biochim. Biophys. Acta*, 16 (1955) 590.
[2] M. ROVERY, M. POILROUX, A. CURNIER ET P. DESNUELLE, *Biochim. Biophys. Acta*, 17 (1955) 565.
[3] W. J. DREYER ET H. NEURATH, *J. Am. Chem. Soc.*, 77 (1955) 814.
[4] M. ROVERY ET P. DESNUELLE, *Biochim. Biophys. Acta*, 130 (1954) 30.
[5] M. ROVERY, M. POILROUX ET P. DESNUELLE, *Biochim. Biophys. Acta*, 14 (1954) 145.
[6] F. R. BETTELHEIM ET H. NEURATH, *J. Biol. Chem.*, 212 (1955) 241.
[7] S. MOORE ET W. H. STEIN, *J. Biol. Chem.*, 211 (1954) 907.
E. F. JANSEN, M. D. F. NUTTING, R. JANG ET A. K. BALLS, *J. Biol. Chem.*, 179 (1949) 189.

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The incorporation of labeled amino acids into the cytoplasmic particles of rat muscle*

Results from a number of laboratories have provided evidence that of the various cell particulates isolated by the SCHNEIDER AND HOGEBOOM[1] technique, the microsomal fraction plays a leading role in the incorporation of labeled amino acids into protein. The high rate of incorporation

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into the protein of this fraction has been demonstrated *in vivo* in liver using a number of amino acids and various species of animals[2,3,4]. On the other hand, mitochondria have appeared to play a quantitatively less important role in this process, since this fraction showed a much lower rate of incorporation than the microsome fraction. There is reason to believe that the microsomal fraction is also of importance in pancreas and kidney[5].

These results have received strong support from *in vitro* experiments[1,6], using labeled amino acids, in which liver particulate fractions have been incubated separately and together. The mitochondrial proteins showed extremely low incorporation as compared with the microsomes, although phosphorylating mitochondria exerted a large stimulatory effect on incorporation into microsomal protein when the two fractions were incubated together. This increased incorporation into the microsomes could be effected in the absence of mitochondria provided that another adenosine triphosphate generating system is supplied.

These investigations, together with recent observations on the role of the soluble fraction[7], have lent support to the concept that the microsomes are responsible for most of the synthesis of protein in the cell, that the primary role of the mitochondria is to furnish energy for the process, and that the soluble fraction functions in the "activation" of the amino acids.

The results of the *in vivo* experiments described here indicate that the picture in muscle is somewhat different. In this tissue, the protein of the mitochondrial fraction incorporates amino acids at approximately the same rate as the microsomal protein.

Male Sprague-Dawley rats weighing approximately 200 g were given intravenous injections of 3.0 mg of DL-leucine-1-^{14}C. After various intervals (2, 3, 5, 10, 30, 90, 180 minutes), the animals were sacrificed and samples of skeletal muscle and liver were rapidly removed and chilled in 0.25 *M* sucrose solution at 0°. The time between the sacrifice of the animal and the chilling of the tissues amounted to approximately 90 seconds. The muscle was fractionated using a procedure based on the differential centrifugation method described by KITIVAKARA AND HARMON[8] with the following modifications. The muscle was homogenized using a thick-walled, narrow-diameter, glass homogenizer with a loose fitting pestle. After the fraction sedimenting at 200 × *g* and the myofibril fraction were removed, a mitochondria-rich fraction was obtained by centrifugation for 1 hour at 4400 × *g*. It appears that in addition to mitochondria, this fraction also contains an opaque particle peculiar to muscle[8,9]. The microsomes were obtained by further centrifugation for 1 hour at 78,000 × *g*. All particulate fractions were washed once. The myofibril, mitochondrial, and microsomal fractions were examined from time to time by dark field and phase contrast microscopy. In addition, the latter two fractions were subjected to electron microscopy. Except for the presence of a small amount of microsomes in the mitochondrial fraction, no appreciable contamination of one fraction by another could be detected.

The particulates of the liver were obtained by the method of SCHNEIDER AND HOGEBOOM[1]. The proteins of the liver and muscle fractions were precipitated, washed, and heated with 5% trichloroacetic acid[10], washed twice with alcohol and ether, and their specific radioactivities were determined.

Since the specific activity of the total protein rather than of the protein-bound leucine itself was measured, it was necessary to correct for the leucine content of the protein of each fraction. This was determined by microbiological assay using a leucine requiring *E. coli* mutant. The leucine content of each fraction was found to be about 10% of the weight of protein (mitochondria 9.4; microsomes, 9.9; myofibrils, 9.7; 200 × *g*, 8.4; supernatant 11.3).

In accord with the results obtained by previous workers[4], when the incorporation of labeled leucine into liver particulates was measured, the microsomal fraction was the most active and the mitochondrial fraction the least active, the latter having less than 25% of the uptake of the microsomal fraction. This difference was most marked at short time intervals. In muscle (Fig. 1), however, the rate of incorporation into the mitochondrial fraction was not appreciably lower than that into the microsomes. Indeed, at short time intervals (5 minutes or less), the incorporation into the mitochondrial fraction of muscle was actually somewhat higher than into the microsomal fraction. Essentially the same results were obtained when phenylalanine-3-^{14}C was substituted for leucine-1-^{14}C.

Experiments are in progress entailing further fractionation of the microsomal and mitochondrial preparations, with particular attention given to the opaque particles associated with the latter fraction. It is hoped that these studies in conjunction with proposed *in vitro* experiments will further clarify the role of these cellular components in the incorporation of amino acids into muscle protein.

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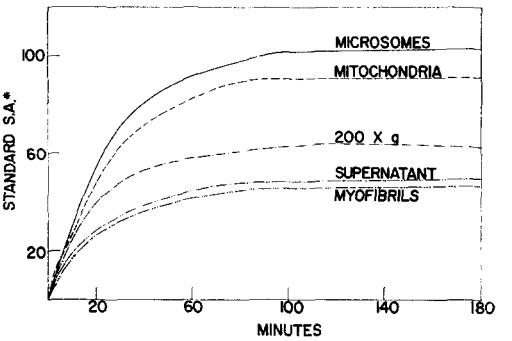

Fig. 1. The rate of uptake of DL-leucine-1-^{14}C by the particulate fractions of rat skeletal muscle.

* Standard specific activity. This is the specific activity obtained corrected for the variations in rat weight and in c.p.m. injected, and was calculated as follows:

$$\text{c.p.m. per } \mu\text{mole of protein-bound leucine} \times \frac{\text{rat wt in g}}{200} \times \frac{0.01 \cdot 10^6}{\text{c.p.m. of leucine injected}}.$$

Department of Biochemistry, Yale University, New Haven, Conn. (U.S.A.)

MELVIN V. SIMPSON
J. R. MCLEAN

[1] W. C. SCHNEIDER AND G. H. HOGEBOOM, *J. Biol. Chem.*, 183 (1950) 123.
[2] T. HULTIN, *Exptl. Cell Research*, 1 (1950) 376.
[3] H. BORSOOK, C. L. DEASY, A. J. HAAGEN-SMIT, G. KEIGHLEY AND P. H. LOWY, *J. Biol. Chem.*, 187 (1950) 839.
[4] E. B. KELLER, P. C. ZAMECNIK AND R. B. LOFTFIELD, *J. Histochem. Cytochem.*, 2 (1954) 378.
[5] V. ALLFREY, M. M. DALY AND A. E. MIRSKY, *J. Gen. Physiol.*, 37 (1953) 157.
[6] P. SIEKEVITZ, *J. Biol. Chem.*, 195 (1952) 549.
[7] M. B. HOAGLAND, *Biochem. Biophys. Acta*, 16 (1955) 288.
[8] A. KITIYAKARA AND J. W. HARMAN, *J. Exptl. Med.*, 97 (1953) 553.
[9] J. W. HARMAN, *Am. J. Phys. Med.*, 34 (1955) 68.
[10] W. C. SCHNEIDER, *J. Biol. Chem.*, 161 (1945) 293.

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Some new solvent systems for the paper chromatography of nucleic acid degradation products

Paper chromatographic methods have proved indispensable for the investigation of the components of nucleic acids[1]. Among the many solvent systems suggested the most generally useful have been the *tert*butanol and *iso*propanol-hydrochloric acid mixtures of MARHAM AND SMITH[2] and of WYATT[3]. These solvent systems have the disadvantage of being somewhat slow in development and it has been found that equally satisfactory results can be achieved with mixtures of methanol, ethanol and hydrochloric acid.

Chromatography has been carried out by upward development on paper strips 16.5 cm × 47 cm. Two holes were punched in each paper and five papers were hung on an all-glass frame which was placed in a glass tank sealed with a plate glass lid.

A mixture of methanol: concentrated HCl: water (70:20:10) (all parts by volume) (Solvent system I) was found to separate the principal bases liberated by complete hydrolysis of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). This solvent system would also separate guanine, adenine, cytidylic and uridylic acids which were liberated when ribonucleic acids were hydrolysed with *N* HCl at 100° C. The chromatograms obtained have been used for quantitative estimations. The solvent rose about 40 cm on Whatman No. 4 paper in 16 hours and Whatman No. 1 gave equally good results.

This solvent system was rather slow in drying and much less hydrochloric acid was needed to separate the products of the hydrolysis of RNA with *N* HCl at 100°. The most suitable system of those tried was a mixture of methanol: ethanol: concentrated HCl: water (50:25:6:19) (Solvent system II). This solvent system moved about the same distance overnight as solvent system I and was the most suitable for quantitative determination. It dried very quickly and gave reproducible and very low blank values. Guanine was eluted with 0.5 *N* HCl, adenine with 0.1 *N* HCl and cytidylic and uridylic acids were eluted with 0.2 *M* sodium acetate. Absorption values were measured at 249, 260, 269.5 and 261 mμ for the four components respectively. The ε values of WYATT[3] were used for guanine (11,000) and adenine (13,000) and values of 9050 and 10,200 were found for cytidylic and uridylic acids at the above wavelengths in 0.2 *M* sodium acetate.

A further series of solvent systems has been investigated and it was found that very little HCl was necessary if a large proportion of acetic acid was present. A mixture of methyl ethyl