Zur Synthese der Teilsequenzen I–IX wurde BOC-Ala-Phe-$NHNH_2$ mit H-Ileu-Gly-OMe bzw. H-Leu-Gly-OMe oder H-Val-Gly-OMe gekuppelt und nach Umwandlung in die Hydrazide mit H-Leu-Met-NH_2 oder H-Leu-Ala-NH_2 umgesetzt. Nach der Abspaltung der BOC-Gruppe wurde mit BOC-Asp(OtBu)-OH, MeOCbo-Asp(NH_2)-$OPhNO_2$, MeOCbo-Glu(NH_2)-$OPhNO_2$ oder MeOCbo-Pyroglu-OH gekuppelt. Die freien Heptapeptidamide resultierten nach Abspaltung der Schutzgruppen mit Trifluoressigsäure.

Zur Synthese von X–XII wurde aus BOC-Gly-OH und H-Ala-Phe-OMe das Tripeptid erhalten, das nach Hydrazinolyse des Methylesters mit H-Ileu-Gly-Leu-Met-NH_2 bzw. der entsprechenden Val- oder Leu-Verbindung umgesetzt wurde. Die erforderlichen Tetrapeptidamide wurden aus BOC-Val-Gly-$NHNH_2$ bzw. BOC-Leu-Gly-$NHNH_2$ und H-Leu-Met-NH_2, das Ileu-Derivat aus H-Leu-Met-NH_2 durch schrittweise Ankondensation von BOC-Gly-OH und BOC-Ileu-OH hergestellt.

Die Verbindungen XIII–XVII stellen Zwischenprodukte dieser Synthesen dar. Da ein Teil der hier beschriebenen Verbindungen zur Synthese von Eledoisinanaloga Verwendung fand, erfolgt eine ausführliche Beschreibung an anderer Stelle. Die analytischen und biologischen Daten der synthetisierten Verbindungen sind in der Tabelle zusammengestellt. Alle Verbindungen mit Ausnahme von IX sind mit Leucinaminopeptidase vollständig hydrolysierbar. Bei den biologischen Daten wird die Kontraktion des Meerschweinchen-Ileums als Grenzdosis in ng/ml angegeben. Der Kaninchenblutdruck bezieht sich auf ein von uns synthetisiertes Eledoisin[9] (Meerschweinchen-Ileum, Grenzdosis 0,15 bis 0,3 ng/ml; Kaninchenblutdruck, Grenzdosis 0,5 bis 1 ng/kg, relative Aktivität zu Bradykinin etwa 10fach).

Der Vergleich der biologischen Wirksamkeiten erlaubt bereits eine Aussage über die für eine Aktivität entscheidenden Aminosäuren.

Die kürzeste noch deutlich wirksame Sequenz ist das C-terminale Pentapeptid[2]. Wesentlich höher wirksam sind die Hexapeptide, die Heptapeptide liegen zum Teil bereits in der gleichen Grössenordnung wie das Eledoisin selbst. Der Asparaginsäurerest lässt sich ohne wesentliche Beeinflussung gegen andere Aminosäuren austauschen (IV, VII und X). Der Isoleucinrest lässt sich bei nur geringem Abfall der Wirkung durch Valin ersetzen (XI und XIV), einen sehr starken Wirkungsverlust bewirkt der Ersatz durch Leucin (II, V, VIII und XV). Ebenso führt der Austausch des Methionins gegen Alanin zu geringer wirksamen Verbindungen (III, VI und XVI). Die Bedeutung des Methioninrestes wird auch durch die kürzlich von CAMERINO et al.[2] publizierten Teilsequenzen bestätigt. Erstaunlich ist die sehr hohe, mit dem Eledoisin vergleichbare Wirkung der Glu(NH_2)- und Gly-Heptapeptide (VII, X und XI).

Summary. A series of synthetic Eledoisin and Eledoisin-related subunits are described. The biological activities of the peptides are discussed.

E. SCHRÖDER und K. LÜBKE

Hauptlaboratorium der Schering AG, Berlin-West (Deutschland), 12. September 1963.

[1] *Über Peptidsynthesen XXIII.* 2. Mitt. über Eledoisin und Eledoisinanaloga. 1. Mitt. siehe [9].
[2] B. CAMERINO, G. DE CARO, R. A. BOISSONNAS, ED. SANDRIN und E. STÜRMER, Exper. *19*, 339 (1963).
[3] V. ERSPARMER und A. ANASTASI, Exper. *18*, 58 (1962).
[4] A. ANASTASI und V. ERSPARMER, Arch. Biochem. Biophys. *101*, 56 (1963).
[5] ED. SANDRIN und R. A. BOISSONNAS, Exper. *18*, 59 (1962).
[6] V. ERSPARMER und G. F. ERSPARMER, Brit. J. Pharmacol. *19*, 337 (1962).
[7] V. ERSPARMER und A. GLAESSER, Brit. J. Pharmacol. *20*, 516 (1963).
[8] F. SICUTERI, M. FANCIULLACCI, G. FRANCHI und S. MICHELACCI, Exper. *19*, 44 (1963).
[9] K. LÜBKE, E. SCHRÖDER, R. SCHMIECHEN und H. GIBIAN, Liebigs Ann., im Druck.

Biochemical Aspects of Phagocytosis in Polymorphonuclear Leucocytes. NADH and NADPH Oxidation by the Granules of Resting and Phagocytizing Cells

It is known[1–8] that, during phagocytosis by guinea-pig polymorphonuclear leucocytes, a dramatic increase of oxygen uptake occurs. The phagocytic event is also accompanied by a slight increment in lactic acid production (+10%, +20%) and by an increase of about sevenfold in the conversion of glucose-carbon 1 to CO_2. It is beyond doubt that the most significant change in phagocytizing polymorphonuclear leucocytes is the stimulation of the hexose-monophosphate shunt of glucose oxidation.

Owing to the lack of a relationship between the enormous increase of respiration and the very low CO_2 production from glucose-carbon-6, the oxygen uptake must be related to oxidation of coenzymes reduced by processes different from tricarboxylic acid cycle, i.e. Embden-Meyerhof and HMP-pathways.

A recent hypothesis[9–10] suggests that a possible sequence of the biochemical events during phagocytosis might be as follows: (a) Stimulation of glycolysis would lower the intracellular pH; (b) this condition would stimulate a NADPH-linked formation of lactate from pyruvate, and consequently the HMP-pathway of glucose oxidation through enhanced regeneration of $NADP^+$; (c) furthermore, NADH produced in glycolysis would now be available for a NADH-oxidase released from disrupted granules. According to EVANS and KARNOWSKY[9–10], the

[1] C. W. BALDRIDGE and R. W. GERALD, Amer. J. Physiol. *103*, 235 (1933).
[2] H. STÄHELIN, E. SUTER, and M. L. KARNOWSKY, J. exp. Med. *104*, 121 (1956).
[3] H. STÄHELIN, M. L. KARNOWSKY, A. E. FARNHAM, and E. SUTER, J. exp. Med. *105*, 265 (1957).
[4] A. J. SBARRA and M. L. KARNOWSKY, J. biol. Chem. *234*, 1355 (1959).
[5] Z. A. COHN and S. I. MORSE, J. exp. Med. *111*, 667 (1960).
[6] B. S. STRAUSS and C. A. STETSON JR., J. exp. Med. *112*, 653 (1960).
[7] G. Y. N. IYER, M. F. ISLAM, and J. H. QUASTEL, Nature *192*, 535 (1961).
[8] W. H. EVANS and M. L. KARNOWSKY, Biochemistry *1*, 159 (1962).
[9] W. H. EVANS and M. L. KARNOWSKY, J. biol. Chem. *236*, PC30 (1961).
[10] M. L. KARNOWSKY, Physiol. Rev. *42*, 143 (1962).

oxygen uptake of phagocytizing leucocytes would be related to oxidation of NADH. On the other hand, IYER et all.[7,11] suggest that oxidation of NADPH (faster than that of NADH) accounts for respiration through a NADPH-oxidase, H_2O_2 forming, which was found in homogenates of resting polymorphonuclear leucocytes, and is probably released from granules during phagocytosis.

It is worthwhile to emphasize that these hypotheses assume that the terminal system of oxidation for reduced coenzymes is brought into play after the lysis of the granules. However, while it is certain that degranulation occurs in phagocytizing leucocytes[12–14], there are no experimental data showing a release of such a NADH- or NADPH-oxidase in addition to the release of the demonstrated lysosomial-like enzymes. This communication reports some experimental evidence suggesting that the respiratory changes during phagocytosis cannot be related to degranulation.

The experiments were performed on guinea-pig polymorphonuclear leucocytes obtained from peritoneal exudates (following injection of 1% NaCl). The phagocytosis experiments were carried out at 37°C for 30 min in plastic tubes using a cell suspension (300–400 millions) in Krebs-Ringer phosphate without Ca^{++}, and 3–5 mg protein of killed Sarcina Lutea in fresh guinea-pig serum. In every experiment the changes of respiratory activity were checked by the conventional manometric technique, and the number of phagocytizing cells was established by microscopical examination. The resting and phagocytizing leucocytes were rapidly washed in 0.34 *M* sucrose and homogenized for 3 min at 0°C in 0.34 *M* sucrose, containing 10 m*M* nicotinamide, with a Potter-Elvejhem type (teflon pestle) homogenizer.

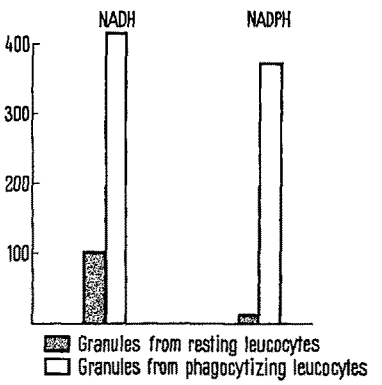
The granular and supernatant fractions were obtained by centrifugation at 20,000 × *g* for 30 min in the cold, after removal of the nuclear fraction and cell debris at low centrifugal force. The granules were then resuspended in nicotinamide containing sucrose.

The supernatant fractions were used for the assay of acid phosphatase and NADH- and NADPH-oxidase activities, and the granular fractions for the assay of NADH- and NADPH-oxidase activities.

The results show that in the supernatant fraction the acid phosphatase activity is strongly increased, whilst the low NADH-oxidase activity is unchanged after the phagocytic event (Table). The NADPH-oxidase is undetectable. The value of NADH-oxidase for the granular fraction of resting cells, expressed as micromoles of oxidized coenzymes is 0.39 ± 0.09/1 mg protein/h (5 experiments); the value of NADPH-oxidase is very low. As summarized in the Figure, the granular fraction of phagocytizing leucocytes exhibits a dramatic increase of both NADH- and NADPH-oxidase activities.

The presence of an increased phosphatase activity in the supernatant fraction after phagocytosis, without any demonstrable increase of the oxidase activities, may be a line of evidence to suggest that the lysosomial-like granules are disrupted, while others, containing electron transport systems, do not lyse. Polymorphonuclear leucocytes are known, indeed, to contain different types of non-mitochondrial granules[15].

Moreover, the finding of greater NADH- and NADPH-oxidase activities in granules obtained from phagocytizing cells, leads us to suggest that the respiration of phagocytizing polymorphonuclear leucocytes is accounted for by an electron transport system localized in a kind of non-disrupted granules. Regarding the mechanism of inter-



NADH- and NADPH-oxidase activities of granules obtained from resting and phagocytizing polymorphonuclear leucocytes. Values are expressed as % variations with respect to NADH-oxidase of granules of resting cells. For conditions see Table. Each vessel contained about 0.5 mg protein. In some experiments the oxidized coenzymes formed were also measured by adding the specific dehydrogenase and substrate and reading at 340 mμ.

[11] G. Y. N. IYER and J. H. QUASTEL, Canad. J. Biochem. Phys. *41*, 427 (1963).
[12] J. G. HIRSCH and Z. A. COHN, J. exp. Med. *112*, 1005 (1960).
[13] Z. A. COHN and J. G. HIRSCH, J. exp. Med. *112*, 1015 (1960).
[14] J. G. HIRSCH, J. exp. Med. *116*, 827 (1962).
[15] H. W. FLOREY and L. H. GRANT, J. Path. Bact. *82*, 13 (1961).
[16] Z. A. COHN and J. G. HIRSCH, J. exp. Med. *112*, 983 (1960).

NADH oxidase and acid phosphatase in the supernatant fraction (20,000 × *g*) of resting (SR) and phagocytizing (SP) polymorphonuclear leucocytes. All activities are expressed per 1 mg protein

		SR	No. of experiments	SP	No. of experiments
NADH-oxidase	micromoles of oxidized coenzyme/h	0.31 ± 0.1	4	0.28 ± 0.1	4
	μl O_2/h	2.6 ± 0.9		2.7 ± 1.0	
Acid phosphatase	μg P/20 min	20.1 ± 4.0	6	59.5 ± 12	6

Conditions: NADH oxidase was measured in a Warburg apparatus using vessels of 5 ml volume. At the end of incubation the remaining NADH was determined in a Beckman DU spectrophotometer at 340 mμ. The reaction mixture contained in 1 ml total volume: sucrose μmoles 136; KCl 21.6; NcCl 21.6; KH_2PO_4-K_2HPO_4 buffer 10.8 pH 7.4; $MgCl_2$ 1; NADH 1.9; nicotinamide 4. Temperature 38°C. Acid phosphatase was measured as reported by COHN and HIRSCH[16].

action of the intragranular electron transport system with the externally reduced coenzymes, as an hypothesis one may assume the involvement of some structural factor or/and of a carrier system (diaphorase, quinone) scarcely participating in the respiration of resting cells.

Riassunto. Gli autori dimostrano che l'aumento della respirazione dei leucociti durante la fagocitosi non è dovuto a fuoriuscita di una NADH-ossidasi dai granuli, ma alla maggiore attività NADH e soprattutto NADPH-ossidasica localizzata in granuli non lisati.

F. Rossi and M. Zatti

Unit Centre 'G. Vernoni' for the Study of Physiopathology, National Research Council and Istituto di Patologia Generale, Università di Padova (Italy),
September 18, 1963.

The Effect of ACTH on Cholinesterase Activity in Plasma, Whole Blood, and Blood Cells of Rats

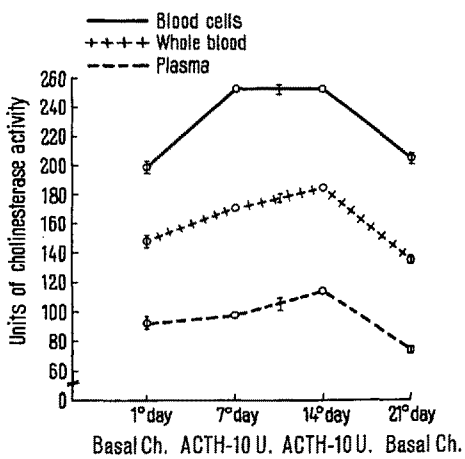
In 1960 one of us proved that when ACTH was administered to normal subjects and asthmatic patients, it produced increased cholinesterase activity in plasma, whole blood, and blood cells[1].

For this present study we used Wistar rats weighing about 250 g each. Blood was extracted by puncturing the jugular vein on four successive occasions: (a) under basic conditions, (b) and (c) after intramuscular and intraperitoneal injection of 10 units of ACTH, (d) under basic conditions. In this way the rats acted as their own controls.

Cholinesterase activity was determined in plasma, whole blood and blood cells by Bigg's colorimetric method[2].

ACTH was administered in the following way: 6 gel units intramuscularly and 4 units in a saline solution intraperitoneally.

Blood was extracted between 20 min and 120 min after the intraperitoneal injection of ACTH and from 3 to 5 h after its injection by the intramuscular route. This technique produced maximum stimulating effect of the corticotrophin on cholinesterase activity. Intramuscular and intraperitoneal injections were also given separately, but with this method we obtained less variation.



Cholinesterase activity in basal conditions and after ACTH.

Extractions were performed weekly and blood tests performed in order to avoid anaemia in the experimental animals.

The averages obtained under basic conditions, and expressed in units of cholinesterase activity were: 92 ± 3.7, 148 ± 3.9, and 199 ± 4.3 in plasma, whole blood, and blood cells respectively (Figure).

The values rose to 97, 171, and 252 after the first administration of ACTH. After the second administration, using the same technique, cholinesterase activity again increased, to 118, 185, and 253. The general average under the action of ACTH was 108 ± 4.5, 178 ± 2.4, and 255 ± 3.3 in plasma, whole blood, and blood cells respectively. Analysis of the statistics showed a highly significant increase, P: 0.001, in the different blood fractions.

Cholinesterase activity was again determined 7 days after the last administration of ACTH and the values were found to have dropped to the basic figures: 72, 135, and 205 units of cholinesterase activity in plasma, whole blood, and cells, respectively.

According to these findings ACTH possesses a stimulating effect on cholinesterase activity both in man and rats.

Résumé. On a déterminé chez des rats l'activité cholinestérasique dans le plasma, sang total et cellules sanguines avant et après l'administration d'ACTH. On a constaté une augmentation hautement significative: P: 0,001 après l'administration de cette hormone.

J. R. Vaccarezza and J. A. Willson

Instituto de Biologia y Medicina Experimental, Laboratorio de Neurobiologia, Buenos Aires, and Laboratorio Central del Hospital Muñiz, Buenos Aires (Argentina),
August 19, 1963.

[1] J. R. Vaccarezza and L. Peltz, Presse Méd. *68*, 723 (1960).
[2] H. G. Biggs, S. Carey, and D. B. Morrinson, Amer. J. clin. Path. *30*, 181 (1958).

Stimulation of Dorsal Axial Differentiation in Ventral Explants of *Rana pipiens* Embryos by $CaCl_2$ and Guanidine HCl

It has been demonstrated that exposure of ectoderm of the early salamander gastrula *in vitro* to saline solutions of pH 4.0 and 9.7 for brief periods can stimulate the differentiation of parts of the brain[1], while brief treatment of ventral mesoderm at pH 12.0 promotes the formation of notochord, muscle and pronephric tubules[2]. The means by which the extreme pH levels cause prospective ventral tissue to form dorsal axial structures possibly may be allied with the provision of soluble protein, and possibly some ribonucleic acid, from some of the yolk platelets

[1] J. Holtfreter, J. exp. Zool. *106*, 197 (1947).
[2] T. Yamada, Biol. Bull. *98*, 98 (1950).