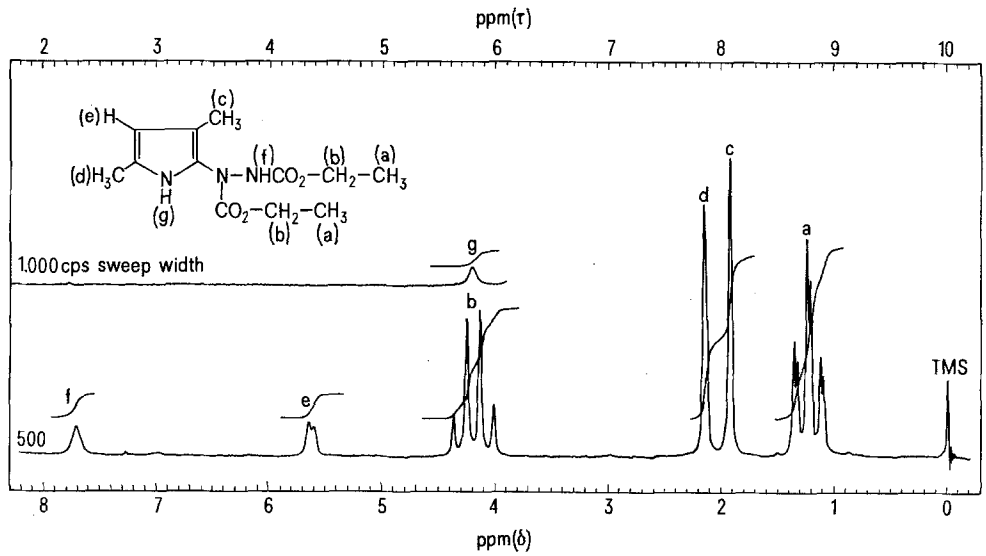

NMR-spectrum of 1 in $CDCl_3$

exhibits signals at 1.20 and 1.25 (triplets, 3H each, –N– and –N'H–$CO_2CH_2CH_3$) 1.9 and 2.15 (singlets, 3H each, β- and α-CH_3) 4.2 (quartet, 4H, –N– and N'H–$CO_2CH_2CH_3$) 5.65 (doublet, 1H, β-H) 7.7 (singlet, 1H, –N–N'H–$CO_2C_2H_5$) and 8.4 ppm (singlet, 1H, N–H).

The IR-spectrum showed bands at 3340 cm^{-1} and 3236 cm^{-1} due to N–H and at 1754 cm^{-1} and 1715 cm^{-1} due to C = O.

Compound	R_2	R_3	R_4	R_5	Product	Yield (%)	m.p.
1	CH_3	H	CH_3	H	1a	87	129–130°
2	CH_3	C_2H_5	CH_3	H	2a	70	105–107°
3	H	CH_3	C_2H_5	H	3a	80	74– 76°
4	CH_3	H	H	CH_3	4a	86	136–138°
5	CH_3	$CO_2C_2H_5$	CH_3	H	5a	79	162–164°
6	CH_3	$COCH_3$	CH_3	H	6a	72	160–162°

DEH– –N–N'H$CO_2C_2H_5$ (N,N'-dicarboethoxyhydrazino)
$CO_2C_2H_5$

Experimental. The purely alkyl pyrroles (1–4, 0.01 mol) in 20 ml of ether/n-pentane (1:1) were treated with the theoretical amount of the azoester. The solid which separated after a few minutes at room temperature was recrystallized from the same solvent. The remaining pyrroles 5 and 6 reacted similarly in ether and the products were re-crystallized from ethanol[14].

Zusammenfassung. Es reagieren die α- und β-Positionen von Pyrrol-Derivaten ohne weiteres mit Äthyl-Azodicarboxylat unter Bildung von Hydrazindicarboxyester-Derivaten, was durch die NMR- und IR-Spektren bestätigt wird.

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Inhibition of Nuclear and Mitochondrial Respiration by Arsenite

Nuclear oxidative phosphorylation has clearly been demonstrated in cell nuclei isolated from the thymus gland[1–4]. Endogenous fatty acids are the major energy source for this ATP generating process[5]. No external substrate capable of stimulating nuclear oxidative phosphorylation has been found as yet[4]. Experiments with inhibitors and uncouplers of the respiratory chain suggest a similarity if the molecular mechanism of the mitochondrial and the nuclear system. Arsenite has been reported[3] to have no effect on nuclear respiration, while this compound is a well known inhibitor of mitochondrial oxidative phosphorylation[6]. Because this observation could be of great importance in finding a significant difference in the molecular mechanism of energy production between these two cell organells, a further study on this subject seemed justified. In this report a comparison is made between the effect of different concentrations of sodium arsenite on the respiration of rat thymus nuclei and rat liver mitochondria.

Nuclei were isolated as published before[5]. Mitochondria were prepared by the method of Hogeboom[7]. Oxygen uptake was measured manometrically in 15 ml Warburg flasks. A piece of accordion-folded filter paper (Whatman-1) with 2 cm sides was inserted in the center well to which 0.1 ml 10% KOH had been added. The nuclei (15 mg protein) were incubated in 2.3 ml of a medium

containing 20 m*M* glutamate, 5 m*M* ADP, 250 m*M* sucrose, 15 m*M* NaCl, 3 m*M* $CaCl_2$ and 50 m*M* *Tris*-HCl (pH 7.4). The mitochondria (2.5 mg protein) were incubated in 20 m*M* glutamate, 5 m*M* ADP, 250 m*M* sucrose, 15 m*M* KCl, 5 m*M* $MgCl_2$, 2 m*M* ethylenediamine tetraacetate (EDTA), 30 m*M* KH_2PO_4 and 50 m*M* *Tris*-HCl (pH 7.4). Protein was determined according to GORNALL et al.[8]. As can be seen from the broken line in the Figure, addition of 0.1 m*M* arsenite does not result in an inhibition of nuclear respiration. This is in agreement with the result of BETEL and KLOUWEN[3], who concluded from experiments with this amount of arsenite that this compound has no effect on nuclear respiration.

From the solid line in the Figure one can see that mitochondrial respiration is inhibited by about 80% by 0.1 m*M* arsenite. A conclusion as made by the former authors[3] based on experiments with arsenite concentrations not exceeding 0.1 m*M*, is not valid. This is shown in the Figure. While 1 m*M* arsenite results in an inhibition of nuclear respiration of about 60%, almost complete inhibition is obtained if higher concentrations of this compound are used.

These experiments suggest that the arsenite anion has more difficulties in penetrating the nuclear membrane as compared to the mitochondrial membrane. No basic difference in the mechanism of nuclear and mitochondrial oxidative phosphorylation may be postulated as yet.

Résumé. La respiration des noyaux isolés du thymus de rat est inhibée par l'arsénite à une concentration 10 fois supérieure à celle qui inhibe la respiration mitochondriale. Ce fait suggère, une difficulté de pénétration à travers la membrane nucleaire et ne rend pas compte de la différence entre le méchanisme de la phosphorylation oxydative des noyaux et celle des mitochondria.

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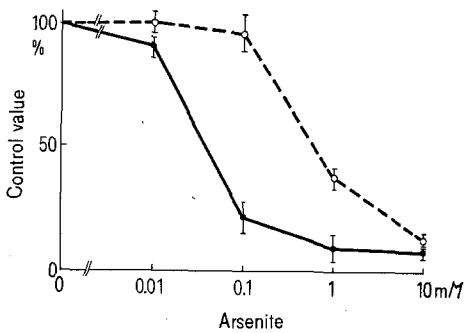

Effect of arsenite on nuclear and mitochondrial respiration. The incubation was performed for 60 min at 37°C in the media described in the text. Without addition of arsenite, the oxygen uptake of the nuclei was 82.5 µl O_2 per flask and for the mitochondria 228.0 µl O_2 per flask. The solid line represents experiments with mitochondria. The broken line illustrates experiments with nuclei.

[1] S. OSAWA, V. G. ALLFREY and A. E. MIRSKY, J. gen. Physiol. *40*, 491 (1957).
[2] B. S. MCEWEN, V. G. ALLFREY and A. E. MIRSKY, J. biol. Chem. *238*, 758 (1963).
[3] I. BETEL and H. M. KLOUWEN, Biochim. biophys. Acta *131*, 453 (1967).
[4] A. W. T. KONINGS, Biochim. biophys. Acta, *189*, 125 (1969).
[5] A. W. T. KONINGS, Biochim. biophys. Acta *223*, 398 (1970).
[6] A. FLUHARTY and D. R. SANADI, Proc. natn. Acad. Sci. USA *46*, 608 (1960).
[7] G. H. HOGEBOOM, in *Methods in Enzymology* (Eds. S. P. COLOWIC and N. O. KAPLAN; Academic press, New York 1965), vol. 1, p. 16.
[8] A. G. GORNALL, C. J. BARDAWILL and M. H. DAVID, J. biol. Chem. *177*, 751 (1949).
[9] Present address: Laboratory of Radiopathology, Bloemsingel 1, University of Groningen, The Netherlands.

Zur Struktur von Scotophobin

Scotophobin, ein Pentadekapeptid, das in Ratten eine Veränderung ihrer natürlichen Verhaltungsweise bewirkt, wurde 1970 von UNGAR et al.[1] isoliert. Für eine Strukturaufklärung standen 300 µg des Peptids zur Verfügung. Nach der Aminosäureanalyse verblieben etwa noch 100 µg zur Sequenzbestimmung. Mikrodansylierung oder Edman-Abbau konnten daher als chemische Methoden nicht verwendet werden. Mit Hilfe der Massenspectrometrie wurde schliesslich die Aminosäuresequenz (I) (Formel) vorgeschlagen.[2] Die C-terminale Aminosäure ist Tyrosinamid. Ungewiss blieb allerdings, ob die Seitenketten der Aminosäuren in Position 2, 5 und 11 Amide tragen oder mit Carboxylgruppen besetzt sind. Da für weitere Untersuchungen kein natürliches Material mehr zur Verfügung stand, konnte nur durch die Synthese der theoretisch möglichen Pentadekapeptide Scotophobin die richtige Struktur zugeordnet werden. Das Resultat von zwei solchen Synthesen[3] deutet darauf hin, dass Scotophobin ein Peptid mit der Sequenz (II) ist (Formel). In einem neuerdings erschienenen Bericht über die Synthese eines Scotophobin-Analogas wird die Struktur von Scotophobin noch einmal zur Diskussion gestellt. WEINSTEIN et al.[4] synthetisierten ein Pentadekapeptid der Sequenz (III) (Formel), das in Position 2 ASP enthält und in den Positionen 5 und 11 jeweils GLU.

Der Rf-Wert sowie die biologische Aktivität (Tabelle I) dieses Peptids stimmen mit dem natürlichen Scotophobin nicht überein, woraus geschlossen wurde, dass Scotophobin nicht die Struktur (III) besitzt.

Wir synthetisierten zunächst das Peramidoscotophobin (IV) (Formel). Die Synthese dieses Peptids wurde bereits beschrieben[3]. Das gereinigte Produkt zeigte eine biologische Aktivität von 300 E/mg[5] (10–12%) verglichen mit dem natürlichen Scotophobin (Tabelle I). Biologische Tests und verschiedene chemische Untersuchungen ergaben, dass (IV) nicht identisch ist mit dem natürlichen Scotophobin.

[1] G. UNGAR, D. M. DESIDERIO und W. PARR, Nature, Lond. in press.
[2] D. M. DESIDERIO, G. UNGAR und P. A. WHITE, Chem. Commun. *9*, 432 (1971).
[3] W. PARR und G. HOLZER, Hoppe-Seyler's Z. physiol. Chem. *352*, 1043 (1971).
[4] A. ALI, J. H. R. FAESEL, D. SARANTAKIS, D. STEVENSON und B. WEINSTEIN, Experientia *27*, 1138 (1971).
[5] Eine Aktivitätseinheit (E/mg) ist diejenige Substanzmenge, welche die mittlere Aufenthaltsdauer in der Dunkelheit von 130 sec auf 60 sec verringert.