with ϕ, Ψ values of experimentally determined conformations. In some cases the data were obtained by X-ray crystallography but there is ample evidence that conformation in crystal and solution are similar[15]. Gramicidin S-A has been studied using nuclear magnetic resonance[16], and although the leucyl residue may be constrained by the cyclic nature of this decapeptide it is noteworthy that the observed conformation agrees with the calculated energy minimum at Ψ and is within 30° at ϕ.

The generally good agreement between calculated and experimental conformations suggests that the extended Hückel molecular orbital approach can be used to determine the preferred conformation of amino acid residues as an initial step towards understanding the forces involved in the folding of polypeptide chains.

Conformations of amino acid residues

Residue	Calculated[a]		Experimental conformations		Reference
	ϕ	Ψ	ϕ	Ψ	
L-Serine	60°	330°	61°	293°	11
	120°				
L-Isoleucine	60°	300°	38°	293°	12
	90°′		61°′	325°′	
L-Valine	60°	300°	38°	293°	13
	90°′		61°′	325°′	
			132°	123°	13
L-Leucine	60°	0°	132°	123°	14
			30°	0°	15

[a] 0° = 360°, angles listed are within 1 kcal/mole^{-1}.

Zusammenfassung. Die Konformation einiger natürlicher Aminosäuren wird auf Grund einer erweiterten Hückel-Molekular-Orbital-Theorie berechnet.

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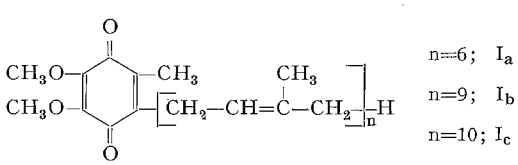
[1] D. KOTELCHUCK and H. A. SCHERAGA, Proc. natn Acad. Sci. *61*, 1163 (1968).
[2] R. J. HOFFMANN, J. Chem. Phys. *39*, 1397 (1961).
[3] L. B. KIER and J. M. GEORGE, Theoret. chim. Acta *14*, 258 (1969).
[4] L. B. KIER, in *Fundamental Concepts of Drug Receptor Interaction* (Ed. D. J. TRIGGLE; Academic Press, New York 1969), chapter 2.
[5] R. HOFFMANN, Tetrahedron *22*, 521 and 539 (1966).
[6] F. JORDAN and B. PULLMAN, Theoret. chim. Acta *9*, 242 (1968).
[7] R. HOFFMANN and A. IMANURA, Biopolymers *7*, 207 (1969).
[8] L. PAULING, R. COREY and H. BRANSON, Proc. natn Acad. Sci. *37*, 207 (1951).
[9] H. A. SCHEREGA, Adv. Phys. org. Chem. *6*, 103 (1968).
[10] J. EDSALL, P. FLORY, J. KENDREW, A. LIQUORI, G. NEMETHY, G. RAMACHANDRAN and H. SCHERAGA, J. biol. Chem. *241*, 1004 and 4167 (1966).
[11] N. TOONEY and G. FASMAN, J. molec. Biol. *36*, 355 (1968).
[12] E. BLOUNT and E. SHEETER, Biopolymers *7*, 1551 (1968).
[13] R. EPAND and H. SCHERAGA, Biopolymers *6*, 1551 (1968).
[14] H. AUER and P. DOTY, Biochemistry *5*, 1708 (1966).
[15] D. CHIPMAN and N. SHARON, Science *165*, 454 (1969).
[16] S. STERN, W. GIBBONS and L. CRAIG, Proc. natn Acad. Sci. *61*, 734 (1968).
[17] Supported by National Institutes of Health Grants No. FR 5409-07 and No. GM16312-01.
[18] Recipient of a Public Health Service Research Career Development Award No. AM1159.

Coenzymes Q: Stimulants of the Phagocytic Activity in Rats and Immune Response in Mice

Coenzymes Q_9 (Ib) and Q_{10} (Ic) are present in shark livers[1], and we identified them in the hexane extracts of shark livers (Lemon shark *Negaprion brevirostris*), that had shown stimulation of the phagocytic activity in rats, increase in the antibody formation of mice vs sheep erythrocytes and modified other parameters of the reticuloendothelial system (RES)[2]. Since the activity of pure coenzymes Q has not been studied in these tests, we decided to investigate their action as stimulants of the RES.



n=6; I_a
n=9; I_b
n=10; I_c

Materials and methods. Pure, commercially available coenzymes Q_6 (Ia) and Q_{10} (Ic) were used for this study. Only pure material was used.

Phagocytic activity. Adult male CFN rats weighing 180 g were injected via the saphenous vein with the material to be tested as an emulsion in a 5% non-pyrogenic glucose solution containing Ninol (lauric diethanolamide) used as an emulsifier. In all cases the amount of Ninol used was 5% by weight of the substance under investigation. The Q_6 and Q_{10} emulsions contained 375 and 250 µg respectively of coenzyme per ml. They were prepared in a 500 ml Waring blender and initially homogenized for 45 sec. The smaller doses were injected first and the emulsion rehomogenized for 15 sec before proceeding to the next larger dose[3]. 10 animals were used for each dose and 10 animals were injected as controls

[1] D. NAZIR and N. MAGAR, Biochem. J. *90*, 268 (1964). – P. GALE, R. ERICKSON, A. PAGE and K. FOLKERS, Arch. Biochem. Biophys. *104*, 169 (1964).
[2] J. P. RANSOM, E. BLIZNAKOV, V. Z. PASTERNAK and J. H. HELLER, C. r. Soc. Biol., Paris *156*, 1022 (1962). – J. P. RANSOM, V. Z. PASTERNAK and J. H. HELLER, J. Bact. *84*, 466 (1962). – J. H. HELLER, E. G. BLIZNAKOV, J. P. RANSOM, D. J. WILKINS and V. Z. PASTERNAK, in *The Reticuloendothelial System*, Proc. IVth Int. Symposium on RES, Kyoto, Japan (Nisha Printing Co., Kyoto, Japan 1965), p. 375. – J. H. HELLER, J. P. RANSOM and V. Z. PASTERNAK, in *Role du système réticulo-endothélial dans l'immunité antibactérienne et antitumorale* (Colloques Internationaux du CNRS, Paris 1963), p. 89. – E. G. BLIZNAKOV, Int. J. Cancer *3*, 336 (1968).
[3] The method used to prepare the emulsion is of critical importance and it should always be the same in order to obtain reproducible results.

with 5% glucose and Ninol only. The phagocytic rate was measured by the intravascular clearance rate of colloidal carbon 48 h after injection of the emulsion as described by BIOZZI et al.[4]. The average time required for 50% of the carbon to disappear from the circulation of the control group was used as 100%. The half time for the test groups is calculated as per cent of the control value; thus, test groups with half times below 80% of the control possess a significantly enhanced activity of the RES.

Immune response. Adult male CF-1 mice weighing 20 g were injected via the tail vein with coenzyme Q_{10} emulsion prepared as described above and 48 h later injected again i.v. with 7×10^5 sheep red blood cells (SRBC) washed 3 times. The control animals were injected with 5% glucose and Ninol. The mice were bled from the retro-orbital venous plexus with heparinized glass capillary tubes 4, 6 and 8 days after the injection of the SRBC. Hemolytic antibody production was determined on the pooled sera from 20 mice using the 50% end point method[5]. Eight serum dilutions were used. The determination of best fitting regression line between probit % hemolysis and the log of the serum dilution was determined by computer analysis. The antibody titer in the test groups is calculated as % of the control group. In Table II the maximum and minimum allowable values based on a least squares fitting of the data points are given in parentheses.

The emulsions were tested in rabbits (single dose 5 mg/kg) for the presence of pyrogens in order to preclude contamination with bacterial endotoxin. The US Pharmacopeia recommended criteria were used for evaluation. They were tested also for induction of proliferative response of the RES determined by weighing the livers and the spleens of mice, sacrificed 3 and 10 days after injection of coenzyme Q_6 or Q_{10} emulsion (single dose 100 and 400 µg/mouse), as described by WOOLES et al.[6].

Results. The results demonstrate that coenzymes Q_6 and Q_{10} emulsions showed no pyrogenic effect. It may be concluded that they are free of contamination by significant amounts of bacterial endotoxin. Further, they produce no significant hyperplastic effect on the RES.

Table I shows that 48 h after an i.v. injection of coenzyme Q_6 or Q_{10} emulsion, the phagocytic activity in rats is highly enhanced at doses as low as 750 µg per rat.

Table II shows that 48 h i.v. pretreatment with coenzyme Q_{10} emulsion produces a two-fold increase of the primary hemolytic antibody titer at doses as low as 150 µg per mouse.

This is the first report of RES stimulation by any member of the coenzyme Q group. Their effect on other RES parameters is now under investigation[7].

Zusammenfassung. Mittels der Tusche-Clearance-Methode wird gezeigt, dass Coenzym Q_6 und Q_{10} die phagozytische Aktivität bei Ratten steigert. Im Vergleich mit Schaferythrozyten wurde bei Mäusen durch Zufuhr von Coenzym Q_{10} eine Verdoppelung der primären hämolytischen Antikörperbildung festgestellt.

Table I. Effect of coenzymes Q_6 and Q_{10} on the rate of colloidal carbon blood clearance in rats

Coenzyme Q_6		Coenzyme Q_{10}	
µg/rat	% of control	µg/rat	% of control
750	60.6	500	72.7
1125	66.8	750	64.7
1500	62.8	1000	63.8

Table II. Primary hemolytic antibody titer in mice pretreated with coenzyme Q_{10} emulsion

Coenzyme Q_{10} (µg/mouse)	No. of mice	Hemolytic units % of control on day after SRBC		
		4	6	8
150	20	244 (228–262)	190 (184–197)	171 (165–177)
300	20	247 (231–262)	155 (154–157)	168 (162–175)
450	20	233 (220–249)	134 (133–135)	131 (126–136)

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[4] G. BIOZZI, B. BENACERRAF and B. HALPERN, Br. J. exp. Path. *34*, 441 (1953).

[5] E. A. KABAT and M. M. MAYER, in *Experimental Immunochemistry*, 2nd Edition (Charles C. Thomas Publisher, Springfield, Illinois 1961).

[6] W. R. WOOLES and N. R. DILUZIO, J. R. Soc. *7*, 160 (1964).

[7] Supported by Research Grants from The John A. Hartford Foundation, T. Mellon and Sons and NSF (Grant No. GH-34).

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Die Solubilisation der Gehirnneuraminidase durch proteolytische Enzyme

Die Neuraminidase (N-Acetylneuraminyl-Hydrolase E.C.3.2.1.18) des Gehirns ist vorwiegend membrangebunden und in der Lysosomen-Mitochondrienfraktion angeordnet[1]. Sie kann auf verschiedene Weise aus dem Hirnhomogenat und, mit höherer Ausbeute, aus der isolierten Lysosomen-Mitochondrienfraktion freigesetzt werden. TETTAMANTI und ZAMBOTTI[2] solubilisierten und extrahierten die Neuraminidase des Schweinegehirns mit isotonischer KCl-Lösung. LEIBOVITZ und GATT[3] fanden im Kalbsgehirn die höchste Aktivität des Enzyms im Überstand der 50000 × *g* Zentrifugation nach Extraktion des Hirntrockenpulvers mit 1% Triton X-100 in 0.1 *M* Natriumacetatpuffer pH 4.3 nach vorhergehender zweimaliger Behandlung des Trockengewebes mit 0.5% Natriumcholat in 0.1 *M* Natriumacetatpuffer pH 4.0.

Wird das in isotonischer KCl-Lösung homogenisierte Hirngewebe der Ratte mit den proteolytischen Enzymen Trypsin, α-Chymotrypsin oder Pronase vorinkubiert,