Q_{O_2} (μl O_2/mg poids sec/h) et cations (mg/g poids frais) du tissu hépatique de Tanche vagotomisée ou non

	Témoin eau douce	Vagotomisé eau douce	Augmentation %	Témoin ClNa 12‰	Vagotomisé ClNa 12‰	Augmentation %	Différence par rapport à l'eau douce
Q_{O_2}	2,21 ± 0,20	2,46 ± 0,18	12	3,21 ± 0,23	3,85 ± 0,50	20	74
% eau	71	76	7	75	79	5	11
Na^+	0,41 ± 0,9	0,74 ± 0,11	80	0,69 ± 0,7	1,08 ± 0,12	56	163
K^+	2,74 ± 0,18	2,55 ± 0,12		2,73 ± 0,21	2,80 ± 0,24		

vagotomisés placés en eau salée que chez les sujets intacts (voir Tableau). Si l'on compare par ailleurs ces mêmes données chez les sujets demeurés en eau douce, on voit que la section des vagues entraîne par elle-même une augmentation de l'intensité respiratoire, et de la concentration en Na^+ du tissu hépatique. Parallèlement, la teneur en eau subit une augmentation croissante alors que la quantité de potassium tissulaire est peu modifiée.

La teneur en sodium augmente donc après vagotomie dans le tissu hépatique de la Tanche mais l'origine de ce cation n'est pas élucidée. Il ne semble pas provenir du milieu extérieur car des expériences réalisées dans l'eau distillée (résultats non publiés) paraissent indiquer un mouvement semblable. D'autre part, il ne provient pas non plus de la masse musculaire puisqu'il augmente aussi au niveau de ce tissu chez les Poissons vagotomisés (animal témoin: 0,27; sujet vagotomisé: 0,35). Il est possible que la vagotomie modifie l'excrétion de cet ion, sinon son absorption; des recherches dans ce sens sont envisagées (tavaux en cours).

Selon MAETZ et MOREL[8], le métabolisme du Na^+ et celui de l'eau ne sont pas toujours liés; en conséquence, il y a lieu également de chercher à élucider si la section des vagues agit sur les deux mécanismes, ou seulement sur l'un d'entre eux, le second étant alors un effet secondaire. L'inhibition du nerf vague ne permet donc pas de prolonger la survie de l'espèce en eau salée; son excitation au contraire serait peut-être un facteur la favorisant. Dans le problème de la régulation hydrominérale, outre les actions hormonales, l'effet du système nerveux parasympathique n'est pas à négliger.

Conclusion. Chez la Tanche, la section des nerfs vagues n'atténue pas le choc osmotique produit par les variations de salinité. La vagotomie provoque au contraire chez ces animaux une augmentation accrue de l'intensité respiratoire et de la teneur en eau et en sodium du tissu hépatique, aussi bien en eau douce qu'en eau salée.

Summary. Vagus nerve section was performed on a stenohaline species: the tench (*Tinca tinca* L.). The oxygen consumption and Na^+ content increased in both tap water and after transfer to salt water. It is suggested that the vagus nerve plays a role in water and electrolyte flux in this fish.

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[8] J. MAETS et F. MOREL, Archs Anat. microsc. *54*, N° 1, 515 (1965).

Trifluoroacetyl as a Protecting Group for Amino Acid Hydrazides

The trifluoroacetyl group is widely employed in peptide chemistry, both for N-protection[1] and as an aid in the gas chromatography of various amino acids and peptides[2]. Previously, N-benzyloxycarbonyl, N-*t*-butyloxycarbonyl and N-trityl substituents have been utilized for the masking of the hydrazide function in peptides where chain extension by the azide method is contemplated at a later synthetic stage[3]. We now report an application of the trifluoroacetyl moiety in a similar fashion. The chief advantage of this technique over existing methods is the ability to use simultaneously a wider variety of blocking agents.

A suspension of L-alanine methyl ester hydrochloride (I)[4,5] in chloroform was neutralized with triethylamine and the resulting L-alanine methyl ester (II) solution was stirred with *o*-nitrophenylsulfenyl chloride[6] to yield

[1] E. SCHRÖDER and K. LÜBKE, *The Peptides* (Academic Press, New York 1965), vol. I, p. 6.

[2] B. WEINSTEIN, *Methods of Biochemical Analysis* (Interscience Publishers, New York 1966), vol. 14, pp. 232, 264, 275.

[3] E. SCHRÖDER and K. LÜBKE, *The Peptides* (Academic Press, New York 1965), vol. 1, p. 64.

[4] All products prepared herein were homogeneous by thin-layer chromatography (support, Silica Gel G; developer, methanol or methanol-chloroform, 9:1; detectors, iodine, ninhydrin or UV-light); satisfactory analyses were obtained for the new compounds III, IV, V, and XI.

[5] H. ZAHN and H. SCHÜSSLER, Justus Liebigs Annlo Chem. *641*, 176 (1961).

[6] L. ZERVAS, D. BOROVAS and E. GAZIS, J. Am. chem. Soc. *85*, 3660 (1963).

[7] The *o*-nitrophenylsulfenyl, trifluoroacetyl and benzyloxycarbonyl prefixes are shortened to Nps, Tfa, and Z, respectively; 'IUPAC-IUB Commission on Biochemical Nomenclature. Abbreviated Designation of Amino Acid Derivatives and Peptides. Tentative Rules,' Biochemistry *5*, 2485 (1966).

yellow N-*o*-nitrophenysulfenyl-L-alanine methyl ester (III); m.p. 75.5–76.5°; $[\alpha]_D^{27.2}$ — 72.0° (c 1, dimethylformamide). The addition of hydrazine hydrate to a methanolic solution of the Nps-ester III[7] formed within minutes crystalline N-*o*-nitrophenylsulfenyl-L-alanine hydrazide (IV); m.p. 183–185° (with effervescence); $[\alpha]_D^{27.1}$ — 51.0° (c 1, dimethylformamide). Solid *p*-nitrophenyl trifluoroacetate[8,9] was mixed with a solution of the Nps-hydrazide IV and triethylamine in dimethylformamide to afford N^1-*o*-nitrophenylsulfenyl-N^2-trifluoroacetyl-L-alanine hydrazide (V); m.p. 203–205°; $[\alpha]_D^{26.7}$ — 43.0° (c 1, dimethylformamide). A solution of the Nps-Tfa hydrazide V[7] and standardized hydrogen chloride in ethyl acetate[10] gave N^2-trifluoroacetyl-L-alanine hydrazide hydrochloride (VI) as a hydroscopic, colorless gel. The Tfa-hydrazide salt VI was coupled to N-benzyloxycarbonyl-L-alanine (VII)[11] in the presence of triethylamine with the aid of either N,N'-dicyclohexylcarbodiimide[12] or 1-ethyl-3-(3-dimethylamino)propyl carbodiimide hydrochloride[13] in methylene chloride or chloroform solutions to yield oily N-benzyloxycarbonyl-N^2-trifluoroacetyl-L-alanyl-L-alanine hydrazide (VIII). The Z-Tfa dipeptide hydrazide VIII[7] in methanol was refluxed with dilute, aqueous sodium hydroxide so as to cleave the Tfa group and to form N-benzyloxycarbonyl-L-alanyl-L-alanine hydrazide (IX), identical with an authentic sample[14–16]. Most importantly, this overall sequence demonstrates that the N^2-Tfa moiety can be carried through a series of typical peptide reaction steps, including introduction in a neutral solvent, hydrolysis of a second and different acid labile primary amino protecting group, and then removal under basic conditions.

Alternatively, a solution of N-benzyloxycarbonyl-L-alanine hydrazide (X)[14] and trifluoroacetic acid in tetrahydrofuran was treated with N,N'-dicyclohexylcarbodiimide to yield quantitatively N^1-benzyloxycarbonyl-N^2-trifluoroacetyl-L-alanine hydrazide (XI); sublimable at 165°/0.3 mm.; m.p. 182–185°; $[\alpha]_D^{26.7}$ — 13.0° (c 1, dimethylformamide). Saponification of the Z-Tfa hydrazide XI with dilute, alcoholic sodium hydroxide at room temperature returned the starting Z-hydrazide X. Hydrogenation of compound XI in methanol with excess acetic acid in the presence of 10% palladium on carbon catalyst furnished oily N^2-trifluoroacetyl-L-alanine (XII). If the acetic acid was omitted, the product was a mixture of N^2-Tfa alanine XII and a second material, possibly N^1-trifluoroacetyl-L-alanine hydrazide (XIII). The isomeric Tfa-hydrazide XIII could have been derived from a N^2-Tfa → N^1-Tfa transfer.

The preparation of N^1-*t*-butyloxycarbonyl and related N^1-Schiff base amino acid hydrazides[17], followed by conversion to the corresponding N^2-Tfa derivatives, would be a logical extension of the work reported here. Cleavage of the N^1-group under mild acidic conditions will produce N^2-Tfa acyl hydrazides that can be routinely coupled to various N^1-acyl amino acids. Attention is called to the point that the hitherto elusive ω-nitroarginyl peptide hydrazides may be synthesized in this fashion, as direct treatment of the nitroguanido group with hydrazine leads to undesirable results[18–21]. These various suggestions are under active investigation[22].

Zusammenfassung. Die Bildung von Z-N^2-Trifluoroacetyl-Aminosäure-Hydraziden wird beschrieben. Die Anwendungsmöglichkeiten dieser Verbindungen in der Peptidsynthese werden durch mehrere Beispiele demonstriert.

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[17] E. SCHRÖDER and K. LÜBKE, *The Peptides* (Academic Press, New York 1965), vol. 1, p. 49.
[18] E. SCHRÖDER and K. LÜBKE, *The Peptides* (Academic Press, New York 1965), vol. 1, p. 168.
[19] K. HOFMANN, in a personal communication (May 31, 1966), stated that some 20 years ago an attempt was made in Bergmann's laboratory to obtain N-benzyloxycarbonyl-ω-nitro-L-arginine hydrazide via the methyl ester; only intractable, gummy materials were obtained, which were not characterized, and the experiments were never repeated.
[20] The only successful preparation by this technique was the conversion of N-benzyloxycarbonyl-ω-nitro-L-arginyl-L-proline methyl ester by the action of anhydrous hydrazine to the corresponding hydrazide, which was analyzed for nitrogen, but not for carbon and hydrogen: I. Z. SIEMION, Roczn. Chem. *38*, 811 (1964).
[21] E. SCHRÖDER, in a personal communication (May 27, 1966), mentioned that the reported hydrazinolysis of N-benzyloxycarbonyl-ω-nitro-L-arginyl-L-proline methyl ester was studied independently, but no positive results were obtained from the experiment.
[22] The authors are indebted to the National Science Foundation for Grants No. GB-587 and GP-3888, which supported this study.

Chromosomal Rearrangements Induced in *Drosophila* Salivary Gland Chromosomes by an Acridine Half Mustard

The monofunctional nitrogen mustard derivative of acridine, 2-methoxy-6-chloro-9-[3-(ethyl-2-chloroethyl) aminopropylamino] acridine dihydrochloride (ICR-170), is an efficient mutagen in *Drosophila*[1–3], *Neurospora*[4] and *Salmonella*[5], in addition to its possessing nucleotoxic activity comparable to bifunctional alkylating agents[6]. Mutations induced by ICR-170 at the dumpy locus in *Drosophila*[1] and *ad-3* region in *Neurospora*[4] have been found not to involve chromosomal deletions. Evidence of ICR-170-induced (a) nonsense, non-complementing mutations in *Neurospora*[4] and (b) reversions of frameshift mutations of *C* gene of the histidine operon in *Salmonella*[5] suggests this chemical may be causing mutations of frameshift type in both *Neurospora* and *Salmonella* and possibly