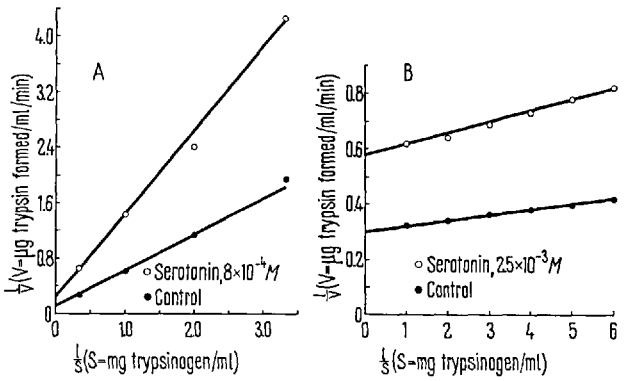
Serotonin, a Strong Inhibitor of the Autocatalytic Activation of Trypsinogen

In previous experiments it has been demonstrated that aliphatic ω-amino and guanidino acids as well as α-keto analogues of tyrosine, tryptophan and phenylalanine inhibit the activation of trypsinogen[1-3]. Recently, TRETTIN and MIX[4] have reported that aliphatic guanidino compounds without carboxylic groups are even stronger inhibitors than the acids themselves. 1-Guanidinopropane was the most powerful inhibitor in their series and was shown to be about 15 times more effective on a molar basis than 4-guanidinobutyric acid, the strongest acidic compound. In the light of these latest results it was decided to investigate also the inhibitory effect of aliphatic amines and of the decarboxylation products of the aromatic and heterocyclic amino acids mentioned above. The study was deemed of special interest because several of the substances to be tested occur within certain cells and tissues where they serve a neuroregulatory function or represent metabolic intermediates.

The effect of the compounds on trypsinogen activation was examined in the autocatalytic system as well as in the enterokinase-dependent reaction. The amounts of trypsin formed in the activation mixtures were determined by an esterolytic method[1] employing *p*-toluenesulfonyl-L-arginine methyl ester as substrate. 4-Guanidinobutyric acid was added in 0.1 *M* concentration to the dilution fluid and to the assay mixtures to suppress any further activation of trypsinogen during the assay procedure[2].

From the Table it can be seen that several alkylamines are powerful inhibitors of the autocatalytic activation outranking both compounds which were included in the series for comparative purposes, i.e. *p*-hydroxyphenylpyruvic acid and 4-guanidinobutyric acid. Serotonin (5-hydroxytryptamine) and tryptamine were the strongest cyclic compounds followed closely by 5-methoxytryptamine. Phenylethylamine and tyramine, on the other hand, were considerably less effective. It is also evident that a free amino group is a necessary prerequisite for the inhibitory activity because acetylation of the amino group or its replacement by a hydroxyl group resulted in loss of all potency. A chain length of four carbon atoms appeared to



Plots according to LINEWEAVER and BURK demonstrating that serotonin is a non-competitive inhibitor of the autocatalytic activation of trypsinogen (A) as well as of the activation by enterokinase (B). Conditions of activation were the same as given in the footnote to the Table except for the variation in the amounts of trypsinogen and for the presence of only 0.66 E.K.U. in the activation mixtures with enterokinase.

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Inhibitory effect of alkylamines on the activation of trypsinogen[a]

Inhibitor	Autocatalytic activation			Activation by enterokinase		
	Concentration of inhibitor (*M*)		K_i	Concentration of inhibitor (*M*)		K_i
	10^{-3}	10^{-4}	(m*M*)	10^{-2}	10^{-3}	(m*M*)
	Trypsin formed (% of control)			Trypsin formed (% of control)		
Serotonin (5-hydroxytryptamine)	25	76	0.70	0	63	3.20
Tryptamine	26	76	–	20	75	–
5-Methoxytryptamine	31	82	–	26	77	–
N-acetyl-5-methoxytryptamine	100	100	–	100	100	–
Tryptophol	100	100	–	100	100	–
Phenylethylamine	62	92	–	85	100	–
Tyramine	69	96	–	61	84	–
1-Propylamine	71	100	–	83	100	–
1-*n*-Butylamine	30	78	0.75	56	85	12.0
1-*n*-Pentylamine	37	86	–	79	100	–
1-*n*-Hexylamine	70	90	–	86	100	–
1-*n*-Heptylamine	82	100	–	79	100	–
1-*n*-Octylamine	81	100	–	76	100	–
4-Guanidinobutyric acid	46	84	1.19	28	77	4.90
p-Hydroxyphenylpyruvic acid	52	89	1.39	0	34	0.72

[a] The activation mixtures for the autocatalytic reaction consisted of 2 ml containing 50 µg trypsin, 2 mg trypsinogen preparation (74% protein) and 0.1 *M* $CaCl_2$ in 0.1 *M* *tris*-HCl buffer (pH 7.7). Incubation was carried out for 12 min at 23°C. The activation mixtures for the enterokinase-dependent process contained 2.64 EKU (enterokinase units according to KUNITZ[5]) and 2 mg trypsinogen preparation in 2 ml 0.02 *M* phosphate buffer (pH 5.8). Incubation was for 8 min at 23°C.

be optimal for full inhibitory strength of the aliphatic amines which is in accordance with the results of INAGAMI[6], who tested the influence of a number of aliphatic amines on the tryptic hydrolysis of benzoyl-L-arginine ethyl ester. It should be noted that butylamine possesses one carbon less than 5-aminovaleric acid, which besides 8-aminocaprylic acid is the leading inhibitory aliphatic amino acid[1]. Similar structural requirements pertain to the guanidino compounds where 1-guanidinopropane also represents the decarboxylation product of the strongest inhibitory acid, i.e. 4-guanidinobutyric acid[4].

In contrast to their marked interference with the autocatalytic process, all alkylamines tested were much weaker inhibitors of the activation by enterokinase. Serotonin and tryptamine were still superior to 4-guanidinobutyric acid, however.

Results of kinetic studies, as plotted in the Figure, revealed that inhibition by serotonin was non-competitive in both activation systems.

The data contained in this communication suggest a possible physiological role for naturally occurring alkylamines as inhibitors of proteolytic enzymes, especially as several aliphatic and cyclic alkylamines are already known to inhibit the esterase activity of trypsin[6] and the fibrinolytic activity of plasmin[7,8], and as tryptamine is able to block the activity of chymotrypsin[9]. The findings, furthermore, indicate lines of future investigations to obtain even more powerful inhibitors of trypsinogen activation. Substitutions on the indole structure might lead to stronger inhibitors of the autocatalytic reaction while modification of *p*-hydroxyphenylpyruvic acid seems to offer hope of finding still more effective inhibitors of enterokinase[10].

Zusammenfassung. Eine Reihe von Alkylaminen wurden auf ihren hemmenden Einfluss auf die Trypsinogenaktivierung untersucht. Hierbei erwies sich Serotonin als der stärkste Hemmstoff, gefolgt von Tryptamin und Butylamin. Die Hemmung der autokatalytischen Aktivierung war bedeutend stärker ausgeprägt als die Hemmung der Aktivierung durch Enterokinase.

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July 20, 1965.

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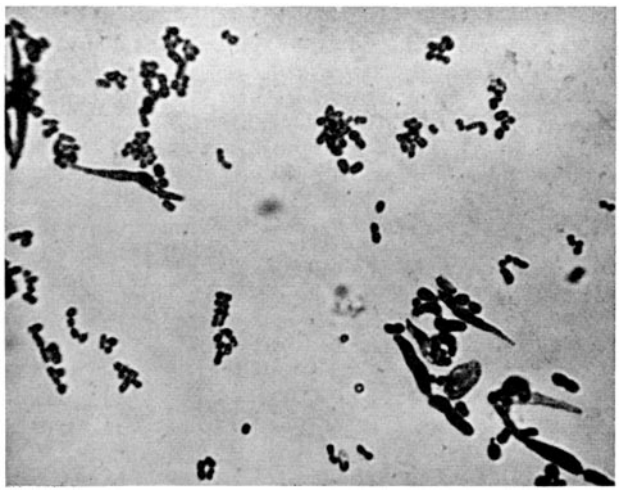
Induction of Morphological Changes in Bacteria by Optochin

Many chemical and physical agents[1-7] are known to change bacterial cells morphologically. The purpose of this report is to present evidence for the inclusion of optochin (ethylhydrocupreine hydrochloride) in the already long list of chemicals with this property.

Two of the strains of *Bacterium anitratum*, which showed elongation and enlargement of cells when exposed to sulphonamides[3], produced similar forms in the presence of optochin. Some of these enlarged cells are seen in the Figure, lying among normal ones. These cells are from a 24 h culture of strain 1 which grew around a paper disc soaked in 0.02 ml of a 1-in-4000 solution of optochin. The method of testing was the same as that used in testing *Bacterium anitratum* for response to sulphonamides[3]. The zone of inhibition of the macroscopic growth of bacterial colonies around the optochin disc was narrow (about 1.3 cm in diameter) and irregular in outline. The colonies of *Bacterium anitratum* growing at the border of this inhibition zone were not smooth and rounded as they were outside the zone of action to the optochin disc, but were rough in appearance, with many elongated cells protruding from the border of the colonies.

Optochin caused fewer cells in a population to change morphologically than did the sulphonamides. Also, the cells did not enlarge as much as when they were exposed to sulphonamides. However, the exact morphological character of the changes induced by either of these agents was the same. First, the cells grew in long nonseptated filaments. Then they enlarged, mostly in the middle, into



24 h culture of strain 1, taken at the border of the inhibition zone of optochin on nutrient agar. Gram stain. Magnification × 830.

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