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.

J. D. GERATZ

Department of Pathology, University of North Carolina School of Medicine, Chapel Hill (N.C. USA),
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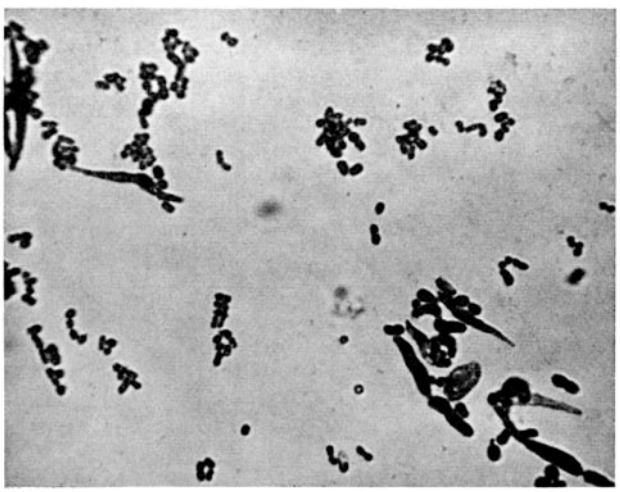
Induction of Morphological Changes in Bacteria by Optochin

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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irregular, spindle-shaped forms. The enlarged parts of the cells showed a tendency to retain methyl violet when stained by the Gram technique. In addition to the elongated and enlarged cells, single or double spheroidal cells were seen.

Similar results were obtained with different culture media (nutrient agar, 5% horse blood agar, chocolate agar, trypticase soy agar) and different temperatures (23°, 25°, 37°C). When incubation was prolonged to 72 h, the cells changed only slightly in size but markedly in texture. They became granulated, irregularly outlined, and they stained poorly.

When the organisms were transferred to the same, or another medium, without optochin, they again grew as short bacilli within 24 h.

We believe that the changes described above are caused by the inhibition of cell division in *Bacterium anitratum* at bacteriostatic concentrations of optochin, and by the continuation of growth of individual cells beyond their normal dimensions.

The appearance of rough colonies is the result of the elongation of the individual cells which form the colonies. In this way the changes in size and shape of the individual cells, as well as the change in the colonial morphology, can be explained by the dissociative action of optochin on cell growth and cell division. Furthermore, the forms described above, which we regard as transitional to the L-forms of bacterial growth, are caused by environmental factors rather than due to some developmental cycle of bacterial growth[8].

Some other gram negative bacteria were examined for their reactions to optochin. No macroscopic or microscopic changes were found in the colonies of various strains of other bacteria, namely *E. coli*, *B. aerobacter aerogenes*, *Proteus* bacilli, *Salmonella typhi*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Pseudomonas pyocyanea*, *Pasteurella pseudotuberculosis*, all grown for 48 h around optochin discs on 5% horse blood agar, either at 37°C or at room temperature.

This simple technique of screening different bacteria for their morphological reactions to certain chemicals, i.e. by using paper discs instead of incorporating the various chemicals into the media in various concentrations, has the advantage of being similar to gradient plates in their efficiency in providing a range of concentrations of the tested chemicals on only one plate[9].

Zusammenfassung. Es wird die Wirkung des Optochins auf die Zellmorphologie des *Bacterium anitratum* beschrieben und aus Versuchsergebnissen geschlossen, dass Optochin zur Gruppe chemischer Substanzen gehört, die zu hochgradigen morphologischen Veränderungen der Bakterienzelle führen.

B. BRZIN

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On the Origin of the Antiheparin Activity of Serum. The Effect of Reserpin

The origin of serum antiheparin activity[1] is still not clear. O'BRIEN[2] argued that this activity is a function of thrombocytes. Since this view has not been clearly confirmed as yet, the present attempt was made to investigate whether antiheparin activity of serum is influenced by reserpin added to the blood, since the latter substance interferes with platelet metabolism to a marked degree[3].

Material and methods. The effect of reserpin was estimated by means of the elastogram (thrombelastograph HELLIGE), performed on blood without and with reserpin. 10 ml of venous blood were withdrawn from each subject in all, two 5 ml portions of this blood received 1 ml of phosphate buffer pH 7.4, but only one of them received 1 mg reserpin. 0.3 ml blood of each portion was used for the actual analysis in the thrombelastograph. After filling the cuvettes, the remainder of these portions was coagulated by incubation in a thermostat for 60 min, followed by 15 min of centrifugation at 1500 RPM, thus yielding reserpinized (S_R) and non-reserpinized, or control, sera (S_C).

The antiheparin activity of these sera was estimated by a previously published method[4]. The actual procedure was simple: 1 ml of fresh sera (S_R and S_C) was added to test tubes containing 2 U of heparin dissolved in 2 ml of physiological saline. After 1 min incubation at 37°C, 1 ml of freshly drawn venous blood (from healthy volunteers) was added to both these test tubes. 0.3 ml was withdrawn and placed in thrombelastograph cuvettes, and 'r_R' and 'r_C' values were measured. This estimation was made in six healthy persons.

Table I. The effect of reserpine (1 mg/5 ml) on the 'r' and 'Σ' values in the thromboelastogram

Test no.	'r'				'Σ'	
	Control blood min	sec	Reserpinized blood min	sec	Control blood	Reserpinized blood
1	6	00	7	00	138	12
2	9	00	9	00	133	20
3	8	30	6	00	144	15
4	6	30	7	45	144	13
5	7	30	6	30	163	16
6	6	30	5	30	138	15

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