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.

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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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Since the same blood and the same amount of heparin was used for both 'r_R' and 'r_C' measurements under the same conditions, the antiheparin activity of the sera investigated is proportional to the 'r_R' and 'r_C' values. This relationship is inverse: the greater the antiheparin activity of serum the shorter the 'r' value. The antiheparin activities of both these sera (S_R and S_C) were compared by expressing the antiheparin activity of reserpinized serum (S_R) as a percentage of the antiheparin activity of serum from blood without reserpin, which was taken as 100% according to the formula: antiheparin activity of serum $_R = r_C/r_R \cdot 100$.

Table II. Antiheparin activity of serum: control and reserpinized samples

Test no.	Control blood (%)	Reserpinized blood (%)
1	100	210.0
2	100	322.2
3	100	590.9
4	100	170.0
5	100	250.0
6	100	425.0
Average:	100%	328.0%

$t = 3.474$, $P < 0.05$

Results. Table I presents 'r' and 'Σ' values from thrombelastograms of control and reserpinized bloods. As can be seen, reserpin had a marked influence on the 'Σ' values, but the 'r' values were not affected, i.e. the effect was the same on thrombocytes. Table II shows relative values of antiheparin activity of reserpinized serum (S_R) as a % of activity of the non-reserpinized (S_C) serum.

Conclusion. Reserpin significantly increased the antiheparin activity of serum from reserpinized blood ($t = 3.474$, $P < 0.05$) and decreased the 'Σ' value in thrombelastogram of this blood.

There is no direct evidence whatsoever as to the nature of the antiheparin activity of serum, but it would appear to be a substance freed from thrombocytes, which process is accelerated by reserpin.

Zusammenfassung. Es wurde gezeigt, dass Zusatz von Reserpin zum Blut die Antiheparinaktivität im Serum deutlich steigert. Da Reserpin zugleich die Thrombocyten beeinflusst, wird vermutet, dass diese für die Antiheparinaktivität verantwortlich sind.

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Specific Inhibition of Mast Cell Disruption in vitro

Successful attempts have been made to use direct or indirect degranulation of basophile cells[1-9], or indirect mast cell degranulation[10], in the diagnosis of hypersensitivity.

Rat mast cell degranulation was employed chiefly in experimental anaphylaxis[11-17] by in vitro exposure to the antigen of mast cells from sensitized animals, or sensitization of normal rat cells with the serum of sensitized rats, which contains a mast cell sensitizing antibody (MCSAb)[14].

We have investigated: (a) the disruption of isolated peritoneal mast cells from sensitized rats in vitro in the presence of antigen; (b) how soon after inoculation it occurs; (c) if it could be specifically inhibited by a suitable antibody.

Albino rats were sensitized by a single inoculation in each foot pad of human γ-globulin (with complete Freund adjuvant 'Difco'), groups of animals were killed weekly over a period of 6 weeks following inoculation, and serum and peritoneal mast cells subsequently removed. Skin test performed on the 7th day after inoculation showed a delayed reaction of hypersensitivity.

Peritoneal mast cell suspension was obtained by a previously described technique[10], and the degranulation test was carried out in the presence of human γ-globulin diluted 1:5.

The inhibition test was performed by adding to the mast cells an antigen-antibody mixture (antigen in a 1:2.5 dilution, antibody – anti human-rabbit serum – in twofold dilutions from 1:2 to 1:32).

Two controls were used: (1) mast cells added to antibody alone, and (2) mast cells added to antigen mixed with normal rabbit serum rather than antibody (in order to test the specificity of the reaction).

We found extensive disruption of mast cells from sensitized rats when exposed in vitro to the antigen at 37°C.

The reaction is already positive in the first week, more pronounced in the second and third weeks, decreases in

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