

of homogenate from 25 g of calf lungs with 250 mg of DHA were identical with the behaviour of the referent synthetic 7 α -OH-DHA. To identify this substance, the following reactions were performed: (a) Dehydration by treatment with 4% HCl in 90% methanol over a period of 24 h yielded $\Delta^{5,7}$ -androstadiene-3 β -ol-17-one; (b) Reduction with NaBH₄ in a water-ethanolic medium yielded Δ^5 -androstene-3 β ,7 α ,17 β -triol; (c) Acetylation using acetic anhydride in a pyridine medium yielded 3,7-diacetate.

Table II. Influence of several inhibitors on the *in vitro* 7 α -hydroxylation of DHA in the livers and the lungs of female rats. 2 g of tissue were incubated with 2 mg of DHA

Inhibitor	7 α -OH-DHA/1 mg of DHA	
	Liver	Lung
	μ g	μ g
Ø (control)	6.0	34.0
N ₂ atmosphere	2.9	4.0
95°C/10 min	0.0	0.0
KCN (10 ⁻² M)	10.4	27.0
HgCl ₂ (10 ⁻³ M)	1.5	0.0
α,α' -Dipyridyl (5.10 ⁻⁴ M)	2.5	—
Na ₄ P ₂ O ₇ (10 ⁻³ M)	—	0.0

Determination of the Structure of the Peptide Moiety of the Antibiotic Albomycin

The albomycin¹ molecule consists of two moieties²: the peptide part containing 3 L-serine residues, 3 N δ -hydroxyornithine residues, and iron bound in complex; and the pyrimidine part containing a sulphur atom. Both moieties are linked through the oxygen atom of the hydroxy group of one of the three serine residues. By acid hydrolysis of the pyrimidine moiety 3-methyluracil is formed, and by alkaline hydrolysis 4-(N'-methyl)cytosine arises. Partial acid hydrolysis of albomycin releases three peptides containing 3-methyl-uracil linked with one, two, and probably three serine residues³. From the partial acid hydrolysate of deferrialbomycin (preparation with carefully removed iron) a great amount of ninhydrin positive degradation products (formed from unstable N δ -hydroxyornithine) was obtained from which, however, it was not possible to construct the amino acid sequence in the peptide part of the antibiotic. Neither albomycin nor deferrialbomycin were cleaved in experiments of enzymatic hydrolysis. Although N δ -hydroxyornithine is transformed into stable ornithine by hydrogenation, deferrialbomycin is hydrogenated in the pyrimidine part of the molecule only since N δ -hydroxyornithine is acetylated in the antibiotic and forms three hydroxamic acid groups.

The most adequate procedure for stabilizing N δ -hydroxyornithine in the cyclopeptide of deferrialbomycin is, according to our experience, its transformation to glutamic acid by oxidation with performic acid as described previously^{3,4}. The sequence of its amino acids can then be solved by partial acid hydrolysis. Six peptides, P 32, P 33, P 71, P 72, P 73, and P 82, were obtained thereby, which were isolated by paper chromatography and electrophoresis. The pattern of these peptides in

The identities of the resulting products were proved by their chromogenic properties and chromatographic mobilities in at least two systems.

Analogically, 40 μ g of the substance obtained by incubating DHA with the homogenate from rat lungs was identified.

The *in vitro* 7 α -hydroxylation of DHA in lung and liver homogenates fails to show any significant dependence on the sex of the rats. The influence of the action of several inhibitors during incubation and the influence of preheating the homogenate is obvious from Table II. The presence of TPNH slightly increased yields from both the lungs and the livers.

The presence of 7 α -OH-DHA in tissue incubates is not occasioned by an autooxidation of DHA, as confirmed by the negative results of blind experiments without the use of tissues.

Zusammenfassung. In verschiedenen Rattenorganen (Lunge, Niere, Milz, Blut, Muskelgewebe) konnte ein System nachgewiesen werden, das imstande ist, *in vitro* Dehydroepiandrosteron an der 7 α -Stellung zu hydroxylieren. Dies Ergebnis weist auf die allgemein mögliche extrahepatale und extraadrenale Steroidhydroxylation hin.

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Figure 1 shows the unambiguously determined sequence of the oxidized cyclopeptide moiety of the antibiotic. X denotes the degradation product formed by oxidation of the pyrimidine part. The previously mentioned peptides

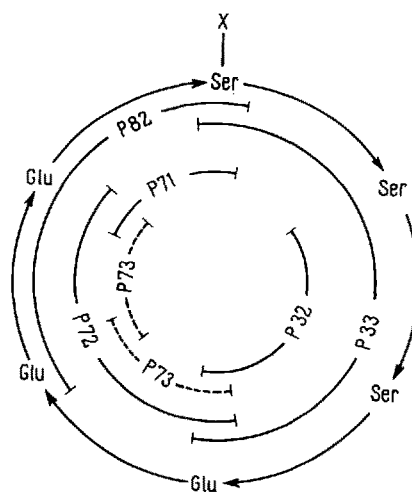


Fig. 1

¹ G. F. GAUZE and M. G. BRAZHNKOVA, *Novosti Med.* 23, 1, 3 (1951).

² O. MIKEŠ, J. TURKOVÁ, and F. ŠORM, *Coll. Czech. chem. Comm.* 28, 1747 (1963).

³ O. MIKEŠ and J. TURKOVÁ, *Coll. Czech. chem. Comm.* 27, 581 (1962).

⁴ J. TURKOVÁ, O. MIKEŠ, and F. ŠORM, *Coll. Czech. chem. Comm.* 27, 591 (1962).

obtained from a partial acid hydrolysate of deferriolbomycin², and containing the pyrimidine derivative besides serine, are in good agreement with this sequence. The hydroxamate groups form ligands linking the Fe^{3+} ion

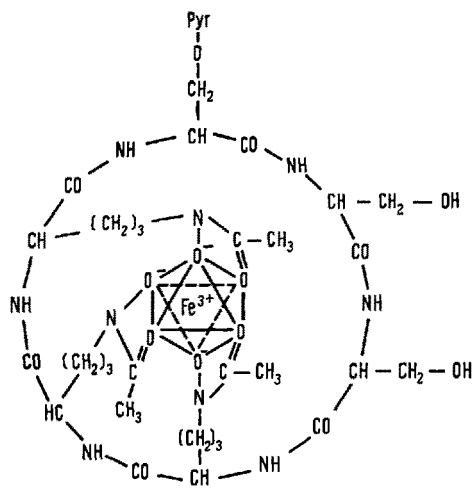


Fig. 2

in stable complex bond, whose linkage is represented by the octahedron in the structural formula Figure 2. Albomycin, in analogy to ferrichrome⁵, besides this iron ion, is able to bind another Fe^{3+} ion on the same complexing centre⁶.

Details of this work are being published in Collection of Czechoslovak Chemical Communications.

Zusammenfassung. Der Peptidteil des Antibioticums Albomycin wurde aufgeklärt durch Überführung der N^δ-Hydroxy-ornithinreste in Glutaminsäurereste und anschließende saure Partialhydrolyse, wobei sechs Peptide resultierten, deren Kombination eine eindeutige Strukturformel lieferten.

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Czechoslovak Academy of Science, Prague (Czechoslovakia),
June 28, 1963.*

⁵ T. EMERY and J. B. NEILANDS, *J. Amer. chem. Soc.* **83**, 1626 (1961).

⁶ J. TURKOVÁ, O. MIKEŠ, J. SCHRAML, O. KNESSL, and F. ŠORM, *Antibiotiki*, in press.

Production of Bulbous and Spheroplast-like Cells in *Bacterium anitratum* under the Action of Sulphathiazol

It is well known that many bacteria, especially the gram-negative bacilli, are induced to form spheroplasts under the action of penicillin and other agents^{1,2}. Also in gram-positive bacteria similar forms can be induced by methicillin³. For the sulphonamides, it is known¹ that they may cause filamentation in some rod-shaped organisms. Other changes of the form of gram-negative bacteria produced by the sulphonamides are, to our knowledge, not yet described. In this communication, the morphological evidence is presented to demonstrate that the changes produced by the sulphonamides in some bacteria could be of the same nature and degree as those produced (and already described) by penicillin.

From the material taken by pulmetomy, a strain of *Bacterium anitratum* was isolated in pure culture. It was sensitive to sulphathiazol and slightly sensitive to penicillin (paper disc method⁴). If the plate was inspected by using low power objectives, pictures such as those in Figure 1 were observed at the border of the inhibitory zone around the disc of sulphathiazol (0.5%). The smear prepared from the small irregular colonies found at the border mentioned above showed fusiform filaments. Some of these filaments were swollen to bulbs, which showed the

¹ A. J. SALLE, *Fundamental Principles of Bacteriology*, fifth Ed. (McGraw-Hill Book Company, New York 1961).

² B. BRZIN, *Acta path. microbiol. scand.* **57**, 188 (1962).

³ B. M. KAGEN, C. W. MOLANDER, and H. J. WEINBERGER, *J. Bacteriol.* **83**, 1162 (1962).

⁴ I. G. SCHAUB and M. K. FOLEY, *Diagnostic Bacteriology*, fourth Ed. (The C. V. Mosby Comp, St. Louis 1952).

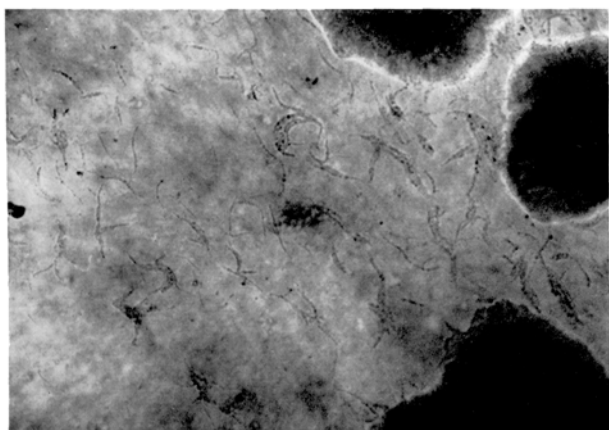


Fig. 1

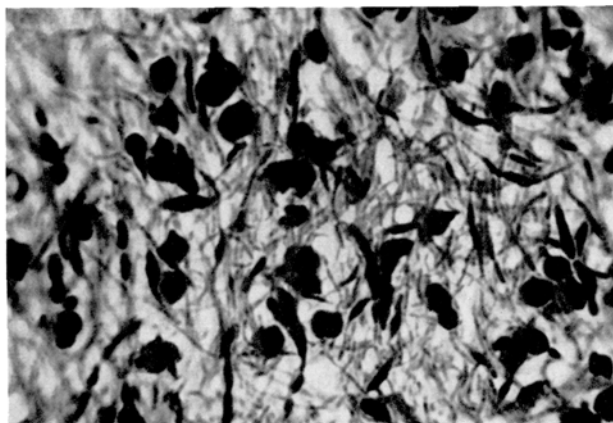


Fig. 2