

obtained from a partial acid hydrolysate of deferriolbomycin<sup>2</sup>, and containing the pyrimidine derivative besides serine, are in good agreement with this sequence. The hydroxamate groups form ligands linking the  $\text{Fe}^{3+}$  ion

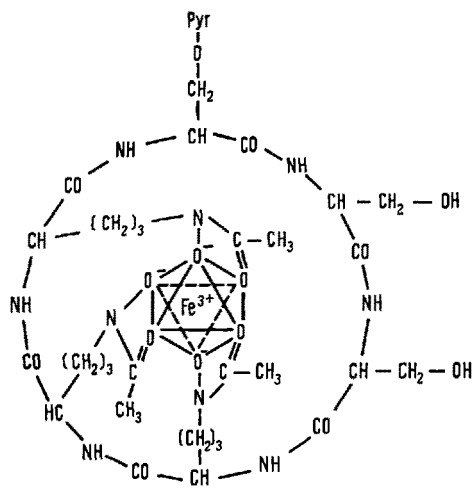


Fig. 2

in stable complex bond, whose linkage is represented by the octahedron in the structural formula Figure 2. Albomycin, in analogy to ferrichrome<sup>5</sup>, besides this iron ion, is able to bind another  $\text{Fe}^{3+}$  ion on the same complexing centre<sup>6</sup>.

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<sup>δ</sup>-Hydroxy-ornithinreste in Glutaminsäurereste und anschließende saure Partialhydrolyse, wobei sechs Peptide resultierten, deren Kombination eine eindeutige Strukturformel lieferten.

J. TURKOVÁ, O. MIKEŠ, and F. ŠORM

*Institute of Organic Chemistry and Biochemistry,  
Czechoslovak Academy of Science, Prague (Czechoslovakia),  
June 28, 1963.*

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

<sup>6</sup> 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<sup>1,2</sup>. Also in gram-positive bacteria similar forms can be induced by methicillin<sup>3</sup>. For the sulphonamides, it is known<sup>1</sup> 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 method<sup>4</sup>). 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

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

<sup>2</sup> B. BRZIN, *Acta path. microbiol. scand.* **57**, 188 (1962).

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

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

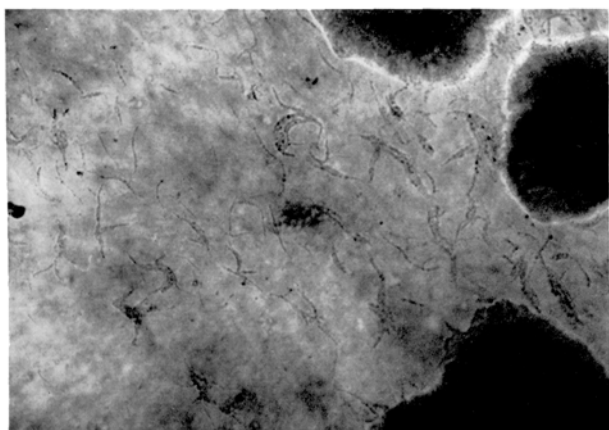


Fig. 1

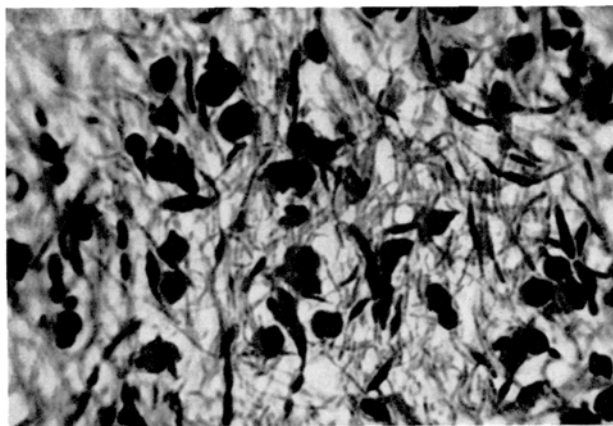


Fig. 2

tendency to retain methylviolet in the Gram stain (Figure 2). Following the next 24 h of incubation, some round spheroplast-like forms were found in the smears and by direct inspection of the plate. The smear prepared from the colonies around the disc soaked with penicillin (50 and 500 E/ml) showed similar pictures. The spheroplast formation was more pronounced around the disc soaked with penicillin 500 E/ml than around that of only 50 E/ml. Smears prepared from the colonies grown around the discs with other antibiotics (streptomycin, chloramphenicol, terramycin and ilotycin) and from the colonies which grew far away from the discs with penicillin and sulphathiazol showed a normal morphological picture of *Bacterium anitratum*, i.e. short bacilli.

Antibiogram test is in our opinion very convenient for use in studying the inductibility of the abnormal forms by the action of antibiotics and sulphonamides on bacteria. In this way all concentrations of the substance tested from 0 up to the concentrations near to that prepared for soaking the disc, are acting at the same time but on different places on the organisms tested. By incorporating the active substance into the medium we may miss the optimal concentration, i.e. the subbactericidal concentration which might induce filamentation and consequently the spheroplast-like forms.

Beside the strain described above, some other strains of *Bacterium anitratum* were found to change their form under the influence of sulphathiazol.

Investigations are in progress to obtain more concentrated spheroplast-like cells or even a 'pure culture' of these by using prolonged influence of the inducing agent (sulphathiazol) in a subbactericidal concentration and a stabilizing agent (Mg salts)<sup>5</sup>.

*Zusammenfassung.* Veränderungen der Form des *Bacterium anitratum* unter dem Einfluss von Sulphathiazol werden beschrieben. Sie sind denjenigen ähnlich, die bei diesen und anderen Bakterien unter dem Einfluss des Penicillins gefunden wurden.

B. BRZIN

*Microbiological Institute, Medical Faculty, Ljubljana (Yugoslavia), June 25, 1963.*

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## The Problem of the Phage-Like Structure of the Avian Leukosis Virus

In the first communication of BEARD et al.<sup>1</sup> concerning the morphology of the avian myeloblastosis virus particles (BAI-strain A-virus<sup>2</sup>) isolated from the blood plasma of diseased animals, two forms of virus particles in the virus population were described: the spherical, and the phage-like type. The shape of the latter seemed 'to lie between that of the virus of the Newcastle disease and the form of the tailed bacteriophages'<sup>1</sup>. Later it was observed<sup>2</sup> that the drying of the virus from saline suspension on an agar surface led to a considerable decrease in the number of the tailed particles, whereas the increase in the salt concentration attendant upon drying of the virus in a collodion membrane was considered a possible, potent factor leading to the alteration of the shape of the virus particle<sup>2</sup>. Even though 'it was very difficult to discuss the tailed form of the leukosis virus wholly as artifacts'<sup>2</sup>, the cause of the virus particle pleiomorphism appeared to have been elucidated. It is now generally held that the real form of the virus particles is the spherical form<sup>2-9</sup>.

During the course of a two-year study of the BAI-strain A-virus isolated from chicken plasmas, we repeatedly encountered the spherical and phage-like forms as earlier described. The striking variations in the proportions of both forms in the virus populations isolated from individual plasmas by identical techniques (Figures 1, 1a, 2, 2a) led us to re-examine the correlation between the proportions of the two forms and the technique of preparation as well as other variables, particularly the stage of the leukemic process.

*Materials and Methods.* BAI-strain A-virus employed throughout this study originates from the myelo-pool of Professor B. G. THORELL, Stockholm, October 14, 1960. The virus was passed<sup>1,2</sup> in random bred white Leghorn chickens, kept at 33°C and fed with standard local chow. The stage of the leukemic process was defined by haematocrit values and blood cell counts. The fresh heparinized (1:500) blood was centrifuged at 1500 × g for 30 min at

4°C, the plasma was filtered through a sintered glass filter G4 (Jena), and subsequently centrifuged twice at 4°C at 1500 × g for 30 min and then at 2°C at 55 000 × g for 40 min (MSE Super). The virus pellet obtained in this way was suspended by homogenization (plexiglass-glass) in 0.15 M ammonium buffer of pH 7.0<sup>10</sup>, and sprayed onto a collodion membrane<sup>10</sup>. The agar technique for drying the virus suspension was used as described in the literature<sup>2</sup>. Negative staining of the virus particles was carried out with phosphotungstic acid<sup>11</sup>. An electron microscope constructed in the Institute for Instrument Research of the Czechoslovak Academy of Science (Brno) was used. The number of the tailed particles was counted in populations of at least 1000 particles observed in one or more micro-droplets. The effect of the preparative technique and of the isolation media on the virus particle shape was always studied on a single initial population. (It is not obvious whether this was done in previous work<sup>2</sup>.) The results were analysed statistically.

*Results and Discussion.* The quantitative evaluation of the ratio of tailed and spherical particles in the individual

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<sup>4</sup> E. A. ECKERT, D. G. SHARP, I. GREEN, D. BEARD, and J. W. BEARD, *Proc. Soc. exp. Biol. Med.* 88, 181 (1955).

<sup>5</sup> E. A. ECKERT, D. G. SHARP, D. BEARD, and J. W. BEARD, *J. Nat. Cancer Inst.* 16, 593 (1955).

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<sup>7</sup> J. W. BEARD, *American Scientist* 46, 226 (1958).

<sup>8</sup> W. BERNHARD, R. A. BONAR, D. BEARD, and J. W. BEARD, *Proc. Soc. exp. Biol. Med.* 97, 48 (1958).

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<sup>10</sup> R. C. BACKUS and R. C. WILLIAMS, *J. appl. Phys.* 21, 11 (1950).

<sup>11</sup> S. BRENNER and R. W. HORNE, *Biochim. biophys. Acta* 34, 103 (1959).