tendency to retain methylviolet in the Gram strain (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)[5].

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 (Jugoslavia), June 25, 1963.

[5] My thanks are due to Dr. M. Likar for helpful discussion and for reading the manuscript.

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

In the first communication of Beard et al.[1] concerning the morphology of the avian myeloblastosis virus particles (BAI-strain A-virus[2]) 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'[1]. Later it was observed[2] 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[2]. Even though 'it was very difficult to discuss the tailed form of the leukosis virus wholly as artifacts'[2], 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[2-9].

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[1,2] 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 $1500 \times g$ 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 $1500 \times g$ for 30 min and then at 2°C at $55000 \times g$ 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[10], and sprayed onto a collodion membrane[10]. The agar technique for drying the virus suspension was used as described in the literature[2]. Negative staining of the virus particles was carried out with phosphotungstic acid[11]. 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[2].) The results were analysed statistically.

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

[1] D. Beard, E. A. Eckert, T. Z. Csáky, D. G. Sharp, and J. W. Beard, Proc. Soc. exp. Biol. Med. *75*, 533 (1950).
[2] D. G. Sharp, E. A. Eckert, D. Beard, and J. W. Beard, J. Bacteriol. *63*, 151 (1952).
[3] E. A. Eckert, D. G. Sharp, E. B. Mommaerts, R. H. Reeve, D. Beard, and J. W. Beard, J. Nat. Cancer Inst. *14*, 1039 (1954).
[4] E. A. Eckert, D. G. Sharp, I. Green, D. Beard, and J. W. Beard, Proc. Soc. exp. Biol. Med. *88*, 181 (1955).
[5] E. A. Eckert, D. G. Sharp, D. Beard, and J. W. Beard, J. Nat. Cancer Inst. *16*, 593 (1955).
[6] J. W. Beard, Poultry Sci. *35*, 203 (1956).
[7] J. W. Beard, American Scientist *46*, 226 (1958).
[8] W. Bernhard, R. A. Bonar, D. Beard, and J. W. Beard, Proc. Soc. exper. Biol. Med. *97*, 48 (1958).
[9] J. W. Beard, Progress in Haematology *3*, 105 (1962).
[10] R. C. Backus and R. C. Williams, J. appl. Phys. *21*, 11 (1950).
[11] S. Brenner and R. W. Horne, Biochim. biophys. Acta *34*, 103 (1959).

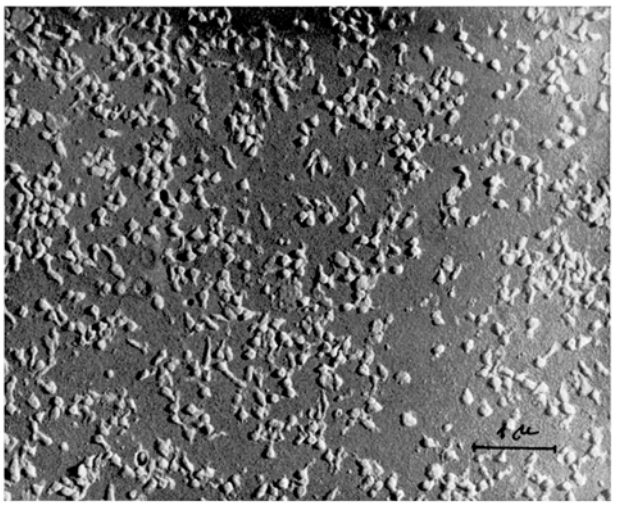

Fig. 1

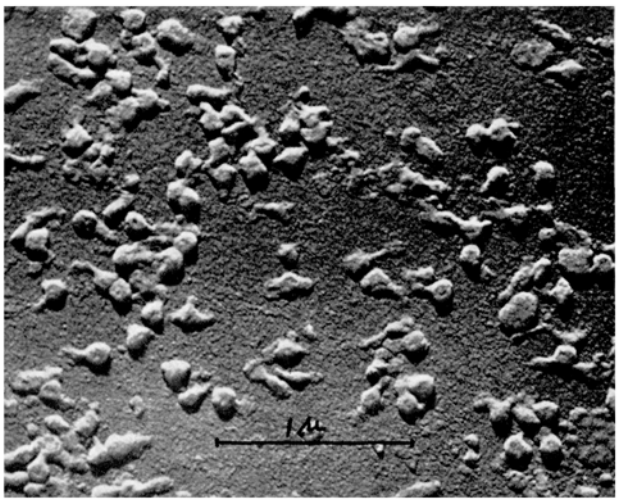

Fig. 1a

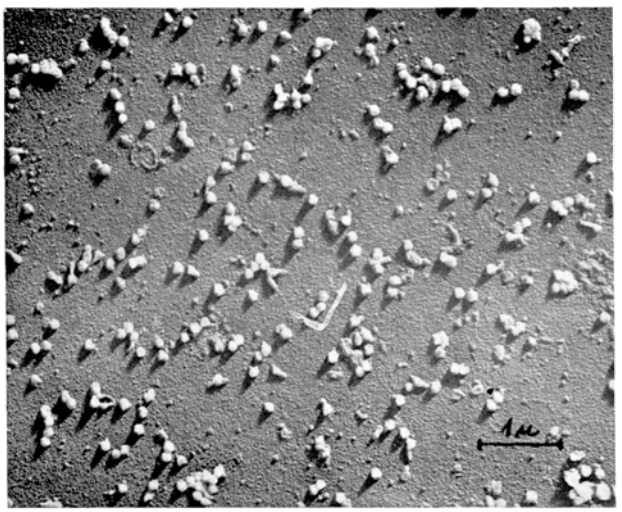

Fig. 2

Fig. 2a

Figs. 1, 1a, 2, and 2a. Examples of extreme incidences of individual forms (phage-like and spherical) of particles in the virus populations isolated identically from different plasmas.

virus populations showed that it was not substantially affected by the technique of drying (Table I). Further freezing of the fresh plasma, repeated three times at −70°C, did not influence the morphological character of the virus population. The effect of the various isolation media (isotonic sucrose, physiological saline, and doubly distilled water) was in agreement with previous reports about the strong hydratation effect of water[2] and the relatively high fragility of the tails[2] during manipulation in the saline and isotonic sucrose media.

The proportions of two virus particle forms, however, could be correlated with the stage of the leukemic process. Plasma from leukemic blood with 24–33% myeloblasts yielded a virus population with 80–85% of the tailed forms (Table II). This relatively short period in the course of the whole leukemic process represents the period of the steepest increment in the number of leukemic cells in the blood (Figure 3). The period is also related to the maximum increment of the ATP-ase activity in blood plasma[12], which is intimately associated with the virus particle[13], and also with the maximum increment in the phosphoenolpyruvate-kinase activity in the unsedimentable plasma fraction[12]. In agreement with this observation, pronounced changes in the ATP/AMP and ADP/AMP ratios in myeloblast populations over the corresponding ratios during the incipient or later stages was found[12]. Later stages (more than 35% leukocytes in blood) exhibit certain irregularities in the relation between the total mass of the myeloblasts and their number indicating cell populations with continuous growth of cell mass but with decreased mitotic activity (Figure 3)[12]. The negative staining technique, in agreement with previous reports[2], revealed a continuous

[12] J. ŘÍMAN and F. ŠORM, in preparation.

[13] E. B. MOMMAERTS, D. G. SHARP, E. A. ECKERT, D. BEARD, and J. W. BEARD, J. Nat. Cancer Inst. *14*, 1011 (1954).