Unusual Cell Form of *Bacterium anitratum* Produced by Sulfonamides

Beside the simple filamentation and subsequent bulging to spindle-shaped and later to spheroplast-like forms which was observed to occur under the action of sulfathiazol in otherwise rod-shaped *Bacterium anitratum*[1], some other changed forms were encountered in further work with new strains of the same organism.

Five strains of *Bacterium anitratum* were used in this study. They were isolated from superficial wounds, from deep suppuration, and from oral mucosa in normal adults.

The method of producing the morphological changes in these strains by sulfonamides has been described previously[1]. Beside sulfathiazol, some other sulfonamides (sulfadiazine, sulfamerazine, sulfamethoxypyridazine, gantrisin, elkosin) were used in this study to see if there were any differences in the morphological reactions of the strains to the influence of different sulfonamides. Special emphasis was put on the examination of very small colonies which were found in and between the less changed colonies of the same strains growing at the border of the inhibition zone around the sulfonamide discs and in the inhibition zone where no growth could be detected with the naked eye. These small colonies were very irregular in form and outline. Some of them were irregularly star-shaped, some with longer filaments protruding from the colony border. The still smaller ones were represented only by irregular bunches of unevenly long filaments. The smallest were composed of a few cells only. When observed by Örskov's direct agar microscopy[2], some single or multiple enlargements were seen in the filaments. These enlargements were granulated and in general darker. They corresponded to the cell enlargements seen in stained smears where they showed the tendency to retain methyl violet when stained by Gram technique, and also methylene blue when stained for 30 sec, or fuchsin 1:10 when stained for 10–20 sec. In addition to elongated and spindle-shaped forms, some club-shaped (Figure 1) and plectridium-like forms were seen. Some occasional triangular or ramified forms were also found (Figure 2). Some of the elongated forms were wound and coiled over their whole length and others only at the end (Figure 3).

We do not consider the changed forms described above to be produced specifically by sulfonamides because they were also found in bacteria exposed to penicillin or to other unfavorable environmental factors which inhibited cell-division but allowed cell-growth to go on at the same time. In *Klebsiella pneumoniae* triangular and clearly ramified forms were found as a result of the low incubation temperature[3]. In *Lactobacillus vaginalis* winding and coiling was found subsequent to the elongation of cells to filaments after a prolonged incubation in thioglycollate broth at 37 °C[4]. Winding and coiling of the elongated filaments was found also in the cultures of *Pasteurella pseudotuberculosis* exposed to penicillin[5]. All these unusual forms are an expression of the stress reaction and subsequent adaptation of bacterial cell growth to the environmental shocks of a chemical or physical nature which result in inhibition of cell division in growing bacteria.

Besides these striking changes, the number of distinctly polar-stained cells (which, however, might be found in *Bacterium anitratum* under apparently 'normal' conditions) was so high in the colonies exposed to penicillin that this seemed to represent a type of cell reaction to unfavorable environmental conditions which allow only little further growth of the individual cells after their division has been stopped. Polar staining might be found also in cases where the inhibition of cell division happened not long before the smears were made and the cell had no time to elongate further.

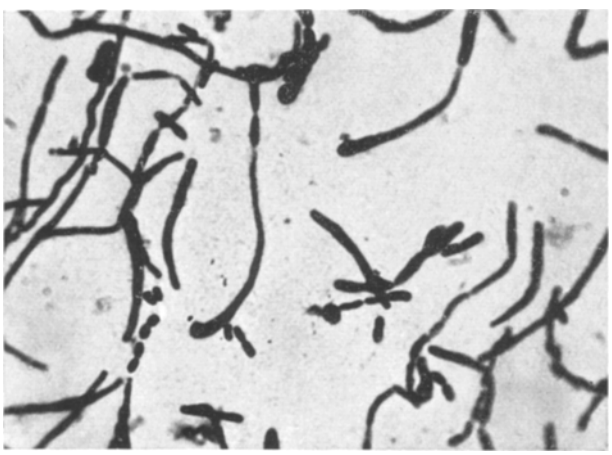

Fig. 1. Club-shaped filaments of *B. anitratum* grown under sulfonamides. Gram stain. Magnification approx. × 1200.

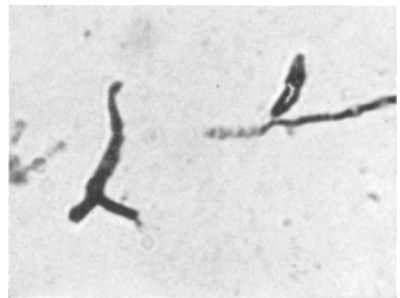

Fig. 2. Ramified filaments of *B. anitratum* grown under sulfonamides. Gram stain. Magnification approx. × 1200.

Fig. 3. Coiled forms of *B. anitratum* grown under sulfonamides. Gram stain. Magnification approx. × 1000.

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Wound forms might be explained by local weakening of the cell wall, the coiled ones by unilateral weakening of cell wall in cells which are growing beyond their normal dimensions. Triangular forms are sometimes found at the beginning of branching, or occur in cases where the branching is later stopped by the bactericidal concentration of the sulfonamides which with time replaces the bacteriostatic concentration, as happens in the sensitivity test.

Ramified forms may be produced by multiple budding of rounded spheroplast-like cells, which were already described in *Bacterium anitratum* under sulfathiazol[1], and by subsequent elongation of these buddings. If the elongations of multiple buddings later fragment at their base but stay in approximately the same position, diphtheroid formations are produced. Perhaps in this way the frequent appearance of diphtheroids (some of which agglutinate in antiserum prepared from the parental strain[6]) from secondary colonies in old bacterial cultures might be explained.

No significant differences were found in the action of different sulfonamides tested.

Zusammenfassung. Neben bereits beschriebenen fusiformen Filamenten und Spheroplasten-ähnlichen Formen entstanden unter dem Einfluss der Sulfonamidpräparate (Sulfathiazol, Sulfadiazine, Sulfamerazine, Sulfamethoxypyridazine, Gantrisin, Elkosin) aus den stäbchenförmigen Zellen des *Bacterium anitratum*, auch andere aberrante Zellformen, wie plektridiumförmige, trianguläre und verzweigte Filamente.

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Succinoxidase Activity of Mitochondria Isolated from the Liver of Rats after Partial Hepatectomy and Hypophysectomy

Whereas a decrease of succinate dehydrogenase activity has been observed in the homogenate of regenerating liver tissue during the first 48 h following partial hepatectomy (PHE)[1], an increase of this activity has been noted in a suspension of mitochondria isolated from regenerating liver[2]. Similar discrepancies, depending on the conditions of assay, have been obtained, for example, in the determination of cytochrome oxidase activity[1–3]. Analogous to the succinate dehydrogenase activity, succinate oxidase activity also falls in the homogenate from regenerating liver[4]. As the respective enzyme system is located exclusively in mitochondria, we have decided to determine succinate oxidase activity in a suspension of isolated mitochondria. Since some processes which take place after partial hepatectomy, such as the incorporation of ^{3}H-thymidine[5], are affected by the removal of the pituitary, succinate oxidase activity was also studied in hypophysectomized rats (HyE).

Fourty-eight albino rats of the Wistar strain, aged 3–4 months, which had had free access to the usual laboratory diet[6], were used. The estimations were performed on unoperated control rats 24 h and 48 h after PHE, i.e. a resection of 65–70% of the liver[7], 24 h after HyE[8], 24 h after HyE and laparotomy (sham operation), and 24 h after HyE and PHE. Mitochondria were isolated from the liver tissue by a modification of the procedure of ALDRIDGE[9,10]. Succinate oxidase activity was determined in the WARBURG apparatus[11–13] from the oxygen consumption by mitochondria suspended in the incubation medium (Krebs-Ringer saline with reduced $NaHCO_3$ content of 0.35 mg/ml)[14] to which sodium succinate had been added (18.3 mg per 3 ml incubation medium). Nitrogen was estimated by the micro-Kjeldahl method[15].

The results are shown in the Table. The left-hand column represents the nitrogen content of mitochondria

Groups	Mitochondrial N mg per 1 g wet tissue	mm^3 O_2 uptake per mg mitochondrial N
Non-operated rats	3.13 ± 0.22	504.70 ± 18.30
24 h after laparotomy	2.76 ± 0.17	404.90 ± 49.00
24 h after partial hepatectomy	2.55 ± 0.14	234.31 ± 52.00
24 h after hypophysectomy	3.22 ± 0.59	520.00 ± 45.07
24 h after hypophysectomy and laparotomy	2.73 ± 0.19	373.00 ± 27.45
24 h after hypophysectomy and partial hepatectomy	3.15 ± 0.65	299.60 ± 60.50
48 h after laparotomy	2.70 ± 0.20	587.50 ± 83.19
48 h after partial hepatectomy	2.84 ± 0.41	190.70 ± 63.70

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