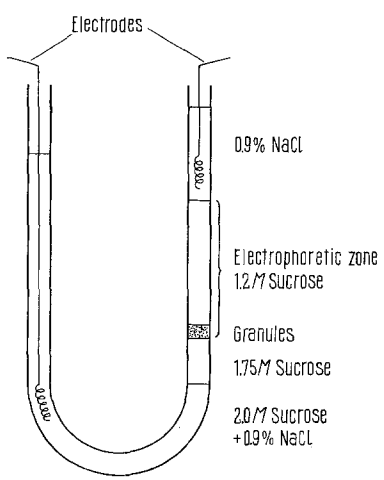
In the resting state of the cell the plasma membrane is electrically polarized, being negative on the inside. The secretion process is initiated by a 'depolarisation' of the plasma membrane of the nerve terminals. Also some calcium dependent steps seem to be involved in the secretion mechanism[2,3]. Since exocytosis would hardly be expected to occur, if the electrostatic forces between the membranes involved were repulsive, it was found of interest to study whether isolated neurosecretory granules carried any electrical charge and if so, whether it was positive or negative.

Methods. Purified neurosecretory granules were isolated by density gradient ultracentrifugation according to the method of DEAN and HOPE[4]. The isolated granules were recovered in 1.4 *M* sucrose. After equilibration to a temperature of 20°C this suspension was layered in a U-shaped glass tube on top of a solution of 1.75 *M* sucrose (see Figure). On top of the granular suspension was layered a solution of 1.2 *M* sucrose (the electrophoretic zone). Electrodes were placed in solutions of 0.9% NaCl in one end of the tube (upper) and in 2.0 *M* sucrose with 0.9% NaCl in the other end (lower). The granular suspension and its mobility in the electrophoretic zone could be visualized through a stereomicroscope. The U-shaped tube was submerged in a water bath (20°C). Electric fields from 40 to 100 V/cm were applied.

Results and discussion. On applying a voltage across the two electrodes the granular suspension would start to migrate towards the positive electrode. If the electrical polarity of the electrodes was changed the direction of the movement would also change. If the electrode in the upper end of the tube was positive the suspension would pass through the electrophoretic zone and gradually be concentrated at the upper end of that zone (at the border of the 0.9% NaCl solution). If the other (lower) electrode subsequently was made positive the granules would start to move downwards again. Introducing 2 m*M* $CaCl_2$ or 4 m*M* NaCl in 1.2 *M* sucrose in the electrophoretic zone reduced the mobility of the granular suspension (measured as the time required for the granules to reach the upper end of the electrophoretic zone). This effect might be due to a decrease in the electric field, caused by the increased conductivity of the zone. In 3 out of 6 experiments $CaCl_2$ was more potent than NaCl in reducing the rate of migration. Since there was no difference in conductivity of the two solutions, the additional effect of $CaCl_2$ might be due to neutralization by that ion of fixed negative charges on the secretory granules. Similar results have been obtained with chromaffin granules of the adrenal medulla[5].



Apparatus for free flow electrophoresis of isolated secretory granules from bovine neurohypophyses.

If the granules carry a net negative charge in the cytosol of the nerve terminals they would be repelled from the negative inside of the plasma membrane during non-secretory states of the cell. On secretion, however, the potential of the plasma membrane is abolished or even reversed, which might make it possible for the granules to approach and fuse with the plasma membrane. Calcium entering the cell on depolarization of the plasma membrane[6] may play a role in modifiyng the surface charge of the secretory granules.

Zusammenfassung. Sekretorische Granula aus der Neurohypophyse von Rindern wurden durch Ultrazentrifugierung isoliert. Frei-Fluss Elektrophorese dieser Partikel zeigte, dass sie eine negative Ladung besitzen. Ca^{++} und Na^{+} verminderten die Mobilität der Granula im elektrischen Feld.

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Spontaneous Mutants of *Staphylococcus aureus* PS 80

Staphylococcus aureus strain PS 80 serves as the propagation organism for the staphylococcal bacteriophage 80. It was obtained from the National Reference Laboratory for Phage Typing, Prague, Czechoslovakia.

Pigment mutants in stored culture. The strain was stored stabbed in nutrient agar slants overlaid with paraffin oil at room temperature in the dark for 1 year.

A suspension was prepared directly from the agar scrapings, diluted and plated on cream agar plates (WILLIS, O'CONNOR and SMITH[1]). After incubation, colonies of different size and colour developed. The majority consisted of the original wild type – i.e. orange coloured colonies, about 2 mm in diameter. The rest of the colonies occurring in a frequency 10^{-1}–10^{-2} were chromogenic mutants of different colony size. They were selected, purified and tested along with normally appearing colonies for different markers (Table I). The tests were performed as described elsewhere (SCHINDLER, MAREŠOVÁ

and SCHINDLER[2]). All clones of mutant phenotype were checked for anaerobic glucose fermentation (ref.[3]).

The original strain *Staphylococcus aureus* PS 80 is orange, produces clumping factor, and staphylocoagulase, acidifies mannitol agar, ferments anaerobically mannitol and lyses rabbit erythrocytes. Thus its phenotype appears as follows: PS 80: Ora, Clu, Cog, Man, Ama, Kin, Her.

Table I. Phenotypes of mutants isolated after storage of *Staphylococcus aureus* PS 80

Type	Clu	Cog	Man	Ama	Kin	Her	
PS 80 (wild)	1	1	1	1	1	1	Ora
3-1T	0	0	0	0	0	0	Whi
1erl	0	0	0	0	0	0	Yel
322L	0	0	0	0	0	1	Whi
3BZ	0	0	0	0	0	1	Yel
4e4	0	0	0	1	1	0	Whi
3er3	0	0	1	0	0	1	Yel
9b3	0	0	1	1	0	1	Whi
3er2	0	0	1	1	1	0	Ora
4-3T	0	1	0	0	0	0	Yel
4-5	0	1	0	1	0	0	Whi
3-1	0	1	0	0	1	0	Whi
9b4	0	1	1	0	0	1	Whi
4e1	0	1	1	1	1	1	Whi
45 3T	0	1	1	1	1	1	Yel
3er2T	1	0	0	0	0	0	Whi
9b1T	1	0	0	0	0	0	Yel
3B	1	0	0	0	0	1	Ora
S-S	1	0	1	0	0	0	Whi
3-4	1	0	1	0	0	1	Yel
1-1T	1	0	1	0	1	1	Ora
2a2	1	0	1	1	0	1	Whi
1er5	1	0	1	1	0	1	Yel
33T	1	0	1	1	1	0	Ora
24	1	1	0	0	0	0	Ora
4-2T	1	0	1	1	1	1	Ora
3-7	1	1	0	0	1	0	Ora
1er3	1	1	1	0	0	0	Ora
4-1T	1	1	1	0	1	1	Ora
2a3	1	1	1	1	0	1	Ora
45 2T	1	1	1	1	1	0	Ora
5e2	1	1	1	1	1	1	Whi

Marker designations are constructed according to the recommendations of DEMEREC et al.[10].: Clu, clumping factor production; Cog, coagulase production; Man, mannitol utilization on Chapman agar; Ama, anaerobic mannitol fermentation; Kin, staphylokinase production; Her, rabbit erythrocytes haemolysis; Tet, tetracycline resistance 20 μg/ml; Ery, erythromycin resistance, 25 μg/ml; Ora, orange colour; Yel, yellow colour; Whi, white colour.
1 = positive; 0 = negative.

Table I shows various isolated mutants and their respective phenotypes. All were resistant to phage 80.

Tetracycline resistant mutants. An investigation was made in a search for tetracycline resistant mutants which might be present in a population of the strain PS 80. 100 ml of a stationary culture were made up to 300 ml with nutrient broth containing tetracycline to a final concentration 20 μg/ml. After incubation for 48 h at 37°C and plating on nutrient agar containing tetracycline 10 μg/ml, resistant mutants were isolated and purified. By replica plating on cadmium nitrate agar ($10^{-3}M$), 2 tetracycline-resistant, cadmium-sensitive mutants were isolated and purified. In 10 tetracycline resistant mutants and in 2 tetracycline-resistant and cadmium-sensitive mutants, penicillinase and the markers or resistance were examined (Table II). The tests were described elsewhere (SCHINDLER[4]). In all 10 isolates, tetracycline resistant mutants carry along with the Tet marker also Cad, Mer, Ery, Lin, Cef, Spi and Yel marker. All are resistant to phage 80. Tetracycline-resistant and cadmium-sensitive mutants were penicillinase negative.

Discussion and conclusions. Some conclusions concerning both genetic aspects and diagnostic practice may be drawn: 1. The storage of staphylococci favours the occurrence of mutants. 2. The dissociation of the Clu and Cog as well as of other markers occurs readily by mutation.

Even after losing both Clu and Cog, Man may be retained. Staphylococcal strains may exist, which do not carry markers (Clu, Cog, Ama), used for species differentiation (Recommendation[3], BAIRD-PARKER[5]). Spontaneous tetracycline-resistant mutants may be present in a sensitive population. They are yellow and multiresistant. Similar strains were reported in 1966 by WILLIS, SMITH and O'CONNOR[6]. Spontaneous chromogenic mutants which mutated in several other markers were reported by PARISI[7]. The loss of chromogenic capacity can be linked with the resistance to the international typing phages (ROSENBLUM and JACKSON[8]). The instability of propaga-

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Table II. Markers in *Staphylococcus aureus* PS 80 and its mutants

	Pna	Cad	Mer	Kin	Clu	Pen	Clo	Tet	Ery	Str	Neo	Kan	Cef	Lin	Nov	Pri	Spi
PS80	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0
80tr 1[a]	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	0	1
80trc	0	0	1	1	1	1	1	1	1	1	0	0	1	1	0	0	1

1, positive (or resistant); 0, negative (or sensitive). [a] Strains 80tr2–80tr10 were the same phenotype as 80trl. Designation of markers: penicillinase, Pna. Markers of resistance to Cd^{++}, Cad; Hg^{++}, Mer; penicillin, Pen; oxacillin, Oxa; chloramphenicol, Clo; tetracycline, Tet; erythromycin, Ery; streptomycine, Str; neomycin, Neo; kanamycin, Kan; lincomycin, Lin; novobiocin, Nov; pristinamycin, Pri; fusidic acid, Fus; cephaloridine, Cef; and spiramycin, Spi.

tion strains, as far as the phage host specificity is concerned, was discussed by PILLICH et al.[9].

Zusammenfassung. Eine Reihe von Mutanten mit verschiedenen Phenotypen wurde nach einjähriger Lagerung des *Staphylococcus aureus* PS 80 isoliert. Sie unterscheiden sich in mehreren Merkmalen vom Wildtyp. Aus der Tetracyklin-empfindlichen Population der PS-80-Zellen wurden Tetracyklin-resistente Mutanten selektiert, die gleichzeitig resistent gegen Streptomycin, Chloramfenikol, Erythromycin, Cephaloridin, Lincomycin und Spiramycin sind.

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A Quantitative Analysis of Cleistothecia Production in *Aspergillus nidulans*

Fungi are not frequently employed in studies of quantitative genetics inheritance. Being generally haploids they have a segregation pattern which is not amenable to the methods of quantitative genetics. However, during heterokaryon formation it is possible to suppose the existance of additive, dominant and heterotic effects of the genes. Such effects must bear similarities to those which occur in diploid organisms since in the heterokaryons there are 2 sets of genes, although in different nuclei. *Aspergillus nidulans* is a filamentous fungus which has been widely used in genetic research. It produces spherical fruiting bodies, the cleistothecia, 100 µm or more in diameter, which arise after 8–10 days incubation at 37°C. In the formation of cleistothecia, several cytoplasmic and nuclear factors are probably involved. Cleistothecia formation is irregular; certain strains produce only conidia, others are regularly sexual and finally others produce conidia and sporadically start to produce cleistothecia[1–5]. Since the variation in the number of cleistothecia produced in the same environmental conditions is due to hereditary factors, it is possible to estimate and to test through a diallel cross model, the general and specific combining abilities and to look for quantitative effects of the genes which take part in cleistothecia production in *A. nidulans*.

Material and methods. Minimal medium (MM) was Czapeck-Dox medium with 1% (w/v) glucose. Complete medium (CM) was a complex medium containing yeast extract, hydrolyzed casein, hydrolyzed nucleic acids, vitamins, etc[6]. Solid media contained 2% agar. The strains of *A. nidulans* all derived from Glasgow stocks, were kept at 5°C on CM slopes. They were purified at 6-month intervals by single colony isolation and auxanographic characterization. The following strains were used: Strain A: *y*, nic_2 $ribo_5$; strain B: *y*, w_2, s_{12}, $pyro_4$; strain C:

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Table I. Mean frequencies of cleistothecia (per mm²) in the 15 crosses

Strains	A	B	C	D	E	F
A		0.21	0.68	0.79	1.43	0.23
B			2.32	6.58	7.27	5.03
C				2.21	1.47	2.19
D					2.43	1.12
E						0.11

Table II. Analysis of variance for the general and specific combining abilities

Source	D.F.[b]	M.S.[c]
General combining ability	5	9.18[a]
Specific combining ability	9	2.86[a]
Error	14	0.41

[a] Significant at 1% level.
[b] Degrees of freedon
[c] Mean square

Table III. Estimates of the effects of the general combining ability for each strain

Strain	Effects $(\hat{g}_i)$
A	−2.00
B	2.51
C	0.62
D	0.44
E	0.33
F	−0.67

Table IV. Estimates of the effects of the specific combining abilities for each strain

Strains	Effects $\hat{s}_{ij}$ B	C	D	E	F
A	−2.57	1.03	0.08	0.82	0.63
B		−1.84	1.35	2.15	0.91
C			0.12	−0.52	1.21
D				−0.62	−0.92
E					−1.83