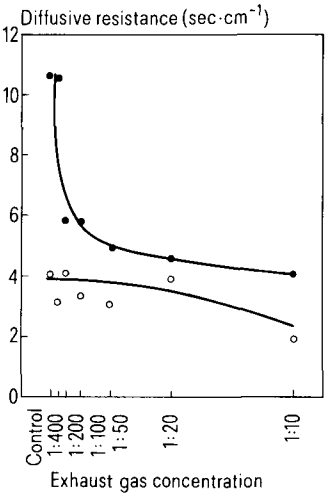
closing of the stomata. But, infact, investigations performed on aspen have shown that exhaust gases induce an opening of stomata.

Pottet clones of young aspen (*Populus tremula*) were exposed to 30 klux, 25 °C, and 65% relative humidity in chambers through which exhaust gases of different concentrations flowed from a gasoline generator. After a period of 2 h exposure to light, as well as additional 2 h to darkness, measurements of stomatal diffusive resistance were taken by means of a porometer. The air for the control chamber and the rarefying air for the individual concentration of exhaust gas were filtered through charcoal. The relationship between clean air and exhaust gas on the one hand, and the flowing through of gas on the other, were carefully controlled by means of a flow meter. Sample tests were taken to determine the amount of CO_2, CO, HC, and NO_x.

As can be seen from the figure, plants show a low stomatal diffusive resistance at light exposure, that is opened stomata, under all conditions of exhaust gas concentrations. In spite of the increased CO_2-concentration, no closing of the stomata can be observed. On the contrary, it can be seen that, with the high exhaust gas concentration of 1:10, the resistance, as compared to the control plant, is significantly lower. During the period of darkness, tests showed the usual high stomatal diffusive resistance, whereas it could be observed that already a high degree of gas exhaust rarefaction of 1:200 causes the stomata to remain open. It therefore appears that the regulatory ability of the stomata is inhibited by certain exhaust gas components. It is known that SO_2 has such an effect[5–7]; however, in conducting these experiments, SO_2, because of its low concentration in exhaust gas, could hardly have had a significant influence upon the regulatory ability.

Field experiments in the vicinity of a motorway confirm the possibility that exhaust gas concentrations in heavy traffic situations may be sufficient to exert an influence on the stomata apparatus. Plants growing immediately along the motorway, as compared to plants growing at a distance of 200 m from it, show lower stomatal diffusive resistance during the hot noon hours, when normally a closure of stomata takes place. However, examinations under the microscope have pointed out that in plants growing along the motorway the closing of stomata is inhibited by particles of dust as well. Additional inhibiting effects upon the stomata, such as these, could further contribute to permanently open stomata. These observations are supported by laboratory tests, pointing out that shortly after exposure to gases, but in the absence of the effect of dust particles, the stomata soon showed a regeneration of its regulatory ability. When plants were subjected to a 4-h exposure to 1:200 exhaust gas in the dark, and subsequently to clean air for a period of 30 min, a significant increase was noted in the stomatal diffusive resistance from 54% in the exhaust gas atmosphere to 66% in clean air, as compared to 100% of the control plants. As a consequence of this failure of stomatal regulation, the water economy in hot and dry weather conditions can be damaged by excessive transpiration.



Stomatal diffusive resistance of clones of *Populus tremula* in dependence of various exhaust gas-air mixtures; ○, in light, ●, in darkness. The resistance is significantly lower in light between control (clean air) and gas mixture 1:10 ($2\ p < 0.001$), and in darkness between control and gas mixture 1:200 ($2\ p < 0.001$). $n = 8$. Exhaust gas concentrations at 1:100:CO_2 600 ppm, CO 40 ppm, NO_x 0.05 ppm, hydrocarbons (hexene aequivalent) 1 ppm.

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Voltage-clamp analysis of the sodium and potassium currents in skeletal muscle fibres treated with 4-aminopyridine[1]

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Summary. External application of low concentrations of 4-aminopyridine blocks potassium currents without affecting sodium currents in pieces of single frog skeletal muscle fibres. The blockade of potassium currents was voltage-dependent, being partially relieved on depolarization.

Among the compounds that interfer with the operation of the potassium channels in excitable membranes, tetraethylammonium (TEA) and its derivatives are the best known[3–6]. Recent studies performed with aminopyridines have shown that 2-, 3-, and 4-aminopyridine block the potassium channels in a variety of nerve membranes[7–12]. 4-aminopyridine (4-AP) is known to prolong the repolarizing phase of the action potential of frog and rat skeletal muscle fibres[13–15], and has been shown to inhibit the potassium conductance in frog muscle fibres in lower concentrations than does TEA[16]. The present experiments examine in more detail 4-AP's action on sodium and potassium currents in fragments of single frog skeletal muscle fibres bathed in a normal ionic medium, under voltage-clamp conditions, in order further to characterize its mode of action.

Methods. Experiments were performed on pieces of single muscle fibres (having a diameter of 90–150 µm and a length

of 6–8 mm) isolated from the semitendinous muscle of the chilean frog *Caliptocephalella Gayi*. Only fibres which appeared undamaged, and which gave a fully propagated twitch when stimulated, were used. These fibres were placed across lucite partitions topped by vaseline separating the 4 pools of the recording chamber[17]. 3 of the pools of the recording chamber remained filled with KCl (110 mM) throughout the experiment, and the 4th one with standard Ringer solution[18].

The voltage-clamp method used was similar to that developed by Hille and Campbell for frog skeletal muscle fibres[17]. Oscilloscope records of membrane currents were fed into a digital analyzer which made it possible to record the output using a standard high-fidelity tape recorder or chart recorder. All records were corrected electronically for leakage and capacity currents. Experiments were performed at a constant temperature of 18 °C. 4-AP was added from a stock aqueous solution of recrystallized 4-AP (m.p. 156 °C) to the pool containing standard Ringer solution. In many experiments, this pool also contained 100–200 nM tetrodotoxin (TTX) to suppress ionic movement through sodium channels.

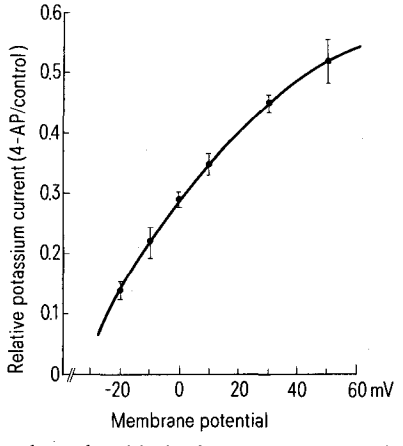

Fig. 1. Voltage-dependent block of potassium currents in fragments of single frog skeletal muscle fibres bathed for 30 min with Ringer's solution containing 12×10^{-5} M 4-AP. Current values were obtained before and during 4-AP treatment in response to clamp pulses of +70 to +140 mV from a holding potential of −90 mV. The membrane potential during the clamp pulse is indicated on the abscissa. Current measurements were done at the end of a 20 msec pulse applied every 30 sec. Curve was drawn by eye through the points, representing the mean±SEM of 5 experiments.

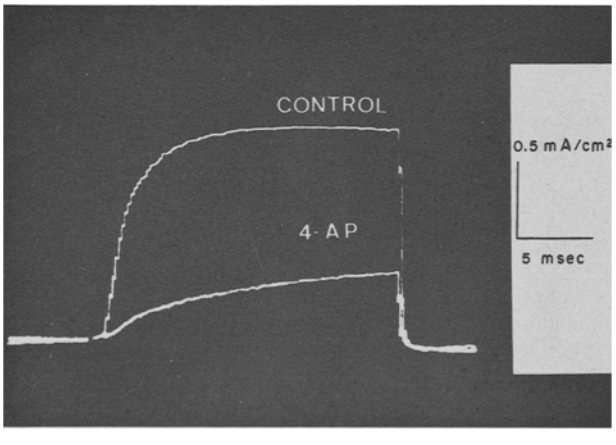

Fig. 2. Potassium currents during a clamp pulse to +120 mV before and after 10 min application of 2×10^{-4} M 4-AP in the presence of 200 nM of TTX. The initial remaining capacitive current was omitted.

Results and discussion. Under voltage-clamp conditions, 4-AP (36.8×10^{-6} to 5×10^{-4} M) selectively suppressed potassium currents in fragments of single muscle fibres. The dose-effect relationship showed no abnormalities. Sodium currents remained unaffected by 4-AP treatment in the range of concentrations used. The maximum sodium current densities were observed during clamp pulses to −20 mV. The largest values for controls and 4-AP treated fibres (5×10^{-4} M) were 3.27±0.51 and 3.19±0.44 mA/cm², respectively (mean±SD of 5 measurements in different fibres). The rate of onset of 4-AP action on potassium currents is rapid and stabilizes in about 3 min. The effects of 4-AP were reversible, and the time required for complete recovery depended both on the concentration of 4-AP used and on the time during which the preparations were exposed to the drug.

The suppression of the potassium current by 4-AP was dependent upon the membrane potential. In figure 1 the ratio of potassium current in the presence of 4-AP to potassium current in controls is plotted as a function of membrane potential. A progressive recovery of the potassium current occurred during severe depolarizing steps while the block was almost complete with moderate depolarizations. The stability and reversibility of the 4-AP effects indicates that the recovery from 4-AP blockade at extreme depolarizations is not an artefact of membrane breakdown.

The partial removal of 4-AP block was not a linear function of the membrane potential, specially beyond the level where potassium channels are fully open, suggesting that the opening of the potassium channels is required for the partial removal of 4-AP block. Similar results were obtained in the presence of 200 mM TTX. Furthermore, the time taken for potassium currents to reach a steady state level was examined over a wide range of membrane potentials. In the presence of 4-AP, potassium currents rose more slowly than in controls, requiring more time to reach a final steady-state level on the same time scale, as is shown in figure 2. The analysis of the slow rise of potassium currents under 4-AP may be explained by a progressive removal of the 4-AP molecules from their binding sites in the potassium channels.

It may be noted that the effects of 4-AP on potassium channels from skeletal muscle fibres exhibit similar voltage-dependent characteristics as those reported in other excitable membrane[12,19,20]. The present findings seem to be in contradiction to those recently reported by Gillespie[15]. No explanation of the difference can be advanced, since there is no clear information on the composition of the bathing solution in Gillespie's note.

In conclusion, the present data confirm and extend earlier results showing that 4-AP is a selective and reversible blocking agent of the potassium currents without any effect on sodium currents. Moreover, the block of potassium currents by 4-AP is a function of both the membrane potential and the 4-AP concentration. The effects of 4-AP on the potassium channels may account for the changes in duration of the action potentials previously reported in skeletal muscle fibres[13,14]. 4-AP, because of its selective action, should be a useful pharmacological agent for studying excitation-contraction mechanisms.

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The 'Spitzenkörper', centre of the reducing power in the growing hyphal apices of two septomycetous fungi[1]

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Summary. Redox dyes, such as methylene blue and neutral red, are stably reduced to their leucobase in the 'Spitzenkörper' of the growing hyphal tips of *Neurospora crassa* and *Monilia fructigena.*

The ultimate tip of vegetatively growing hyphae of Septomycetes contains a centrally located apical body, the so-called Spitzenkörper[2-4]. This spherical organelle, now known to be composed of microvesicles and membranous tubules[5], was first characterized by its high stainability with haemotoxylin dye[2], contrasting, however, with its absence of affinity for nucleic acid stains[6]. Practically nothing is known of its functional role except that it temporarily disintegrates when irradiated with strong light[6], and that it has been assumed to play a crucial role in the maintenance of the apical polar growth[5].

As we have recently found that the ultimate tips of vegetative hyphae of both the chytridiomycete *Allomyces* and the septomycete *Neurospora crassa* are highly reducing to a few selected semi-vital redox stains[7], we thought it would be interesting to try to localize more precisely the site of that apical reducing power in representatives of those fungi, the Septomycetes, all known to present a 'Spitzenkörper' in their hyphal tips[5].

Vegetative hyphae of *Neurospora crassa* (Lindegren strain A) were harvested either from the progressing front of mycelium growing for 16-20 h at 25 °C from the lower part of slants of malt agar inoculated with macroconidia, or from young mycelia developed from conidia germinating in liquid Vogel's medium[8]. The bunches of parallely elongating hyphae were bathed on glass slides in 10^{-4}-10^{-3} freshly prepared solutions of redox dyes (analytical grade) in distilled water below coverslips which were only momentarily lifted off to permit reoxidation of the leucobases in the mitochondrially-rich subapical zones of the hyphae. A similar method was applied to the pH indicators used and the lipid reagent (Sudan III in alcoholic solution) applied after pumping off excess water. Similarly, vegetative hyphae of *Monilia = Sclerotinia fructigena* Aden and Ruhl were grown on membranes aseptically placed on the surface of malt + casamino-acids agar plates[9]. Bits of mycelial margin were removed after 3 days growth at 25 °C and treated as the *Neurospora* materials. Microphotographs were made with Ilford pan F 120 on a Wild M 20-EB microscope.

Topological ultrastructural controls were obtained from current electron microscope studies in our laboratory using both *N. crassa*[10] and *M. fructigena*[9] and the technique described for *Allomyces*[11] to obtain longitudinally oriented thin sections of hyphae.

We first repeated our previous experiments with neutral red on *N. crassa* and extended them to the hyphal apices of *Monilia fructigena.* Using stepwise vertical focussing on the ultimate tip of *Neurospora* hyphae, we got a first glance at the densest location of the switch from red to yellow in a corpuscle corresponding in its size and location to the apical 'Spitzenkörper' (figure 1a). In an especially favourable case, in which the stain was vitally still faintly colouring in red the whole apical cytoplasm, the yellow tinge was restricted to the only apical granule detectable (figure 1b) which corresponded positionally to the 'Spitzenkörper', as checked by electron microscopy (figure 2b). In apices of *Monilia,* the relatively extensive yellow 'capping' of otherwise uniformly red coloured hyphae could also be located by adequate focussing in the spherical centre of the ultimate hyphal tips (figure 1c).

To decide whether the switch from red to yellow of neutral red was really due to the highly reducing power (lower than the $E'_o = -0{,}30$ of this stain at pH 7.0)[7] of the hyphal tips, especially of their 'Spitzenkörper', and not to local alkalinisation (an unexpectedly needed pH 8), we devised a few pH determinations using indicators overlapping for their colour changes: bromothymol blue stained yellow (pH 6.0) the apices (vivid yellow in the tip granule) with a subapical slightly greenish hue (pH 6.2) in a few relatively wide hyphae; with bromocresol green, all apices were mostly greenish ('Spitzenkörper' whitish) and never blue (pH 5.5), bromocresol purple turned to reddish orange (pH around 5.0) while Congo red provided a generalized reddish staining (pH at least pH 5.0); all these tests confirm the average of pH 5.0-6.0 (closer to 5.0) preliminarily visualized from the yellow colour obtained in bathing vegetative hyphal tips in an extracted water solution of the universal Merck indicator. In any case, such acidic pH, apparently reinforced in the ultimate tip as also suggested by the yellow staining by alizarin (sulfonated dioxy-anthraquinone) of the 'Spitzenkörper' compared to an orange tinge developing farther back in the hyphae, should exclude an alkaline-due yellow switch and confirm our previous assumption of a reductive reaction of neutral red at the level of the hyphal tip.