changed during an experiment lasting at least 1 h. Under these conditions muscle $\dot{D}O_2$ was 14.9 ± 0.5 µl/min/g and the resting $\dot{V}O_2$ was 594.5 ± 21.0 µl/h/g. The hematocrit value for undiluted blood was $60.4 \pm 2.1\%$. Various dilutions obtained by substitution of the dextran solution for blood resulted in hematocrit values between 44.4 and 18.0%. Under these conditions the perfusion flow rate spontaneously increased up to a maximum of 208.3 µl/min/g. Depending on the extent of hemodilution, $\dot{D}O_2$ either increased (moderate hemodilution) or decreased (severe hemodilution) with respect to its initial value, within the range 7.0–35.4 µl/min/g. $\dot{Q}$ and arterial O_2 concentration were respectively 202.3 µl/min/g and 17.5 ml O_2/100 ml at maximum $\dot{D}O_2$, and 120.7 µl/min/g and 5.8 ml O_2/100 ml at minimum $\dot{D}O_2$.

A distinct relationship was found between $\dot{D}O_2$ and $\dot{V}O_2$ (fig.). $\dot{V}O_2$ increased proportionally to $\dot{D}O_2$ in the range of 7.0 to about 16 µl/min/g. The O_2 extraction coefficient remained submaximum in this $\dot{D}O_2$ range (which is in accordance with the ability of noradrenaline to increase it while stimulating $\dot{V}O_2$[1,3]). The rise of $\dot{D}O_2$ above the level of 16 µl/min/g did not, in itself, increase muscle respiration. Thus, at $\dot{D}O_2$ values beyond 16 µl/min/g and up to 35.4 µl/min/g the O_2 extraction coefficient decreased from 0.617 to 0.314.

$\dot{V}O_2$ was not found to be a simple function of $\dot{Q}$, since at the same $\dot{Q}$, different $\dot{V}O_2$ were achieved when the muscle was supplied with different amounts of O_2. Conversely, to any given $\dot{V}O_2$ value corresponded different $\dot{Q}$ values: for example, at the $\dot{V}O_2$ corresponding to $\dot{D}O_2 = 15$ µl/min/g, $\dot{Q}$ varied from about 65 to about 160 µl/min/g, the last value being obtained at 25% hematocrit level.

Discussion. Previous studies indicated that $\dot{V}O_2$ of the rat skeletal muscle may change according to changes in $\dot{Q}$. Our results show, however, that rather than the $\dot{Q}$, the product of $\dot{Q}$ and arterial O_2 concentration (i.e. O_2 delivery - $\dot{D}O_2$) is the decisive factor determining $\dot{V}O_2$ in resting skeletal muscles, because the same $\dot{V}O_2$ may be obtained at different $\dot{Q}$, depending on arterial O_2 concentration. This is true, however, only when $\dot{D}O_2$ to the muscle is less than 16 µl/min/g. Above this value the muscle $\dot{V}O_2$ becomes independent on $\dot{D}O_2$.

This finding is in contrast with the data reported by Honig et al.[5], who observed that after denervation the rat muscle $\dot{V}O_2$ increased with the increase of $\dot{D}O_2$ even above 16 µl/min/g. These results have been interpreted as a physiological limitation of resting muscle $\dot{V}O_2$ by O_2 transport, due to the inhomogeneity of capillary function and uneven distribution of O_2 within the organ. At present it cannot be decided whether or not such a limitation really takes place in the normal resting muscle, because methods for measuring the heterogeneity of O_2 distribution to muscle cells are still inadequate[6]. In any case, our data show that the mean $\dot{D}O_2$ to the innervated, vascularly isolated muscle, perfused physiologically by undiluted blood achieves the value above which the $\dot{D}O_2$ ceases to further control the muscle $\dot{V}O_2$ (fig.).

The objection still exists that the vascularly isolated muscle may have more open capillaries than the intact resting muscle and thus it may have a larger $\dot{V}O_2$, because it is known that $\dot{Q}$ and functional capillary density are controlled independently[7]. This does not seem to be the case in our experiments, however; our unpublished data demonstrate that the vascular isolation used in this work does not change functional capillary density and capillary spatial distribution in the gracilis muscle (271.1 ± 25.0 and 265.7 ± 17.8 capillaries/mm^2 in intact and isolated muscle, respectively).

In conclusion, we assume that the metabolic rate of the resting rat skeletal muscle is not limited by O_2 transport in the range corresponding to physiological values, like in the dog[5,8,9] and the cat[10]. Adams et al.[11] came to the same conclusion. They observed no change of whole body $\dot{V}O_2$ of the rat when the total O_2 transport was increased above the normal level.

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Sensitivity of *Botrytis cinerea* Pers. to elemental sulfur in the presence of surfactants

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Summary. The sensitivity of *Botrytis cinerea* Pers. to elemental sulfur is enhanced in surfactant solutions (Triton X-100 or SDS). The role of surfactants in the change of permeability to sulfur is discussed.

Botrytis cinerea Pers. is a major grape pathogen in Swiss vineyards. Growers know that it is insensitive to elemental sulfur, and this fungicide has never been used to control the Grey Mould of grapes.

Investigations on the metabolism of endogenous and exogenous elemental sulfur in *Phomopsis viticola* Sacc.[2,3] and *Monilia fructicola*[4] showed that this product interferes with some vital biochemical pathways, such as cell respiration and the resulting oxidative phosphorylation. Thus we can postulate that the sensitivity to elemental sulfur is correlat-

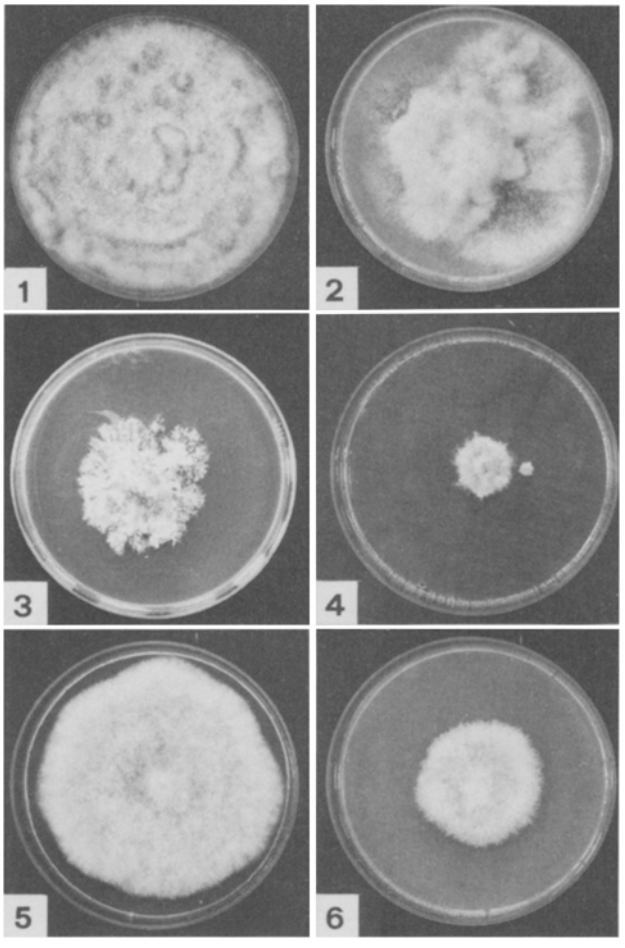
ed with the ability of this product to penetrate the cell. Since both cell wall and plasma membrane may be involved in cell permeability, we decided to circumvent this potential permeability barrier by adding surfactants to elemental sulfur applied to *B. cinerea.*

B. cinerea, strain BC 13, sensitive to cyclic dichlorphenyl-N-imide fungicides, was cultivated on potato dextrose agar (PDA, Oxoid) under laboratory conditions. Conidia were harvested by shaking aerial mycelia cut from sporulating cultures in distilled water, then separated from mycelia by filtration through glass wool. The conidial suspension was adjusted with distilled water to 10^6 conidia per ml. The surfactants used were sodium dodecyl sulfate (SDS, Fluka) and Triton X-100 (TX-100, Calbiochem). Colloidal elemental sulfur was prepared as described by Miller et al.[5]. All these compounds were used at a final concentration of 10^{-3} M in water-agar (WA) or PDA. The effects of elemental sulfur and surfactants on germination were observed on slide-cultures (WA) and on colonies growing on PDA in Petri dishes. Miller et al.[5] showed that the fungicidal activity of elemental sulfur is correlated with the amount of hydrogen sulfide (H_2S) produced by fungi in the presence of sulfur. On this basis, penetration of elemental sulfur into the cell was measured quantitatively by determining the amount of H_2S emitted by a suspension of conidia grown in the presence of colloidal sulfur (^{35}S), with or without surfactant.

Conidia and sulfur mixtures were placed in the glass apparatus described by Pezet et al.[6]. H_2S produced by the conidia was trapped on a round filter paper impregnated with lead acetate. The radioactivity of the resulting Pb-^{35}S – sulfur was quantitatively analysed by integrating the counts during a transit of the filter (300 mm/h) under a TLC-scanner detector (Dünnschicht-Scanner II, Berthold, Switzerland, proportional counter). The standard curve was established from known amounts of elemental sulfur (^{35}S) transformed into H_2S, by the method of Fromageot[7], and trapped as described above. The linear correlation between the radioactivity of ^{35}S and the integration of the counts of $Pb^{35}S$ recovered was 0.997 from 3 to 300 nCi of ^{35}S. The effect of elemental sulfur and surfactants on cell respiration was determined by measuring the oxygen consumption of a suspension of spores incubated at 20 °C (±0.01 °C) (Clark electrode, PO_2 Analyzer Bachofer).

The results are presented in the table and the figure. They show that surfactants alone had no effect on the percentage of germination of conidia after 20 h of incubation on slide cultures placed in a damp chamber and a slight to medium effect on mycelial growth. Synergism was observed with the mixture of sulfur and surfactants, in comparison with sulfur or surfactants alone. Sulfur in TX-100 is the best inhibitory mixture of mycelial growth, whereas in SDS it strongly affects conidial germination. The different natures of the surfactants used, nonionic (TX-100) or anionic (SDS), and the structural differences between conidia and mycelium of *Botrytis cinerea,* are probably an explanation for these results.

These experiments demonstrate that sulfur is more fungitoxic when it can penetrate the cells. The surfactants used disturb the structure of the cell plasma membrane and consequently its permeability[9,10]. Synergism observed between sulfur and surfactants suggests that fungicidal activity of elemental sulfur is correlated with its ability to reach mitochondria, where it is reduced by interfering with hydrogenation and dehydrogenation reactions which occur in the cell respiratory pathway[4,11].



Influence of surfactants (10^{-3} M) and elemental sulfur (10^{-3} M) on the mycelial growth of *Botrytis cinerea* Pers. *1* Control on potato dextrose agar (PDA). *2* PDA + S°. *3* PDA + TX-100. *4* PDA + TX-100 + S°. *5* PDA + SDS. *6* PDA + SDS + S°.

Effect of colloidal elemental sulfur (S°) (10^{-3} M) in water, sodium dodecyl sulfate (SDS), or Triton X-100 (TX-100) aqueous solutions (10^{-3} M) on germination, germ tube length, rate of reduction of sulfur, oxygen consumption of conidia and mycelial growth of *Botrytis cinerea* Pers.

	Water (W)	S° + W	SDS	S° + SDS	TX-100	S° + TX-100
Percent of conidial germination[a]	90	60	90	17	90	55
Germ tube length (μm)[b]	90	77.5	90	27.5	90	57
Sulfur reduced by 10^8 conidia (μg)[c]		2.64		4.55		3.19
Oxygen consumption (nmoles O_2/min/10^8 conidia)	2.2	1.72	1.35	0.17	1.65	0.59
Mycelial growth[d]	8.5	8.5	7.9	4.1	3.8	1.9

[a] Average of 3 estimates on 100 conidia each. Spore germination is considered effective when the germ tube is as long as the diameter of the spore. (Slide-cultures on water-agar, 20 h of incubation). [b] Average of 100 conidia (slide-culture on water-agar, 20 h of incubation). [c] Measured only when sulfur was added. [d] Colony diameter (cm) of cultures on Petri dishes (PDA) after 7 days of incubation in laboratory conditions (maximum for control is 8.5 cm).

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Localization of pyruvate carboxylase in the cells of *Neurospora crassa*

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Summary. The cell wall of *Neurospora crassa* was digested enzymatically and the cytosolic and the mitochondrial fractions were separated. The activity of pyruvate carboxylase (EC 6.4.1.1) was detected entirely in the cytosolic fraction. This indicates that the location of pyruvate carboxylase of *N. crassa* is in the cytosol, but is not in the mitochondria; this is different from the situation in animal tissues.

Pyruvate carboxylase is known to exist in a wide variety of organisms, including mammals[1,2], birds[1,3,4], and bacteria[5–9]. Fungal pyruvate carboxylase has been reported in *Aspergillus niger*[10], *Neurospora crassa*[11,12], *Penicillium camemberti*[13], *Rhizopus nigricans*[14] and yeasts[15–17]. In some cases, the enzyme was purified and molecular architecture was studied[4,6,8,9,15,17]. Pyruvate carboxylase in most animal tissues is chiefly a mitochondrial enzyme[1,18]. In yeast, on the other hand, this enzyme is reported to be localized in the soluble cytosol[16]. In the course of the study on the relationships between the *suc* gene and pyruvate carboxylase of *Neurospora crassa*, we noticed that the location of the enzyme in *Neurospora* is not in the mitochondria but in the cytosol.

Materials and methods. Mycelium of the wild type strain KG1967a of *Neurospora crassa* was grown in a 1.5 l Roux bottle containing 1 l of Vogel's minimal medium[19] supplemented with 2% sucrose and 0.3% sodium acetate under aeration at 34 °C for 1 day. About 8–9 g of wet mycelium were obtained from a bottle. Mitochondria were obtained by essentially the same method as that used by Greenawalt et al.[20]. Cell wall was digested by β-glucuronidase (crude solution from *Helix pomatia*) from Sigma. Wet mycelium weighing 8–9 g was treated in 33 ml of reaction medium containing sorbitol, 0.63 M; citric acid – K_2HPO_4 buffer (pH 5.8), 0.1 M with respect to citric acid; 2-mercaptoethylamine·HCl, 0.03 M; EDTA, 0.4 mM; and 300,000 units of β-glucuronidase for 1 h at 30 °C. Treated cells were washed twice with 60 ml cold, 0.9 M sorbitol, and collected by centrifugation.

The pellet was resuspended in 60 ml of 0.02 M potassium phosphate buffer, pH 7.5, containing 0.25 M sucrose, 5 mM EDTA and 0.15% bovine serum albumin (BSA buffer). The suspension was homogenized using a glass homogenizer with a Teflon pestle. The crude homogenate was diluted with BSA buffer to 150 ml and centrifuged at 1000 × g for 15 min to remove unbroken cells. The supernatant was centrifuged at 10,000 × g for 20 min, and its supernatant fraction (C1S) was recentrifuged at 180,000 × g for 25 min and the final supernatant was obtained (fraction C18S). The pellet obtained between 1000 and 10,000 × g, which contained essentially all mitochondria, was washed with BSA buffer and suspended in 12 ml of 0.02 M potassium phosphate buffer, pH 7.5 (fraction M1P). The suspension was treated with a Tomy model UR-150P sonicator for 1 min at the power setting of 5. The treated suspension (fraction M1P-S) was again centrifuged at 180,000 × g for 25 min and the supernatant was obtained (fraction M18S).

Pyruvate carboxylase activity was determined spectrophotometrically by the citrate synthase-coupled method described by Payne and Morris[5]. Citrate synthase activity was determined by the method of Parvin[21]. Fumarate hydratase activity was determined by the method of Hill and Bradshaw[22], measuring

Activities of the 3 enzymes in various preparations from disrupted *Neurospora* cell. The naming for each preparation is described in the text

	Preparation	Specific activity (μmol mg^{-1} h^{-1})	Total protein (mg)	Total activity (μmol h^{-1})
Pyruvate carboxylase from 9.1 g wet mycelium	C1S	1.80	221	398
	C18S	2.23	161	359
	M1P	0	54	0
	M1P-S	0	49	0
	M18S	0	26	0
Citrate synthase from 8.1 g wet mycelium	C1S	37.6	184	6920
	C18S	43.0	148	6360
	M1P	37.1	63	2340
	M18S	221.4	31	6860
Fumarate hydratase from 8.1 g wet mycelium	C1S	59.0	184	10860
	C18S	70.8	148	10480
	M1P	57.7	63	3640
	M18S	268.8	31	8330