strain under equivalent cultural conditions about 15 h after transfer to sporulation medium[5].

The procedures were done twice with both vegetative and 6-day sporulated cultures of *Saccharomyces oleaceus* Santa Maria and *S. mellis* (Fabian and Quinet) Lodder and Kreger-van Rij. Proline was not detected in the vegetative cells of either yeast; but it was prominent among the amino-acids in sporulated cells. However, no proline was found in four experiments using vegetative and sporulated cells of *Schizosaccharomyces pombe* Lindner, nor in an experiment with *Torulopsis famata* (Harrison) Lodder.

Changes in amino-acid content after transfer of 6-day sporulated cells of *S. cerevisiae* to fresh growth medium were also followed. In about 4 h the spores were distinctly swollen. A day later most had germinated, and growth was in progress. Whereas most amino-acids gave evidence of increase during two days in growth medium, proline diminished to 200 μg/g.

It would have been preferable to use for these experiments large volumes of sporulation medium, taking successive samples during six days, instead of running each chromatogram with an individual 100-ml. culture. But as sporulation was poor in large sporulation cultures, the less-accurate method had to be used, and this may account in part for the fluctuations in amount of glutamic acid and glutathione indicated by successive determinations. But as each point is based on at least four, and in many cases ten, experiments it is possible that marked changes occur in the content of these substances during active sporogenesis.

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Stimulation of Sporulation in *Penicillium* by Anhydroglucose

IN submerged shaken cultivation in a synthetic medium containing potassium nitrate, potassium dihydrogen phosphate, and magnesium sulphate, trace elements and pure (analytical reagent) glucose, *Penicillium griseofulvum* Dierckx (Strain *ARL.* 375) grows vegetatively but does not sporulate even when it has used up all the available nitrogen. Abundant sporulation at a relatively early, exponential, stage of growth was observed when pure glucose was replaced by a crude commercial glucose derived from maize starch by acid hydrolysis[1]. Crude glucose was also found to stimulate submerged sporulation in 5 other species of *Penicillium* out of a further 21 species which were tested. The nature of the sporing factors in crude glucose was therefore investigated.

It was found that the sporing stimulus depended on the simultaneous presence of two substances, of which the first proved to be calcium (Ca^{++}), occurring in amounts sufficient to give a level of approximately 5×10^{-4} M in a medium containing 5 g/l. crude glucose. Calcium was already known to induce submerged sporulation of *Penicillium griseofulvum*, but only at levels above 5×10^{-3} M, and then only at a late, post-exponential stage of growth when the nitrogen in the medium is either exhausted or nearing exhaustion. The amount of calcium present in the crude glucose added to our standard medium does not, however, normally induce sporulation, except in the presence of a second substance occurring in crude glucose. This was found to be a neutral organic heat-stable compound which appeared to be an acid reversion product of glucose. In fact variable amounts of a similarly active substance were found to be produced by autoclaving pure glucose with acid. The substance was separated from crude glucose by adsorption on charcoal and elution with ethanol, and the active fraction was identified as a 1,6-anhydroglucose by preparative paper chromatography. When this fraction, together with 1×10^{-4} M Ca^{++}, was added to a submerged culture of *P. griseofulvum* in a medium containing pure glucose, abundant sporulation was induced early in the exponential phase of growth, although neither Ca^{++} nor the anhydroglucose fraction produced this sporing effect alone.

The nature of the organic sporing factor was confirmed by synthesizing 1,6-anhydro-β-D-glucopyranose by alkali treatment of β-phenylglucoside[2]. This substance gave the characteristic induction of sporulation in *P. griseofulvum* when added, with 1×10^{-4} M Ca^{++}, to a culture growing in medium containing pure glucose. Maximal sporulation occurred when the anhydroglucose concentration was between 2×10^{-4} and 6×10^{-4} M and was not increased at higher concentrations. No sporulation occurred in the absence of added Ca^{++} even with very high concentrations of anhydroglucose in the usual growing medium. When the potassium nitrate was replaced by ammonium succinate, however, very high concentrations of anhydroglucose did induce (late) sporing without added Ca^{++}. This does not seem to be due to traces of Ca^{++} already present, and it indicates, as do certain accompanying morphological changes, that anhydroglucose may have specific biological effects apart from its interaction with Ca^{++}. A mixture of the pyranose and furanose forms of 1,6-anhydro-D-glucose, produced by the pyrolytic distillation of starch[3], proved to have the same sporulation-inducing effect as the pure pyranose form, when tested (with Ca^{++}) in the same conditions. Smaller but significant inductions of sporulation were also given in the presence of Ca^{++} by 1,6-anhydro-β-D-galactopyranose and by 1,6-anhydro-α-D-galactofuranose.

These observations show that the stimulus to sporulation of *P. griseofulvum* (and probably of other species of *Penicillium*) caused by crude glucose is due to the presence of anhydroglucose and traces of Ca^{++}. Further confirmation is provided by the absence of any sporing effect from crude glucose produced by enzyme hydrolysis instead of by acid hydrolysis of starch. Studies of the effects of anhydroglucose and related compounds on metabolism and sporulation of fungi are now in progress. The ability of 1,6-anhydro-β-D-glucopyranose to increase so powerfully the spore-inductive effect of Ca^{++} provides another example of a biological effect elicited by carbohydrate reversion products[4,5]. Interactions between Ca^{++} and hexose phosphates have been observed in the stimulation of perithecia formation in *Chaetomium*[6].

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