DISSOCIATION OF FUNGAL CLUSTERS BY TRYPSIN TREATMENT

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INTRODUCTION

BACTERIAL cultures are remarkable in that the cells can be seen through the microscope and counted. The consequent cell division can be followed by cell counting and it is possible to get synchronous cultures. In fungi showing cell differentiation the cells are present as clusters and cell division and macromolecular synthesis cannot be followed by the conventional methods. In our studies on cell division and macromolecular synthesis in *Aspergillus nidulans*, a fungus of the mold type, attempts were made to dissociate the cell clusters into single cells without damage. Trypsin treatment of the clusters was carried out and the resultant single cellular cultures have been studied.

Aspergillus nidulans are propagated by continuous subculture of conidiospores on growth medium. When grown on agar dish, circular colonies are obtained from single spores with conidiation in 48 hrs. In liquid medium with shaking, growth is observed in the form of spherical balls with brush-like surfaces. It has been observed that each spherical cell cluster originated from a single conidiospore[5] due to consequent cell divisions in three dimensions. Attempts were made to break up these clusters to get a unicellular culture and to study DNA replication, synthesis of mRNA, amino acyl-sRNA and peptides with the use of inhibition studies, on an adenineless mutant strain.

MATERIALS AND METHODS

The adenine requiring mutant (FGSC 216) was obtained from the Fungal Genetics Stock Centre (U.S.A.). It was grown in shake cultures. 10 ml. of complete medium[4] was dispensed in each of ten flasks of 50 ml. capacity and were inoculated with about 10^8 spores in 0·1 ml. of suspension, in 0·89% saline and were incubated at room temperature on a metabolic shaker adjusted to 110 strokes per minute. At intervals of three hours the cultures from one of the flasks were taken and centrifuged. A small amount of the residue was smeared as a thin film, on a drop of lactophenol base fixed to microscopic slide. inactivated by heat and stained with cotton blue. The stages of growth observed on the slides are presented in Figs. 1-5.

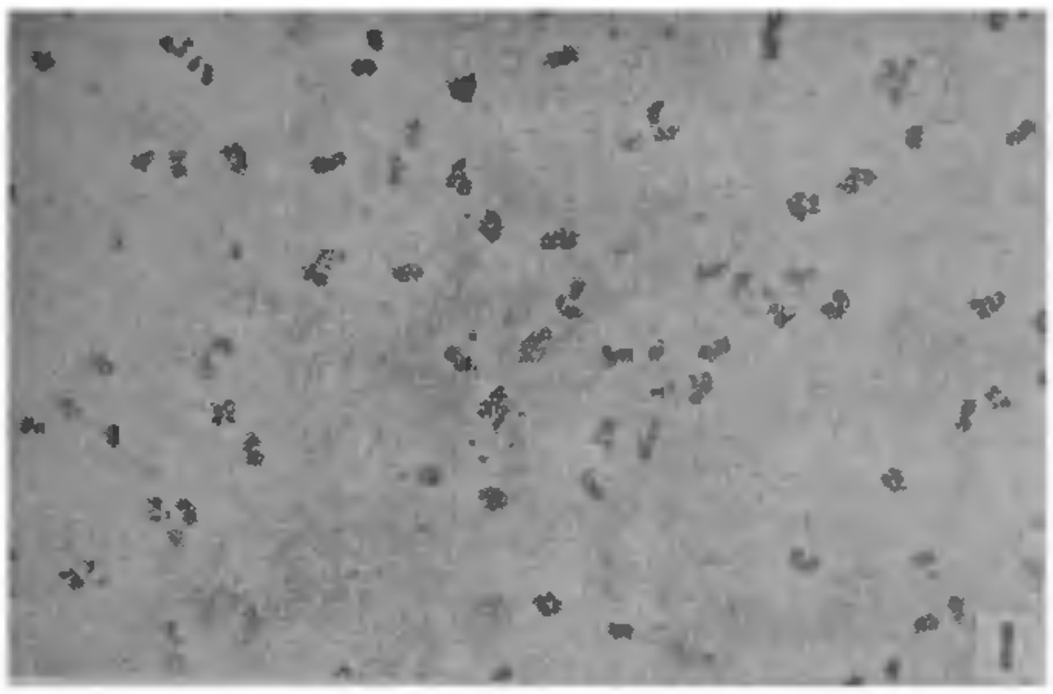
The cells present in 10 ml. of a 24-hr. culture of *A. nidulans* maintained on a metabolic shaker were harvested by centrifugation at 3,000 r.p.m. for 15 minutes. The sedimented cells were washed with water, suspended in Tris-HCl buffer at pH-7·6, and treated with trypsin to a final concentration of 0·25% for 15 mts. At different intervals of time aliquots were centrifuged and the residues smeared as thin films on microscopic slides, inactivated by heat and stained. Photomicrographs of various stages of hyphal disintegration are presented in Figs. 6 and 7. The singled cells were counted on a hæmocytometer under a microscope and the counting verified by plating on the complete medium. Plating method is of paramount importance in determining the number of viable cells present in the medium at a particular instant.

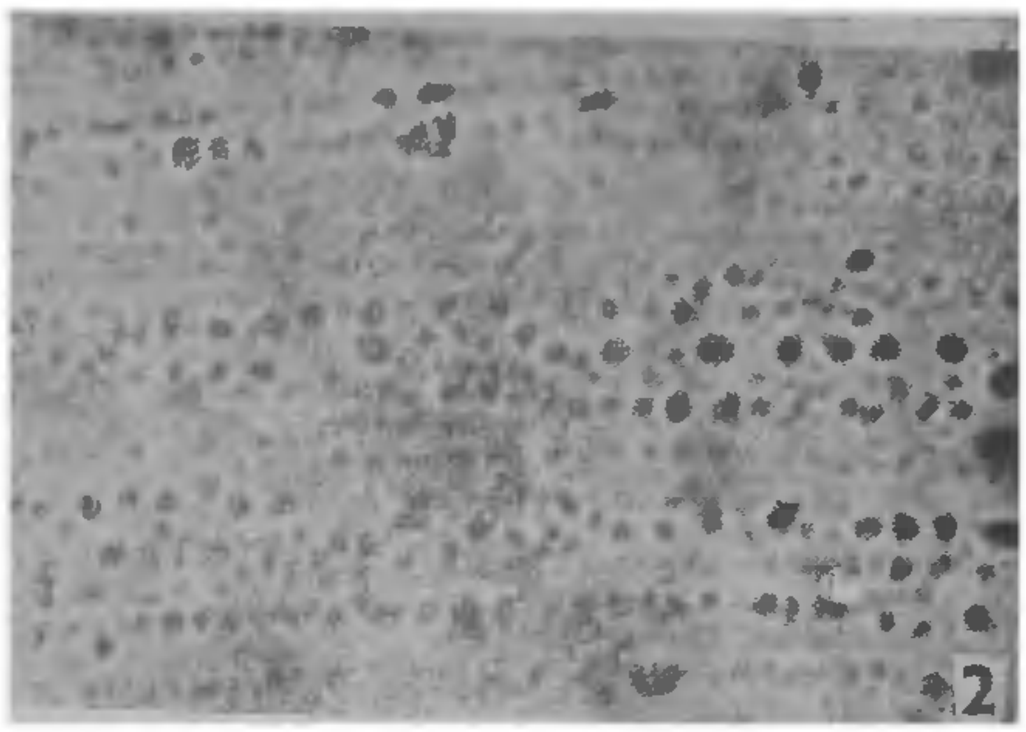
RESULTS AND DISCUSSION

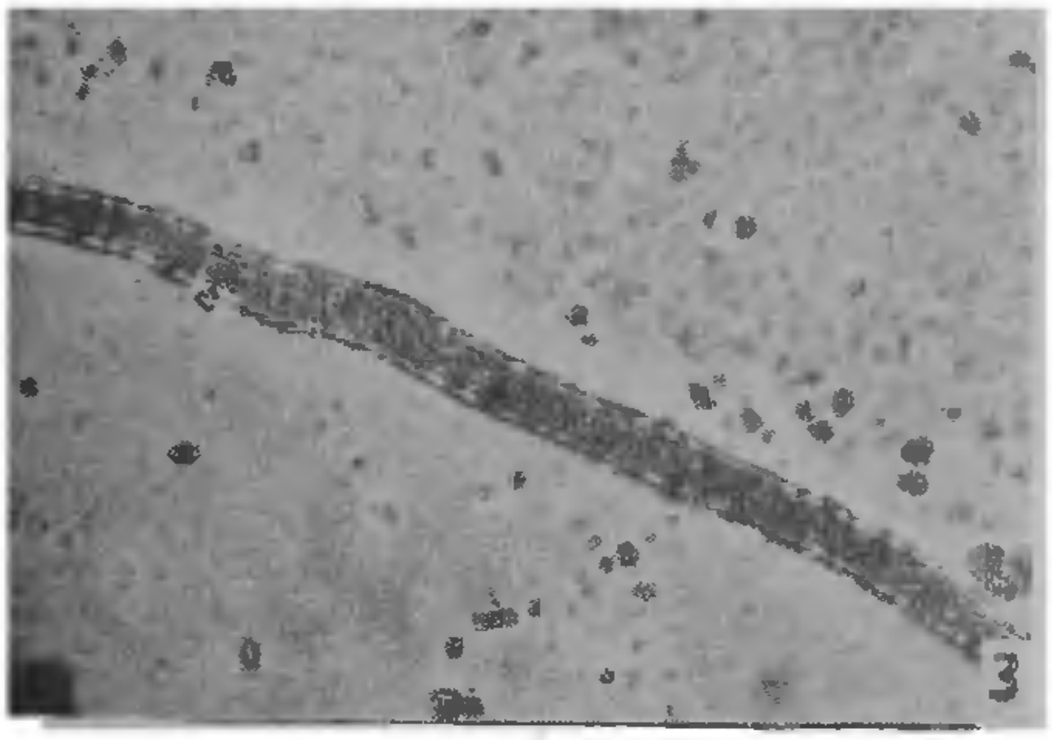
The cell number obtained by plating, agreed satisfactorily with the cell count obtained after trypsin treatment. Continued analysis of the supernatant of tryptic digestion revealed the presence of appreciable amounts of fatty acids and sugars, and of small amounts of proteins, di- and tri-peptides suggesting their involvement in constituting the cell-binding materials. These materials might have cemented the cells into the highly intertwined hyphal structures, which were then dissolved out during trypsin treatment.

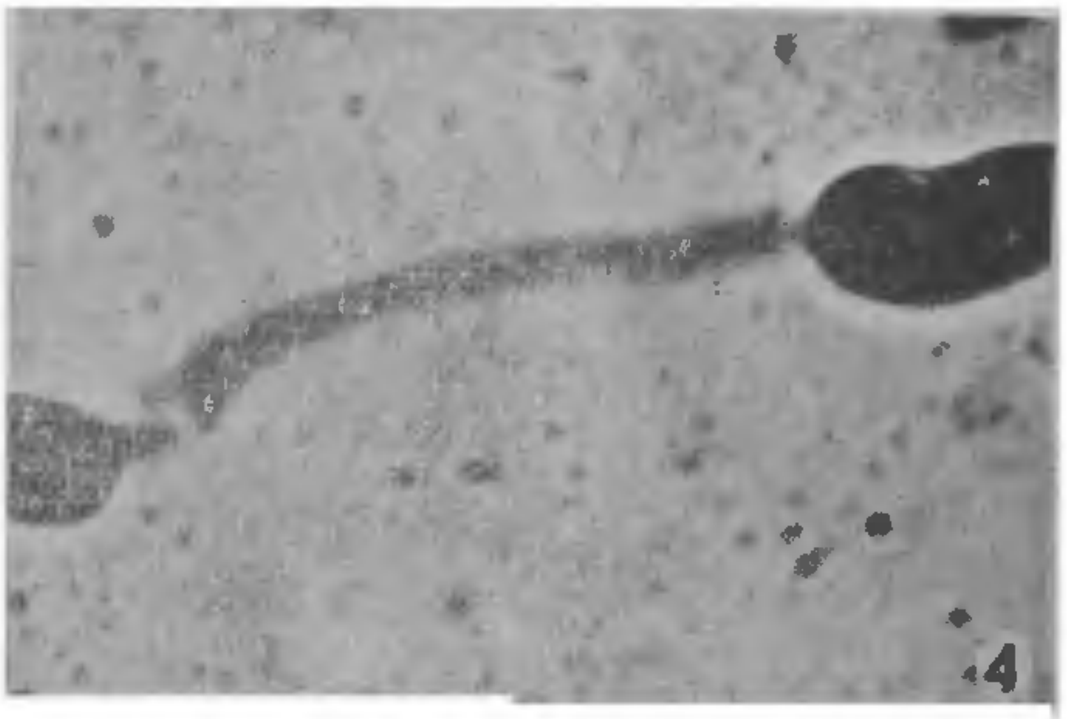
The onset of cell lysis is indicated by the appearance of nucleic acids in the supernatant after trypsin digestion. In the slides presented here, the release of the nucleic acids was not observed in 15 minutes, when the cell clusters were broken up to give a uniform single cell suspension. Also any conspicuous decrease in the viable count compared to the direct count under a microscope is indicative of an accelerated rate of cell death[6] which was not observed in our experiments.

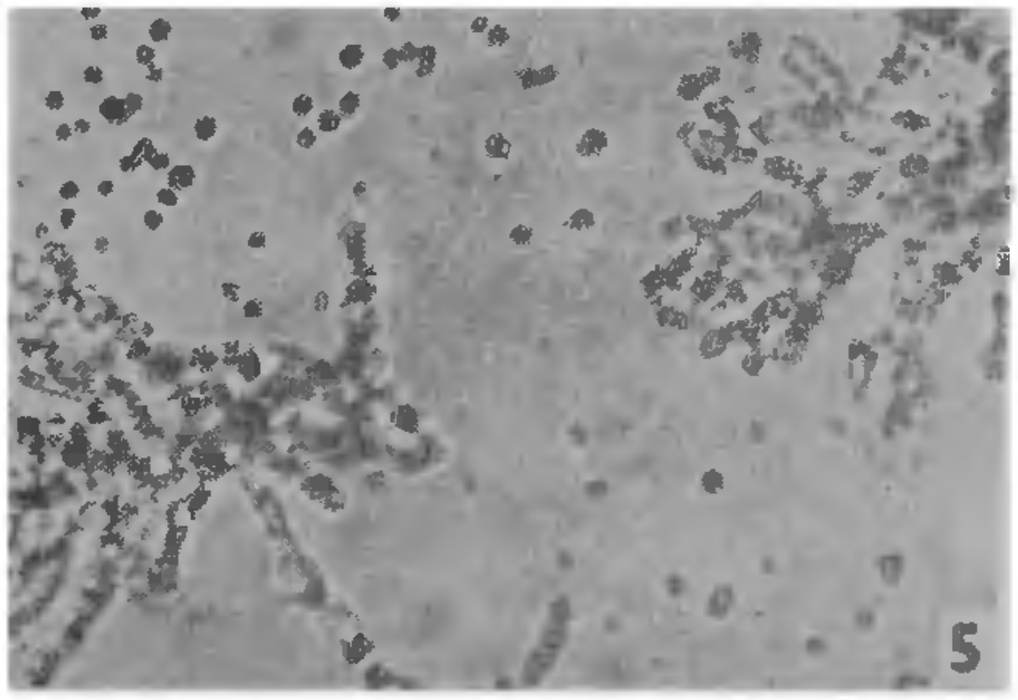
The different stages that the cells underwent towards the formation of chaotic hyphal structures were observable under the microscope. The germination of conidiospores, cell

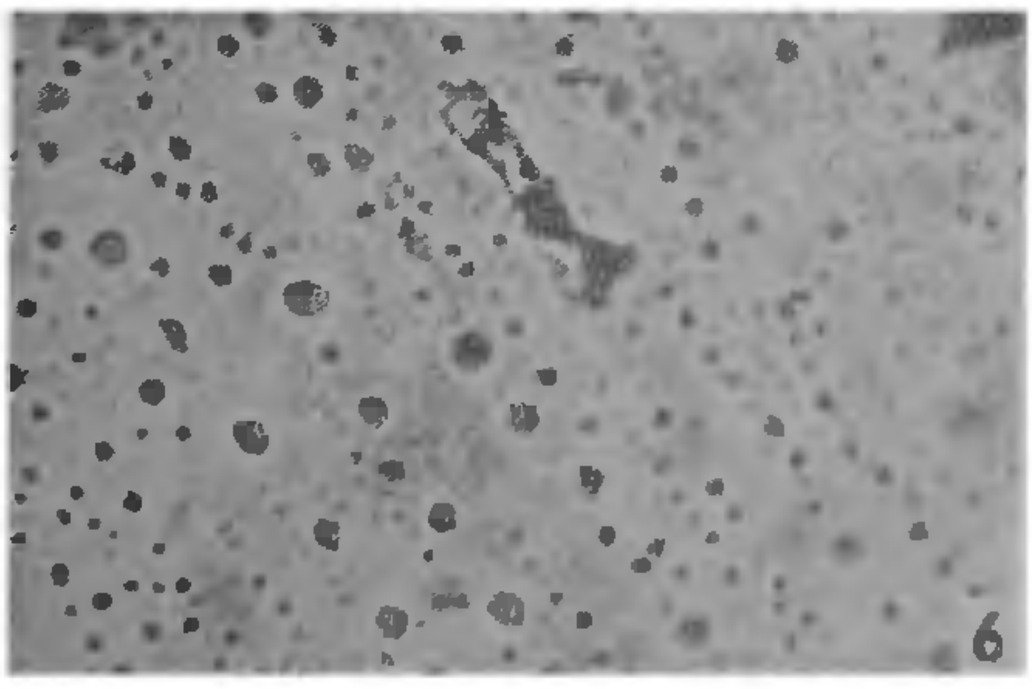


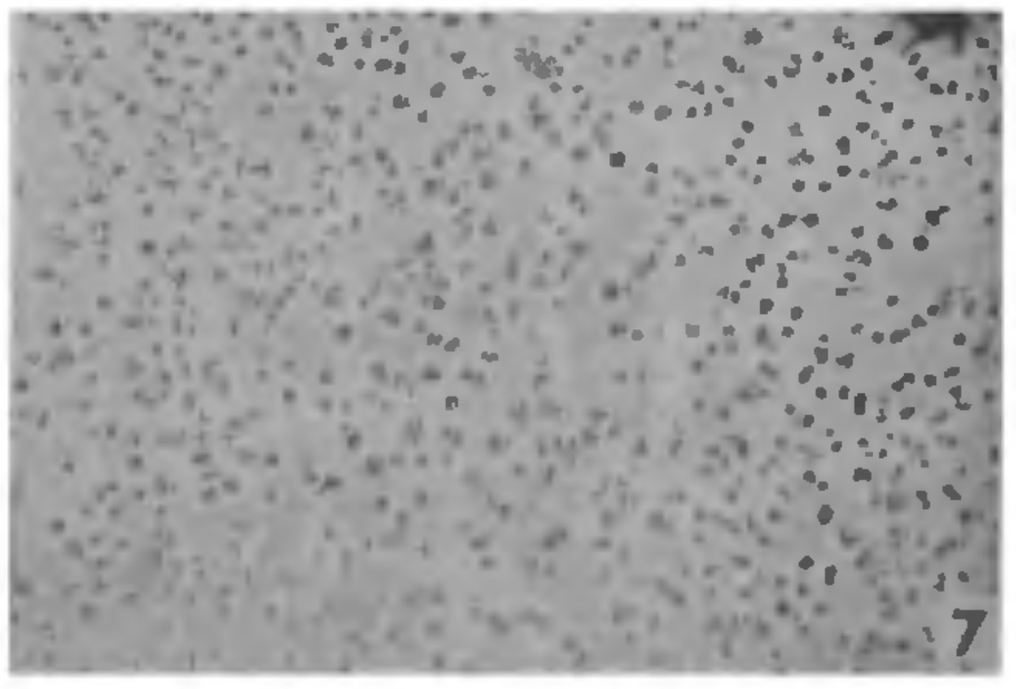












FIGS. 1–7. **Fig. 1.** The cells are present as clusters of two and three and rarely of four suggesting that they might have resulted from single cells by the budding mechanism seen after 20 hrs. of growth. **Fig. 2.** The cells grow into beaded structures by unidirectional division, as observed after 24 hrs. **Fig. 3.** The aligned cells are tightly packed by certain slender fibres as observed after 30 hrs. **Fig. 4.** The beaded filament is encased in an enveloping sheath as observed at 36 hrs. **Fig. 5.** The filaments constitute the chaotic hyphal mass after 48 hrs. growth. **Fig. 6.** Dissolution of cell binding materials by trypsin leaving behind the cells on the ghost-like back-bone material. **Fig. 7.** The single cells obtained by trypsin digestion for 15 mts.

division by budding mechanism, formation of beaded structures and complex hyphal structures are illustrated in Figs. 1-5. Dissolution of the cell-binding materials on trypsin treatment for 15 mts., is observed in Fig. 6 and the resultant uniform unicellular culture is observed in Fig. 7.

The photomicrographs presented reveal that a unicellular suspension of fungal clusters could be obtained by trypsinization as used in tissue culture studies with higher animals.[1-3] This provides a method of following the cell division in fungus and it is hoped that synchronously dividing cultures could be obtained by this method. With the unicellular suspension as the starting material synthesis of DNA, RNA and proteins in the subsequent generations can be followed using selective inhibition studies.

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SOME STUDIES ON THE WORK HARDENING OF A CHROMIUM-NICKEL AUSTENITIC STAINLESS STEEL

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ABSTRACT

It was noticed that the gradients at the initial portion of the True Stress-True Strain curves of an austenitic stainless steel are very sensitive indicators to low temperature annealing treatments than are the yield points.

INTRODUCTION

IT is well known that austenitic stainless steels show a high amount of work hardening. The present study was carried out on a chromium-nickel austenitic stainless steel which was cold-worked to various extents at room temperature and then annealed at various temperatures for a given period of time. True Stress-True Strain curves were drawn for all the samples and both the Yield Points and the gradients of the True Stress-True Strain curves at the initial linear portion close to the Yield Point were determined.

EXPERIMENTAL STUDIES

Chemical analysis of the stainless steel sample revealed its composition to be :

Chromium 19·4, Nickel 11·5, Carbon 0·16, and the rest iron, all in weight percentage.

Rolled bars of the alloy of 15 mm. dia. were initially given a homogenising treatment at 1100° C. for one hour in an argon atmosphere and then quenched in oil. Standard tensile specimens (about thirty pieces) were turned out of these homogenised bars according to DIN 50, 125 specifications. These specimens were then mounted one after the other in a 10 Ton Losenhausenwerk Universal Testing Machine and loaded steadily at the rate of 100 kg. per minute. Reduction in cross-section of the specimen was measured at the centre of its gauge length with a sensitive Kunkel dial gauge. The deformation at the rate of loading adopted was gradual and very uniform over the entire gauge length and very dependable results could be obtained as revealed by repeated studies. True Stress-True Strain curves were plotted in each case from the load-deformation values.

The specimens were divided into Groups A, B, C, D and E depending on the initial deformations given to them which corresponded nearly to 7·5%, 11·9%, 16·5%, 23·8% and 27·6% reduction in cross-section respectively. Each group consisted of at least six specimens. The specimens of each group were, after the initial appropriate deformation, annealed in argon for 1½ hours at 100°, 200°, 300°, 400° and 500° C. respectively—one specimen at each

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