MACROMOLECULAR CHANGES DURING ASEXUAL DIFFERENTIATION IN *ASPERGILLUS NIDULANS* UNDER LIQUID SHAKE CULTURE CONDITIONS

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ABSTRACT

The levels of nucleic acids, proteins and carbohydrates in *Aspergillus nidulans* follow the same pattern under optimal growth conditions (pH 6·5) as well as at pH 5·0 when the cultures remain purely vegetative and fail to undergo reproductive differentiation. Maximum concentration of DNA was found during the vegetative phase, which decreased during conidiation. RNA and proteins were maximum after 36 hours of growth. On the other hand, carbohydrates increased linearly during 48 hours of growth. At pH 5·0 the macromolecules followed the same pattern, but the amount of any of these was less at a given stage of growth as compared to the amount from the mycelium grown under optimal conditions of growth and differentiation.

INTRODUCTION

In the last two decades, a wealth of information has accumulated regarding the understanding and relationships between gene action and macromolecular synthesis and it is now well established that differentiation results from an interplay between an unchanging genome and a labile cytoplasm. Filamentous fungi are most suitable for biochemical analysis of differentiation because here morphogenesis can easily be controlled by simple manipulations of certain physical and chemical factors in the environment[1–4].

Aspergillus nidulans reproduces sexually as well as asexually under optimal submerged shake culture conditions[5,6]. In order to understand the mechanism of metabolic control of submerged conidiation, investigations were carried out with purely vegetative and conidiating cultures and the levels of nucleic acids, proteins and carbohydrates were estimated during various stages of growth and differentiation.

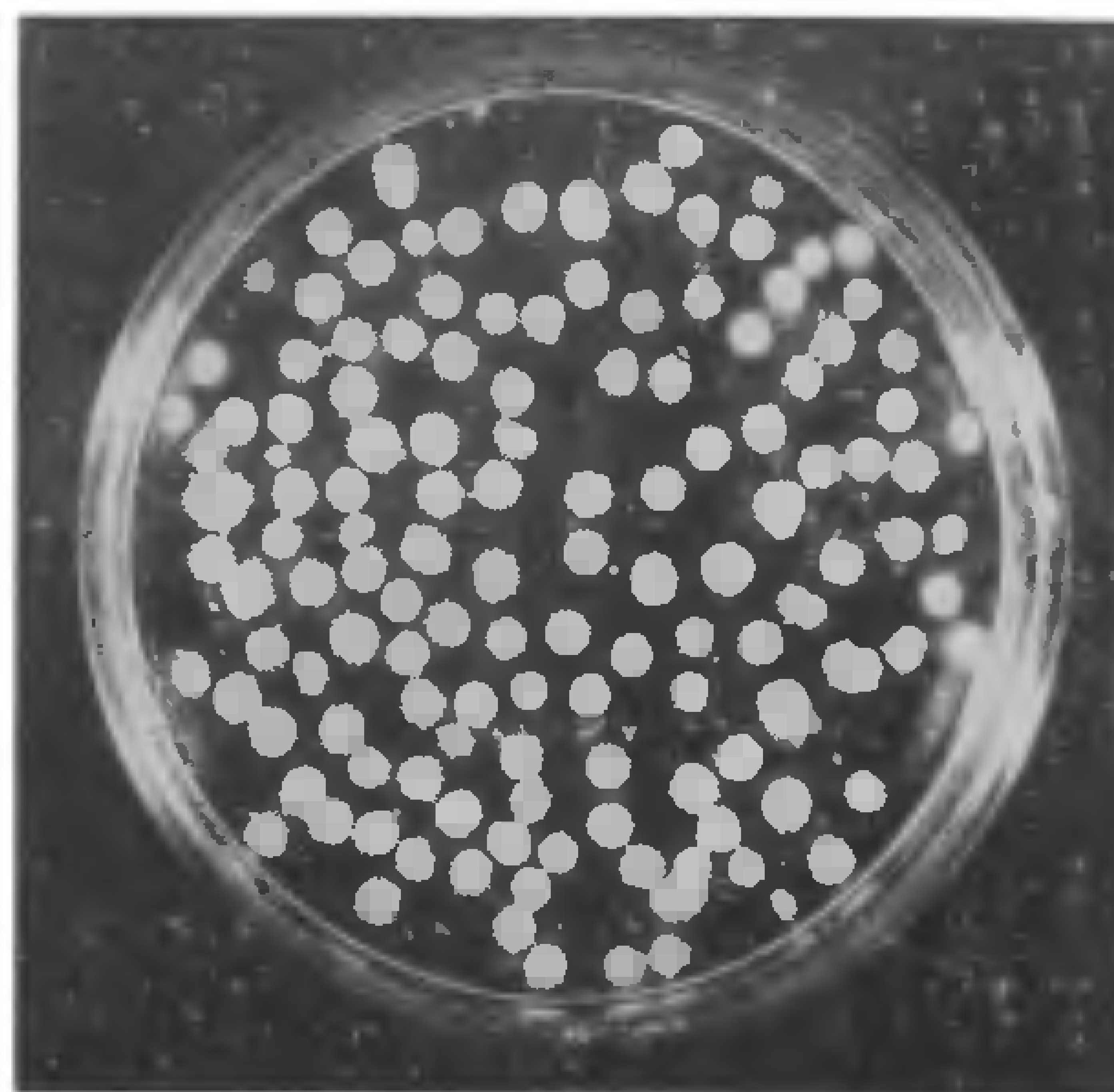

FIG. 1. Pellets formed under submerged shaken culture conditions.

MATERIALS AND METHODS

A biotin and riboflavin requiring strain (*riboA*1, *biA*1) of *A. nidulans* from the Departmental Stock (initially obtained from Glasgow) was used during the course of the present investigations. Compositions of complete (CM) and minimal (MM) media and shaken culture conditions have been described earlier[5,6].

Mycelial pellets (Fig. 1) were harvested at 12 hour intervals up to 48 hours on Whatman filter-paper No. 1 and were washed thoroughly twice with 50 ml of distilled water. Excess of water was removed by pressing the pellets between four layers of muslin cloth. One gram of wet weight of mycelial pellets was homogenized in a pre-cooled mortar and pestle with one gram of acid washed sand. The homogenate was centrifuged at 5,000 × g for five minutes. The supernatant thus obtained, was made up to 25 ml with distilled water. The extract was stored at 4° C until used. Deoxyribose and ribose nucleic acids were estimated using diphenylamine and orcinol reagents, respectively[7]. Anthrone reagent[8] was used for determining carbohydrates and proteins were estimated by using Folin's phenol reagent[9].

RESULTS AND DISCUSSION

Cultures of the *riboA*1, *biA*1 strain were raised in minimal media supplemented with riboflavin and biotin at pH 6·5 and 5·0. From the mycelial extract, DNA, RNA, protein and carbohydrates were estimated at 595, 665, 750 and 620 nm respectively against suitable blanks in a Spectronic-20 (Bausch and Lomb) spectrophotometer.

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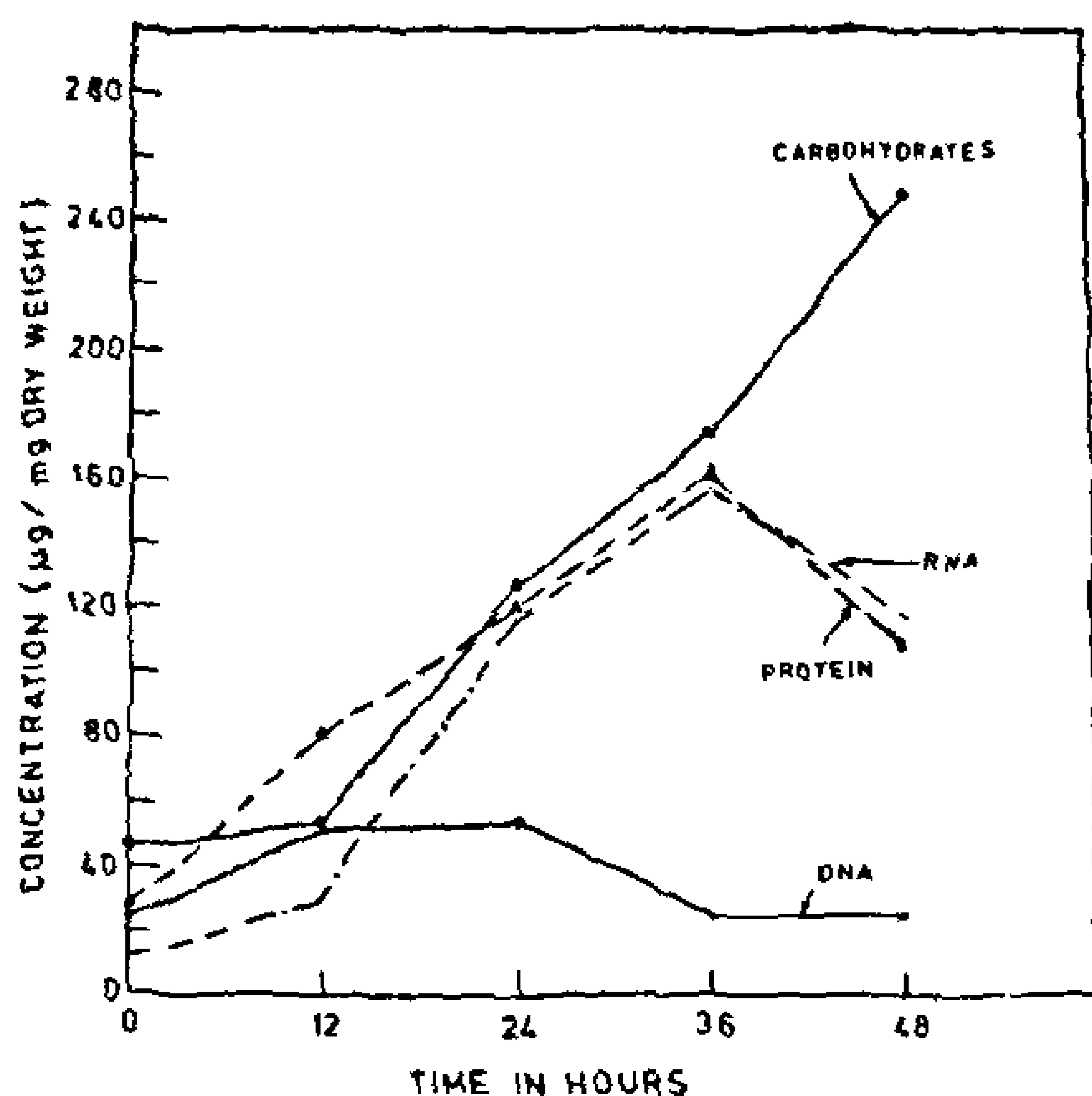

FIG. 2. Levels of macromolecules in mycelia grown under optimal conditions (pH 6·5) in minimal medium.

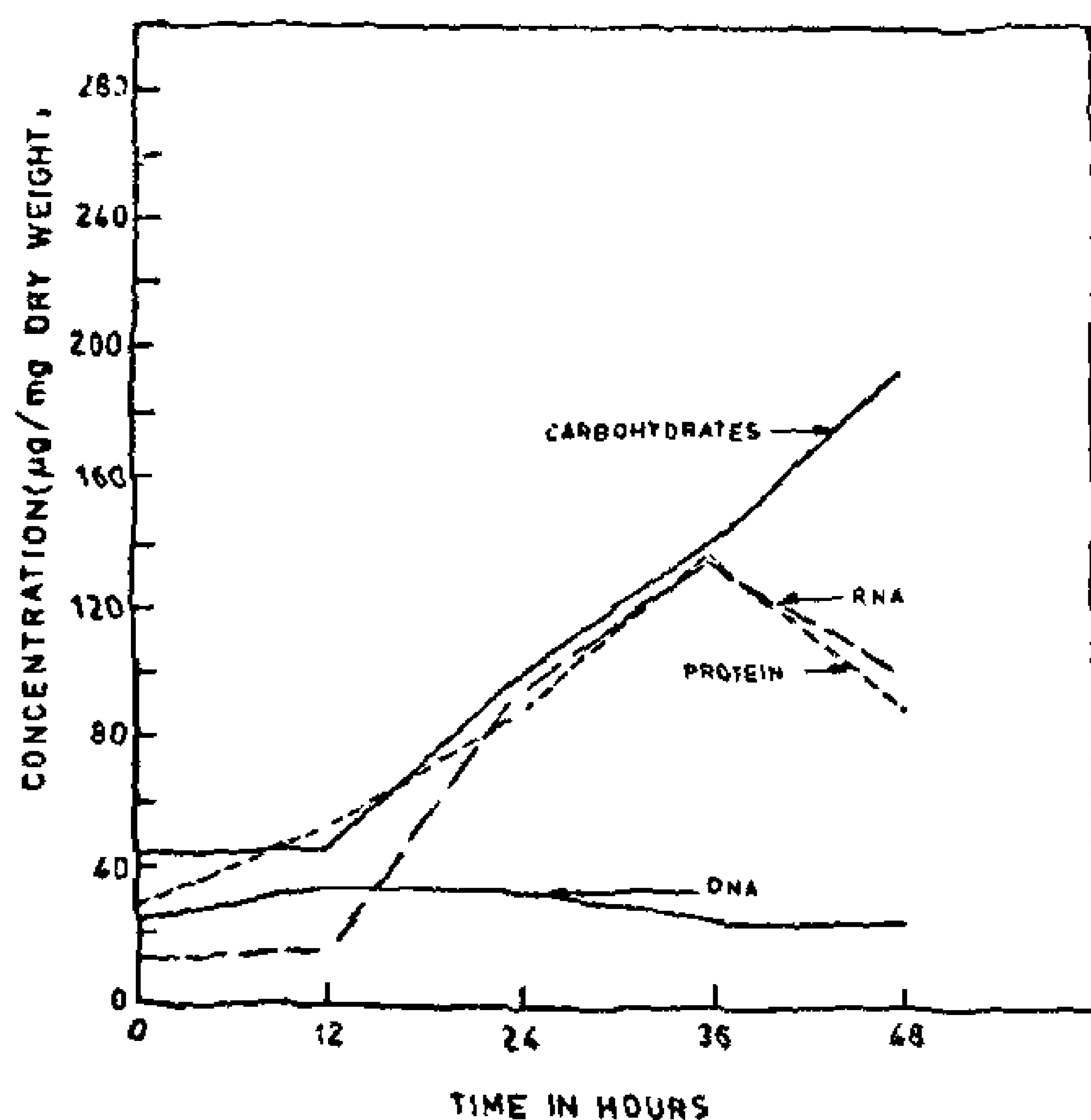

FIG. 3. Levels of macromolecules in mycelia grown at pH 5·0 in minimal medium.

Observations presented in Figs. 2 and 3 revealed that in general all the macromolecules followed the same pattern under optimal growth conditions (pH 6·5) and at pH 5·0 when the cultures fail to undergo reproductive differentiation and remain purely vegetative. DNA was maximum per mg dry weight of the mycelium during vegetative growth and decreased during conidiation. RNA and proteins were maximum (in terms of µg per mg dry weight of the mycelium) in conidiating cultures, that is, after 36 hours of growth, showing thereby that they were synthesized at a faster rate. However, on further incubation a decline in their concentration was recorded. On the other hand, carbohydrates increased linearly during this period.

At pH 5·0, although the macromolecules followed the same pattern, the amount of any of these was less at a given stage of differentiation as compared to the amount from the mycelium grown under optimal conditions, that is at pH 6·5.

During the course of submerged growth in *A. nidulans*, DNA was maximum in its concentration during vegetative phase and its decline in concentration during asexual reproduction can be explained by the rapid rate of cell growth, differentiation and division, unaccompanied by rapid nuclear divisions during this period. The concentration patterns of RNA and proteins may reflect transcription and translation during conidiation of various genes that are involved in metabolic pathways leading to asexual differentiation. A further decline in their concentrations during the autolytic phase is perhaps due to the activity of the lytic enzymes produced by the mycelium. Though the maximal amount of carbohydrates is synthesized during the vegetative phase, it follows a linear pattern. With an increase in the incubation period there is an increase in the total carbohydrate concentration. Macromolecules follow a similar trend at pH 5·0 too. Studies with *Neurospora crassa*[10] and with a non-filamentous aquatic fungus *Blastocladiella emersonii*[11] have shown similar changes in the levels of macromolecules at the time of onset of differentiation. In *N. crassa* the HMP shunt is important for normal vegetative growth and for preparation for asexual differentiation. It provides pentoses for nucleic acid syntheses. The DNA content rises until the early conidial stages and falls subsequently. Sexual differentiation in *A. nidulans* is very sparse and perhaps incomplete under liquid shake culture conditions and, therefore, determinations of macromolecular levels during sexual differentiation in this fungus may not be very meaningful.

ACKNOWLEDGEMENT

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MONOAMINE OXIDASE ACTIVITY IN THE PROSTATE GLAND OF *TAPHOZOUS LONGIMANUS* HARDWICKE (MICROCHIROPTERA : MAMMALIA)

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Abstract

The paper describes the histochemical distribution of monoamine oxidase (MAO : E.C. : 1.4.3.4.) in the prostate gland of sexually mature *Taphozous longimanus*. Very intense activity was discerned in the acini epithelial cells and secretion; stromal cells and luminal fluid exhibited moderate to weak activity. It is suggested that MAO may have a protective role in circumventing the induction of deleterious effects of biogenic amines on the spermatozoa.

Introduction

A VARIETY of metabolic enzymes have been histochemically and biochemically demonstrated in the prostate gland of many mammalian species under normal and pathological states[1-4]. It has been shown that 30% of human seminal plasma constituents originate from prostate gland[4]. These secretions are believed to meet the requirements of spermatozoa to ensure their motility and viability.

Swami and Lall[5] were the first to report the histochemical distribution and possible role of acid phosphatases and adenosine triphosphatase in the prostate gland of sexually mature *Taphozous longimanus*.

Monoamine oxidase (MAO : E.C. 1.4.3.4) converts several biogenic amines into their acid derivatives and has been related to androgen synthesis and testicular development[6]. The occurrence of this enzyme activity has not been histochemically demonstrated in the prostate gland of mammals. The present report deals with the histochemical site and distribution of MAO in the prostate gland of sexually mature *T. longimanus*.

Material and Methods

Sexually mature males of *T. longimanus* (weight 23·0–25·5 gm) used in the present study were locally collected. They were maintained in batches of 2–3 in steel cages with wire nettings and had *ad libitum* access to sugared water. A total of 7 males were used. The animals were sacrificed by cervical dislocation. Prostate glands were quickly dissected out, freed from blood and connective tissues and washed in chilled mammalian ringer (at 4° C).

Fresh frozen sections were cut at 10 μM. MAO activity was demonstrated histochemically according to Gleener *et al* method[7] using tryptamine hydrochloride (Fluka) as substrate. The sections were incubated for 15 min at 37° C. Presence of blue formazon deposits indicated sites of MAO activity. Sections incubated in a substrate free medium served as controls.

Enzyme activity was visually appraised and scored as described earlier[5].

Results

Positive MAO activity was seen in the cytoplasm of the epithelial cells. Secretion granules inside the cells and in the lumen; and stroma exhibited very intense enzyme reaction. Nuclei indicated intense MAO activity. Luminal fluid showed mild enzyme reaction (Figs. 1–3).

Discussion

The present study is the first to demonstrate histochemically the distribution of MAO in the prostate gland of a chiropteran species—*T. longimanus*. The presence of MAO in the acinal epithelium and the luminal fluid of the prostate gland suggests that synthesis and elaboration of this enzyme takes place in this organ. The presence of MAO endows to the prostate the ability to degrade biogenic amines that might otherwise detrimentally affect the spermatozoa.