

of 5.7 min, but only 5% fed on day 25 taking 14.2 min and no fly fed after day 25. These facts show that tsetse flies are inhibited from feeding where the pelt is more than 5 mm thick. Popham et al.¹¹ have shown that sounds emitted by gorged flies stimulate unfed flies to move and enable flies that have failed to feed to move to where blood may be more easily obtained. Unanaesthetized lizard, mice and gerbils attacked and killed 75 (61%) of 123 *G. austeni* placed in their cages – the agility of these small mammals preventing flies feeding on them. Guinea-pigs successfully killed 60

(78%) out of 77 specimens of *G. morsitans* and rabbits killed 17 (65%) out of 26 *G. austeni* used. These observations support others (unpublished) made in Kaduna, Nigeria in which wild lizards (*Agama agama*) attacked and destroyed *G. palpalis*. If reptiles and mammals behave similarly under natural conditions their absence from the records of Weitz¹² is understandable. These experiments show that although tsetse flies are attracted by radiant heat and to all parts of a host's skin, they only feed where the pelt is less than 1–5 mm thick.

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Heterotrophic growth in the blue-green alga *Anacystis nidulans*

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Summary. *Anacystis nidulans* was grown heterotrophically in the dark for the first time. Pyruvate served as the carbon source. The cells did not make pigments when grown heterotrophically, but synthesis of pigments resumed upon transfer of the cells to the light.

Blue-green algae were conventionally considered as obligate photoautotrophs. Many of the forms, including *Anacystis nidulans*, were however shown to take up organic compounds in light^{1–6}. It was shown that filamentous forms such as *Anabaenopsis circularis*⁷, *Anabaena cycadeae*⁸, *Calothrix brevissima*, *C. membranica*, *Nostoc commune*, *Nostoc muscorum*⁹, *Phormidium luridum*, *Plectonema boryanum*⁹ and *Tolypothrix tenuis*¹⁰ and the unicellular form *Aphanocapsa*¹¹ could grow in the dark. The unicellular form *Anacystis nidulans* and the filamentous form *Anabaena variabilis* were considered as obligate photoautotrophs, since they did not respond to external additions of carbon sources¹².

Recently we have reported that dark-starved cells of *A. nidulans* could utilise pyruvate, α -ketoglutarate, succinate and glucose as respiratory substrates¹³. Employing plasmid-curing chemicals, such as acriflavin and acridine orange, we have isolated 'albino' cells of *A. nidulans* which were successfully cultivated at least for a few generations in an autotrophic medium supplemented with glucose¹⁴. The latter 2 experiments indicated the possibility of growing

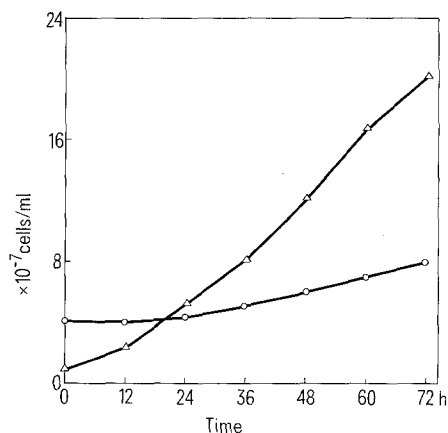


Fig.1. Growth of *Anacystis nidulans* in autotrophic (Δ — Δ) medium in light (5000 lx) at 38 °C in a shaker and in heterotrophic medium in the dark at 32 °C as still culture (\circ — \circ).

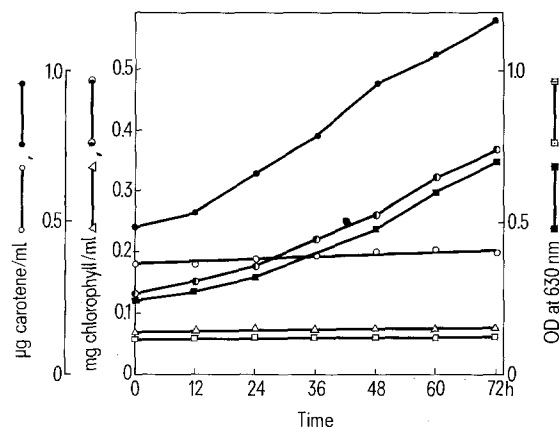


Fig.2. Synthesis of pigments in light and dark grown cells. Chlorophyll (l \bullet — \bullet , d \triangle — \triangle); carotenoids (l \bullet — \bullet , d \circ — \circ); phycocyanin (l \bullet — \bullet , d \square — \square); l, light grown cells; d, dark grown cells. Chlorophyll was extracted from the cells using 80% acetone and the pigment was estimated following the specific absorption coefficient of 82.0 at 663 nm. Phycocyanin was measured following the optical density of cells at 630 nm.

A. nidulans heterotrophically in the dark by employing suitable carbon sources.

A. nidulans (14011) was initially grown in Allen's medium at 38 °C with continuous shaking. The culture was illuminated with day-light fluorescent lamps to yield 5000 lx intensity. Exponentially growing cells were harvested and inoculated in heterotrophic medium containing essentially all salts of Allen's medium with 0.5% sodium pyruvate and grown in the dark (figure 1). The cells grown heterotrophically in the dark were subcultured 2 or 3 times successfully and were found to be growing without production of

pigments (figure 2). On transfer to autotrophic medium and light, these cells started synthesizing pigments again (figure 3).

The lack of pigment synthesis by *A. nidulans* in the dark is in contrast to the results reported with most blue-green algae grown in the dark. However *Plectonema boryanum* grown in the dark on glucose using a dialysis-flow through culture technique made no chlorophyll¹⁵. The results presented here clearly show that *A. nidulans* can be grown heterotrophically in the dark and is a good organism to study the regulation of pigment biosynthesis in procaryotes.

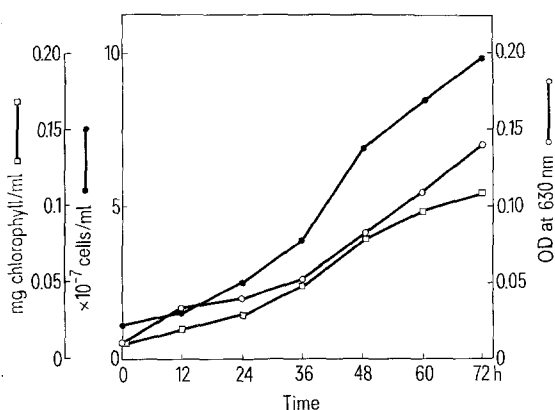


Fig. 3. Growth and pigment synthesis by dark grown cells in light. Cell multiplication (●—●); chlorophyll (□—□); and phycocyanin (○—○). Chlorophyll was extracted from the cells using 80% acetone and the pigment was estimated following the specific absorption coefficient of 82.0 at 663 nm. Phycocyanin was measured following the optical density of cells at 630 nm.

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Diethylpyrocarbonate interferes with lipid-protein interaction and glucose transport in the human red cell membrane¹

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Summary. Diethylpyrocarbonate largely diminishes both discontinuities in red cell glucose transport and also in red cell membrane ANS fluorescence at about 17–20 °C.

Diethylpyrocarbonate has been successfully employed in protein chemistry for the characterization of reactive histidines which form N-ethoxyformyl-derivatives. Dependent on reactivity, the reagent may also modify lysine, tyrosine rests and sulfhydryl groups^{2–5}. We report on experiments using the reagent in membrane biochemistry of the red cell, since in glucose transport imidazole rests of histidine, ϵ -amino groups of lysine have been found to play an essential role^{6,7}. The investigations on glucose transport were supplemented by structural studies on red cell membrane and extracted membrane lipids, in order to achieve a better understanding of these residues in the architecture of the membrane.

Materials and methods. Erythrocytes of freshly collected human blood in ACD solution (11 g sodium citrate, 35 g glucose, 4 g citric acid with aqua bidest. ad 1000 ml) were preloaded with 200 mM glucose in isotonic NaCl solution by 4 washings. Incubation procedure and analytical methods have been described previously⁸. Red cell mem-

branes were obtained by the method of Dodge et al.⁹ as described previously^{10,11}. The red cell membrane lipids were extracted according to the method of Dawson et al.¹². Lipid estimations were carried out by the method of Zöllner and Kirsch¹³, cholesterol was determined according to Watson¹⁴.

Results. As seen in figure 1, glucose transport is influenced by temperature, and a discontinuity of $\log v_0$ (v_0 =initial velocity without inhibitor) at around 20 °C is observed, as has been reported previously¹⁵. This discontinuity is correlated with the transition temperature, due to a structural change of the membrane lipids¹⁰. Since diethylpyrocarbonate is a protein reagent, an influence on the discontinuity of v_0 in glucose transport or on the transition temperature would not normally be anticipated. Nevertheless, there is a distinct change in slope of the straight line obtained for v_i (v_i =initial velocity in presence of inhibitor) of glucose transport for the whole temperature range. The discontinuity has disappeared. This interesting behaviour was fol-