CHARACTERIZATION OF CHANGES INDUCED IN PHYCOBILISOMES DURING ADAPTATION TO HIGH TEMPERATURE IN PHORMIDIUM FOVELARUM

DISSERTATION SUBMITTED TO
JAWAHARLAL NEHRU UNIVERSITY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF DEGREE OF

MASTER OF PHILOSOPHY

1989

PARVEEN KUMAR GARG

SCHOOL OF LIFE SCIENCES
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CERTIFICATE

This is to certify that this dissertation entitled "**Characterisation of changes induced in Phycobilisomes during adaptation to high temperature in *Phormidium fovelarum***" embodies original research work carried out by Shri Parveen Kumar Garg under the guidance of Prof. Prasanna Mohanty at the School of Life Sciences, Jawaharlal Nehru University, New Delhi. This work has not been submitted in part or in full for any other degree or diploma of any university.

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P.K. GARG

ABBREVIATIONS

A^{o}	Angstrom unit
ADP	Adenosine 5'-diphosphate
APC	Allophycocyanin
ATP	Adenosine 5'-triphosphate
BGA	Blue green algae
CFo	Coupling factor 0 of ATP synthase
CFI	Coupling factor 1 of ATP synthase
Chl	Chlorophyll
Cyt	Cytochrome
Cyt b-559	Cytochrome b-559
DCCD	Dicyclohexyl carbodamide
DGDG	Digalactosyl diacylglycerol
DCPIP	2,6-dichlorophenol indophenol
ESR	Electron spin resonance
Fd	Feredoxin
HEPES	N-2-Hydroxyethyl piperzine-N-2- ethane Sulforic acid
KDa	Kilo dalton
LHCP	Light harvesting chlorophyll protein
MGDG	Monoglactosyl diacylglycerol
NAD	Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
NADPH	NADP reduced.

OEC	Oxygen evolving complex
PBsomes	Phycobilisomes
PC	Phycocyanin
PCY	Plastocyanin
PE	Phycoerythrin
PEG	Polyethyl glycol
PQ	Plastoquinone
PS I	Photosystem I
PS II	Photosystem II
TMPD	N-tetramethyl-p-phenylene diamine
Wm^{-2}	Watt per meter square
WOC	Water oxidation complex.

INTRODUCTION

The process of photosynthesis is responsible for the synthesis of simple carbohydrates, such as sugars (in the green leaf, photosynthetic bacteria and algae) in the presence of sunlight, carbon dioxide and water absorbed from air and soil respectively. During photosynthesis oxygen is liberated into the atmosphere. Later in the cell, simple and complex carbohydrates are converted into lipids, nucleic acids, proteins and other complex organic molecules.

In order to have an idea about the various aspects of the complex process of photosynthesis let us first see the various structures involved in this process and then see how these structure perform their functions.

CHLOROPLAST

In higher plants, the chloroplast boundary is formed by an envelope composed of two continuous membranes. The inner compartment etc. is filled with an aqueous matrix, the stroma, within which flattened membrane sacs "the thylakoids", are embedded (Menke, 1962). Two types of thylakoids, grana thylakoid and stroma thylakoid, are distinguished (Menke, 1962). Later studies revealed that all grana and stroma thylakoids in a chloroplast form a continuous membrane system which is organised

into a highly complex network enclosing one single anastomising chamber, usually referred to as thylakoid lumen (Paolillo, 1970: Thomson 1974). Thylakoids are centers for trapping of solar energy and its conversion to chemical energy. The stromal matrix houses a host of enzymes responsible for reduction of carbondioxide to carbohydrates.

In photosynthesis light provides the energy for the production of ATP and the generation of reduction power. First step in photosynthesis is the absorption of photons by an array of light harvesting protein pigment complexes. The protein-pigment complexes are located in two different pigment beds known as photosystem I and photosystem II. Both the photosystems are connected by a series of electron carriers arranged according to their redox potential (Fig.I). Photosystem II (PS II) light harvesting complex is mainly located on the appressed (stacked grana lamellae) regions of the thylakoid membrane which shuttles to PSI complex located mostly in non appressed (unstacked stroma lamellae) region of the thylakoid membrane (Gounaris et al. 1984; Murphy 1986; Anderson 1987) Fig. 2a and 2b.

STRUCTURE AND FUNCTION OF THYLAKOID MEMBRANE

Thylakoid contains all the functional elements that are needed for trapping and transducing light energy into the form of chemical energy. These include light harvesting antennae; two

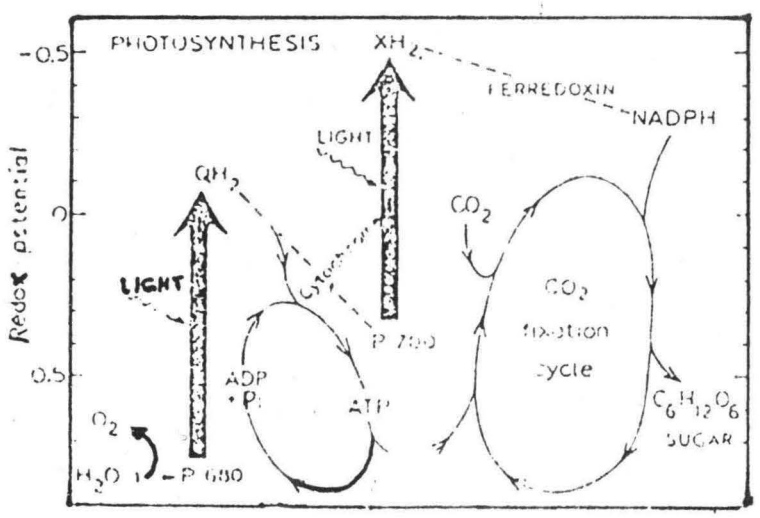

Fig.1 A skelton scheme of photosynthesis indicating light and dark reaction (from Avron, 1981).

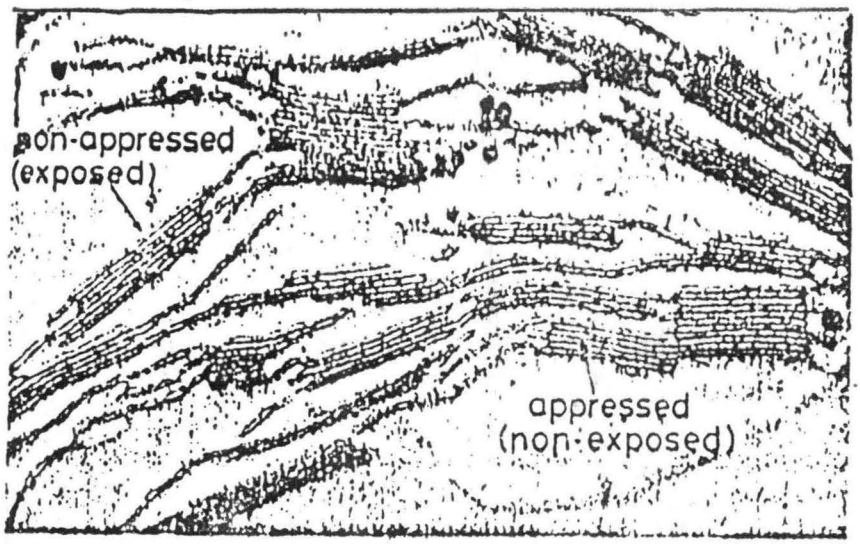

Fig.2a Electron micrograph of appressed and non appressed regions of the thylakoids of Pea Chloroplasts (from Barber, 1985).

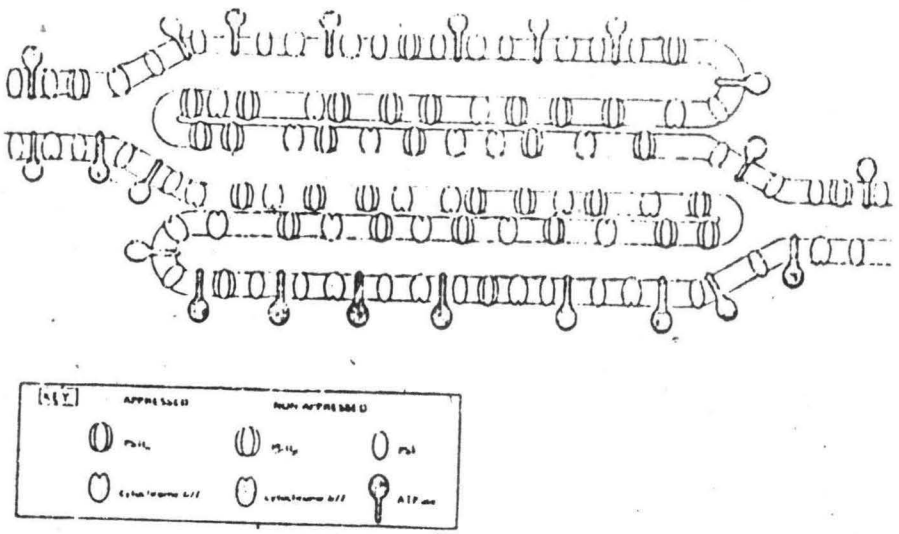

Fig.2b. Distribution of thylakoid complexes between appressed membranes of grana partitions, and non-appressed membranes (Stroma thylakoids, grana margins and grana end membranes). (from Anderson,1984).

types of reaction centers PS-II and PS-I; diverse electron carriers; a system for synthesizing ATP and a well organized oxygen evolving complex (OEC). Biochemical and structural studies have revealed that these components are packed into at least five major types of discrete intramembrane protein complexes (Murphy, 1986; Anderson, 1987) (i) Photosystem I complexes; (ii) Photosystem II complexes. (iii) cytochrome b_6/f complex; (iv) CF_o-CF_1 ATP synthase complex and (v) chlorophyll a/b light harvesting complex (Fig. 3).

Three extrinsic proteins, like feredoxin, feredoxin NADPH oxidoreductase and CF-1 complex of ATP synthase, which are attached to the outer surface of the thylakoid membrane are also helpful in the electron transport and photophosphorylation activity.

The lipids of thylakoid membrane provide a fluid medium which allow diffusion processes to occur. About 75% of acyl lipids are monogalactosyldiacyl glycerol (MGDG) and digalactosylglycerol (DGDG), 15% are phospholipids and the remaining 10% phosphatidyl glycerol (Quinn and Williams, 1978).

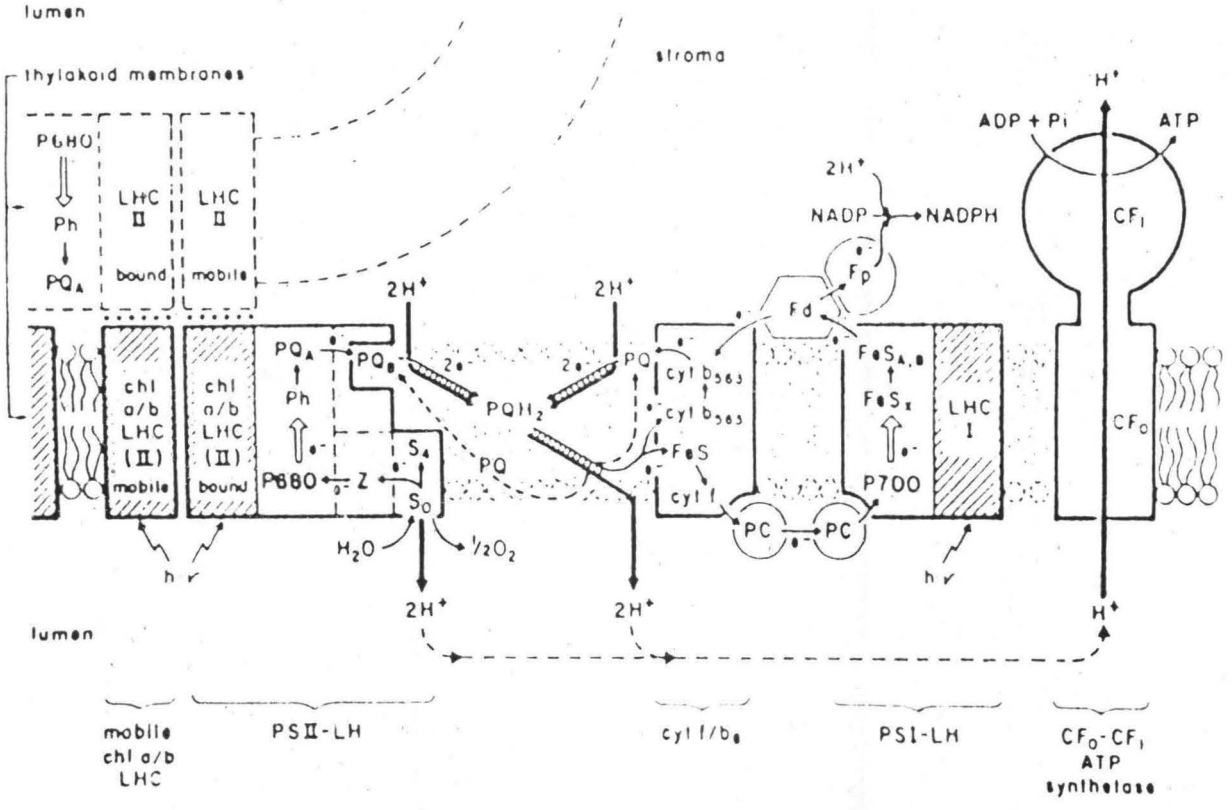

Fig. 3 The organisation of chloroplast membrane components participating in the electron coupling reaction of photosynthetic electron transport chain.

(From Stachellin and Arntzen 1983)

INTRINSIC PROTEINS

Photosystem II Complex

Photosystem II complex of higher plants is made up of three smaller complexes viz. (i) the core complex, (ii) light harvesting complex and (iii) oxygen evolving complex (OEC). The core complex preparation seems to consist of 32 KDa herbicide binding protein (D1), another 34 KDa herbicide binding protein (D2) and the alpha and beta subunits of cytochrome b-559 along with two pigment proteins of 55 KDa and 50 KDa respectively (Satoh, 1985). The D1 and D2 polypeptides are structurally and functionally comparable with the L and M subunits of the reaction center of purple bacteria (Barber, 1987; Satoh, 1988). The PSII complex as isolated by Nanba and Satoh (1987), having D1/D2 and Cyt. b-559 complex, is capable of charge separation, indicating that probably these polypeptides are the major constituents of the core complex of photosystem II reaction center (Barber, 1987).

Light harvesting complex is made up of two polypeptides having molecular mass of 45 kDa and 72 kDa (Thornber, 1975, 1986). Both are found to be a/b chlorophyll containing proteins and functions not only as antenna, but also contributes to the stacking of thylakoids (Kaplan and Arntzen, 1982). It has also been shown that other polypeptides of 19, 24 and 34 kDa are associated with the water-splitting reaction.

PHOTOSYSTEM I COMPLEX

Photosystem I complex of higher plants is made up of two smaller complexes i.e. core complex and light harvesting complex. Core complex I (CCI) contains P-700, β carotene, Chl *a* molecules and polypeptides of 70, 25, 20, 18, 16, 10 and 8 kDa, termed as subunits I-VII, respectively (Okamura et al., 1982). The function of subunit I (70kDa) in binding Chl *a* carotene and P700 is universally agreed. The four smallest subunits are suggested to be Fe-S proteins, and more recently, subunit I also has been proposed to contain an Fe-S center (Thornber, 1986).

LHC I contains four polypeptide subunits, some of which are associated with Chl *a* and *b* having a ratio of 3:5 (Mullet et al., 1930).

CYTOCHROME b_6/f COMPLEX

Cytochrome b_6/f complex, the physiological acceptor of electrons from plastoquinone (PQ pool) serves as an intermediate carrier between PS II and PS I. It acts as a plastoquinone-plastocynain oxidoreductase. These complexes are present in both grana and stroma thylakoid with a slightly greater density in grana regions (Allred and Stachelin 1985). Hurt et al. (1981) have isolated five major polypeptides of this complex with molecular weight of 34, 33, 23, 20, 17.5 kDa and one cytochrome-

f, two cytochrome b-563, the Rieske-Fe-S centers and some bound plastoquinone-9.

CHLOROPHYLL a/b LIGHT HARVESTING COMPLEX (LHC)

The LHC encompasses 50% of the chlorophyll of mature chloroplasts. An enrichment in Chl b is a distinctive hall mark of this complex. It contains several polypeptides in the 20-30 kDa range. In spinach LHC it contains 7 molecules of Chl a and b, 5 molecules of diacyl lipid, and 1 carotenoid per 26 kDa of polypeptide.

CF_o-CF_1 ATP SYNTHASE

Oxidation of water leads to the evolution of oxygen, along with release of protons to the inside of thylakoids, generating electric field across the membrane. Collapse of this electrochemical gradient by proton transport through the membrane bound coupling factor ATPase leads to the formation of ATP (Fig. 4).

Very little is known about the hydrophobic CF_o portion of the CF_o-CF_1 complex, although several proteins (15, 12 and 8 kDa) associated with CF_o base piece have been identified (Pick and Racker, 1979; Nelson et al., 1980). Polypeptide, with 8 kDa molecular weight binds to DCCD (Dicyclohexyl carbodamide) and constitutes a proton conducting channel across the membrane.

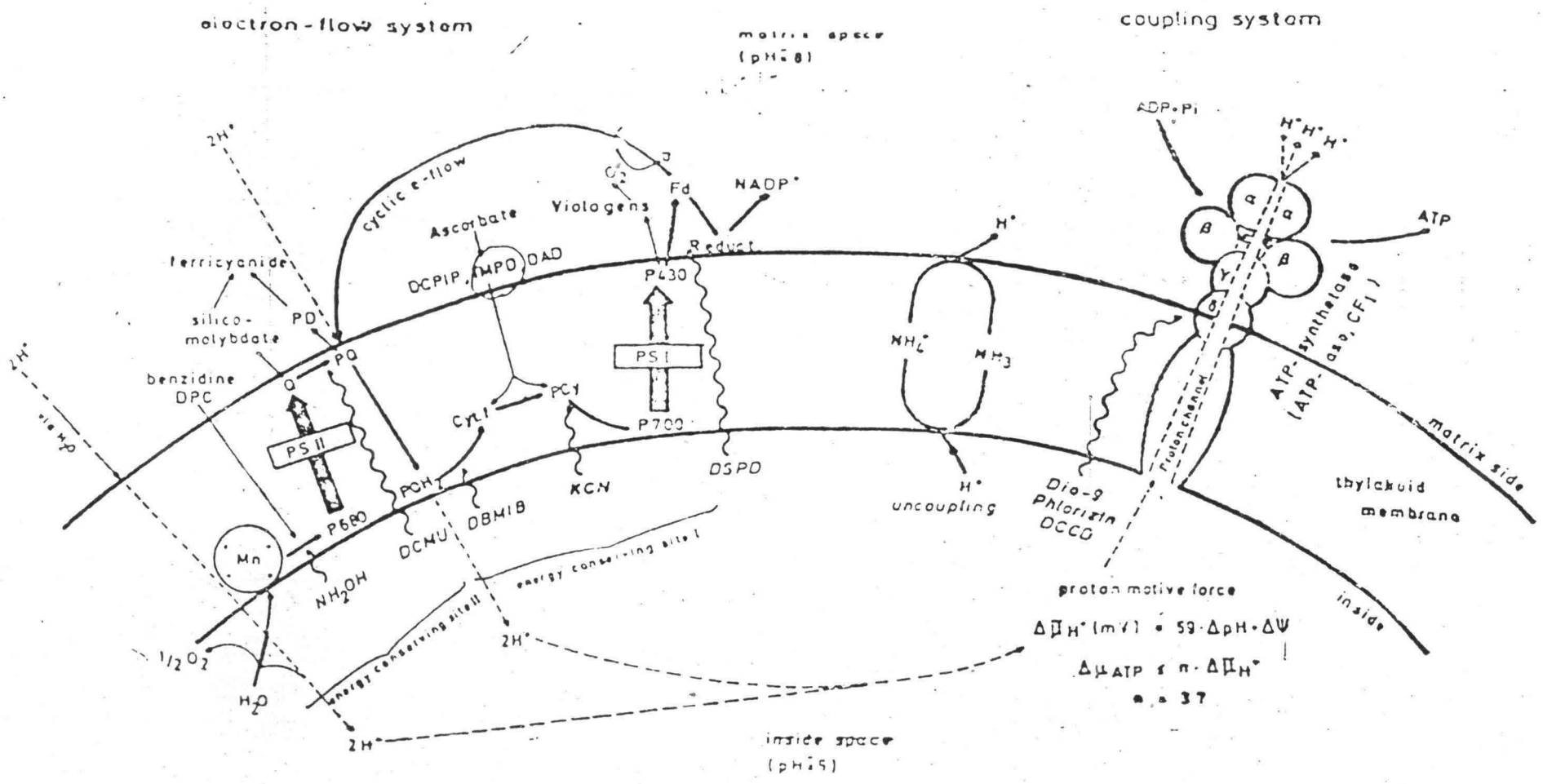

Fig.4 A master scheme for photosynthetic electron flow coupled to ATP formation alongwith the different sites of action for artificial electron donors, acceptors and electron flow inhibition

Second polypeptide (12 kDa) helps in the structural maintenance of third polypeptide. First polypeptide acts as a binding protein between CF_1 and CF_o channel. CF_1 units of intact thylakoids are located exclusively in non-appressed stroma membrane regions. ATP synthase (CF_1 complex) with native molecular weight of 32.5 kDa, can be resolved into five subunits $\alpha, \beta, \gamma, \delta$ and ε with molecular weights 59,54,37,17.5 and 13 kDa. These subunits participate in ATP synthesis/ATP hydrolysis activity (Nelson, 1982).

EXTRINSIC PROTEINS-INSIDE

Plastocyanin (PCY) is a one-copper atom containing protein loosely bound to the inner thylakoid surface (Haehnel et al., 1981). It appears blue in its oxidized state and has a molecular weight of about 10.5 kDa. The core of the molecule is hydrophobic and largely aromatic, while the polar surface has negative charge at physiological pH which is clustered on the side of the molecule. The Cu-atoms are located near one end of the molecule (Colman et al., 1978).

WATER OXIDATION COMPLEX (WOC)

The photosynthetic oxygen evolution from oxidation of water is linked to PS II activity. Kok et al. (1970) formulated the generally accepted concept of "S-state changes (change in oxidation state of Mn) in which each individual oxygen evolving

state undergoes a successive series of increasing oxidation states from S_o to S_4, on excitation of its associated PS II reaction center complex (Fig. 5). When it reaches to S_4 state, it releases oxygen and returns to S_o state. Each of the light induced transitions from S_1 to S_2 and from S_2 to S_3 result in release of one proton and from S_3 to S_o release of two protons. The primary physiological electron donor to P-680 of PS II reaction center is a tyrosine residue of polypeptide Z (Babcock, 1987; Brudvig, 1987). Several extrinsic water soluble polypeptides and cofactors such as Mn^{2+}, Cl^- and Ca^{2+} are involved in the oxygen evolving system (Colmen and Govindjee, 1987: Renge, 1987; Krestschmann et al., 1988). It has also been suggested that ligand histidine residue(s) specifically interact with Mn^{2+} WOC (Tamura et al. 1989).

POLYPEPTIDES

Three water soluble polypeptides having molecular weights of 33, 24 and 17 kDa have been shown to be associated in the water oxidation (Murata and Miyao, 1987, Homann, 1987, 1988). These are peripheral proteins at the luminal side of the thylakoid membrane (Akerlund et al. 1982).

24 kDa polypeptide is associated with the physiological donor Z to P_{680} since Z is linked to metal clusters (Mn) therefore this polypeptide links PS II to oxygen evolving system (OES).

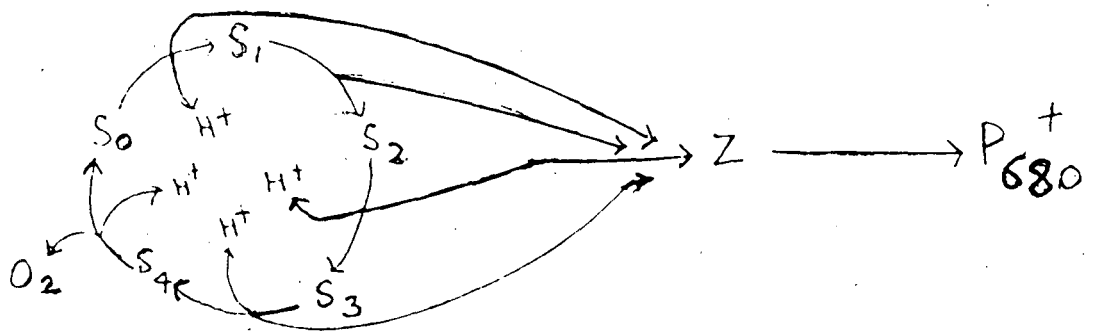

Fig.5 Scheme of the reactions at donor side of PS II. So, S_1, S_2, S_3, S_4 are states of oxygen evolving complex (after Kok, 1970).

33 kDa polypeptide is peripheral in nature, distinct from 24 kDa polypeptide which is absent in algae mutants that lack oxygen evolving capacity. These two polypeptides are involved in clustering of Mn^{2+} ions.

17 kDa polypeptide is helpful in the Ca^{2+} binding and in Cl^- association with oxygen evolving system (Murata and Miyao, 1987).

Manganese is involved as an ion which undergoes oxidation changes in oxygen evolution (Hansson and Wydrzynski, 1989; Volkow, 1989). Four Mn atoms are necessary per P_{680} in PS II oxygen evolution (Zimmermann and Rutherford, 1986).

Chloride binding to Mn cluster is necessary for S_1-S_2 transition (Homann, 1987). The requirement of chloride may be helpful in the adaptive value of plants (Coleman and Govindjee, 1987).

Calcium is necessary for tight binding of Mn and photoreactivation of OEC. In cyanobacteria Ca^{2+} is necessary for retention of a polypeptide in PS II. The 16 kDa polypeptide stimulates oxygen evolution by the addition of Ca^{2+}.

REACTION CENTERS AND THEIR LOCATIONS

The relative number of PS I and PS II reaction centers vary widely in different plants and within the same plant grown under different conditions. The size of antenna per reaction center

also varies significantly with culture conditions or with the stage of chloroplast development (Akoyunoglou and Argyroudi, 1978).

In green plants and green algae, both, the reaction centers and antenna pigments are integral components of thylakoid membranes. In blue green algae, reaction centers are embedded in the thylakoid membranes whereas antenna component lies outside the thylakoid membrane.

A MODEL FOR ELECTRON TRANSPORT

As stated earlier electron transfer from PS II complex of appressed region to the PSI complex of non-appressed occurs through mobile electron carriers like PQ and PCY. The generally accepted model for electron transfer between PS II and PS I in higher plant chloroplast is depicted in Fig.3.

PARTIAL PHOTOCHEMICAL REACTIONS

Partial photochemical reactions are studied with the help of established artificial exogenously added electron donors, electron acceptors and by the use of electron transport inhibitors. The sites of electron donation and acceptance and inhibition of different artificial electron-acceptors, donors and inhibitors are shown in the Fig. 4.

These exogenous donors and acceptors are used to study the PS I and PS II or full chain catalyzed photochemical activities of thylakoid membrane reactions. The study of partial reactions is valuable tool in assessing the photochemical potential of chloroplast as well as of intact cells.

ANOXYGENIC VS. OXYGENIC PHOTOSYNTHESIS

Green and purple bacteria perform anoxygenic photosynthesis, so named because this photochemical process does not lead to the production of O_2. In this bacteria only one type of reaction center is present. Organisms performing oxygenic photosynthesis invariably possess two photosystems as stated earlier, PS I and PS II. Reaction center of PS II (P_{680}) having a redox potential of +0.81 V is a powerful oxidant and obstracts electron from water with the evolution of oxygen. Excitation of P_{700}, the reaction center of PS I with a redox potential of +0.5 V (Okamura et al. 1982) mediates a cyclic electron flow resulting in ATP synthesis. It is generally believed that when PS II and PS I are excited simultaneously, electron abstracted by PS II from water is transported to PS I via plastoquinone to replace PS I. Electrons used for electron flow between PS II and PS I is accompanied by ATP synthesis.

All photosynthetic eukaryotes perform O_2 evolving photosynthesis and contain PS II and PS I complexes. These

properties are also shared by cyanobacteria. The structure of a reaction center complex appears to have been observed among all of these organisms. In contrast, there is a wide variety of antenna complexes with distinctive compositions and spectroscopic properties. Therefore, discussion of photosynthetic apparatus of algae and higher plants includes consideration of reaction center complexes whereas discussion of the same in case of cyanobacteria and various algae emphasizes antenna complexes. A detailed description of cyanobacterial pigment system is described below:

CYANOBACTERIA

The blue green algae are unique among all organisms in possessing a chlorophyll *a* based oxygen evolving photosynthetic apparatus built into a prokaryotic cellular organisation. Cyanobacteria possess not only the photosynthetic pigments, chlorophyll *a*, but also phycobiloprotein complexes contributing to the characteristic colour of the organism. The salient features of these prokaryotes include complementary chromatic adaptation, the presence of gas vacuoles, the sites of dinitrogen fixation, the heterocysts and a typical cell wall resembling those of a gram -ve bacteria.

These microorganisms are the major biological components harvesting the solar energy in the ecosystem. They are also involved in the rapid recycling of the major elements and play a

vital role in scavenging man made pollution. In recent years, the cyanobacteia are also being extensively used as protein rich food, animal feed and for the extraction of vital chemicals.

CYANOBACTERIAL PHOTOSYNTHESIS

Unlike higher plant thylakoid membranes, cyanobacterial thylakoids are not differentiated into grana and stromal thylakoids. Cyanobacterial thylakoids are lamellar in nature and traverse in cytoplasm. These thylakoids contain two photosystems, PS I and PS II which differ from higher plants in the types of pigment-proteins used as light harvesting antennae. Higher plant photosystems depend on Chl b - containing complexes such as Chl a/b LHC, whereas cyanobacterial photosystems depend upon phycobiliproteins organised into phycobilisomes. The chl a/b LHC of higher plants is an integral membrane protein complex whereas phycobilisomes are large, water soluble, peripheral membrane protein complexes that protrude from the membrane surface. PS I and PS II complexes of cyanobacteria resemble those of higher plants both in terms of composition and general organisation, even though their size is smaller due to the absence of bound chlorophyll b containing light harvesting complexes. The PS I and PS II ratio in both cyanobacterial thylakoids is approximately 2.3 (Kursar and Alberts, 1983), versus about 1 for higher plants. Although methods have been developed to isolate not only PS II, but also PS I, cytochrome

b_6/f and CF_o/CF_1 comnplexes from cyanobacteria, virtually no information is available on the actual size and distribution of the latter three complexes in their thylakoid membranes.

Engelmann (1883, 1884) provided the first evidence for the participation of phycobiliproteins in photosynthesis. It was subsequently determined that energy absorbed by these pigments was responsible for most of the photosynthetic activity in blue green algae (Emerson and Lewis 1942). The energy is subsequently conveyed from pigments absorbing at shorter wavelength to those doing so at long wavelength, until it finally reaches chlorophyll (Duysens, 1951). This is assumed to occur by resonance transfer.

CHLOROPHYLL a

All photosynthetic organisms that evolve oxygen possess Chl a as their major pigment. It is believed that Chl a occurs in vivo in several spectroscopic forms: Chl a 660, Chl a 670, Chl a 680, Cnl a 685, Chl a 690 and Chl a 700-720. The number indicates their red absorption maxima of each of the spectral forms (French, 1966; Robinowitch and Govindjee, 1961). The strongly fluorescing short wavelength forms of Chl a are mainly present in PS II. The weakly fluorescing long wavelength forms are mostly present in PS I. In all the higher plants Chl b is present; however, it is absent in cyanobacteria.

CAROTENOIDS AND XANTHOPHYLLS

Almost all the photosynthetic organisms contain the yellow and orange pigments, called carotenoids and xanthophylls respectively, which act as accessory pigments in photosynthesis. The action spectra of photosynthesis demonstrates that light absorbed by carotenoids is utilised with varying degrees of efficiency in photosynthesis. The light energy absorbed by the carotenoids is not used directly but transferred to Chl a where it is efficiently used in the photosynthetic process (Clayton, 1962).

PHYCOBILINS

In cyanobacterial system, the PS II contain only small fraction of Chl a. The major light harvesting pigments are phycobilins. These pigments are assembled in granular aggregates (Phycobilisomes) that are bound to the cytoplasmic surface of the thylakoid. Phycobilisoms (PBsomes) act as light harvesting antenna in the way of the light harvesting chlorophyll protein complexes (LHCP) for photosystem reaction center in higher plant chloroplasts. Light energy trapped by PBsomes is channelled to photosystem II reaction center through a step wise radiation less energy transfer process known as resonance transfer.

The light absorbing components or chromphores of phycobilisomes are open chain tetrapyrroles called as

phycobilins. The phycobilins are covalently attached to apoprotein of phycobiliproteins. Four different tetrapyrroles are known to function as prosthetic groups of cyanobacterial biliproteins. Phycocyanobilin is chromphore of phycocyanin and Allophycocyanin. Phycoerythrobilin is the chromphore of phycoerythrin. Phycourobilin is the chromphore of R-Phycoerythrins. The native biliproteins absorption peaks in the 600-700 nm region were assigned to phycocyanobilin, between 540-570 nm to phycoerythrobilin and those at 495-500 nm to phycourobilin (Fig.6).

PHYCOBILIPROTEINS

Phycobiliproteins are essential accessory protein pigment complexes for harnessing the light energy in prokaryotic cyanobacteria and eukaryotic cryptomonads and rhodophytes. These chromoproteins have been subject to extreme conservation, presumably as a result of their role in energy transfer. Phycobiliproteins represent a considerable fraction (upto 50%) of the total cellular proteins of red algae and cyanobacteria. Phycobiliproteins account approximately 80-85% of the total phycobilisome proteins. The biliproteins are water soluble photosynthetic accessory pigments of which phycocyanin (λmax 615-620 nm), phycoerythrin (λmax 565, 540 nm), allophycocyanin (λmax 652 nm) are well documented (Fig. 6). Along with these, allophycocyanin B, C-phycoerythrin and R-phycoerythrin are also present in some cases.

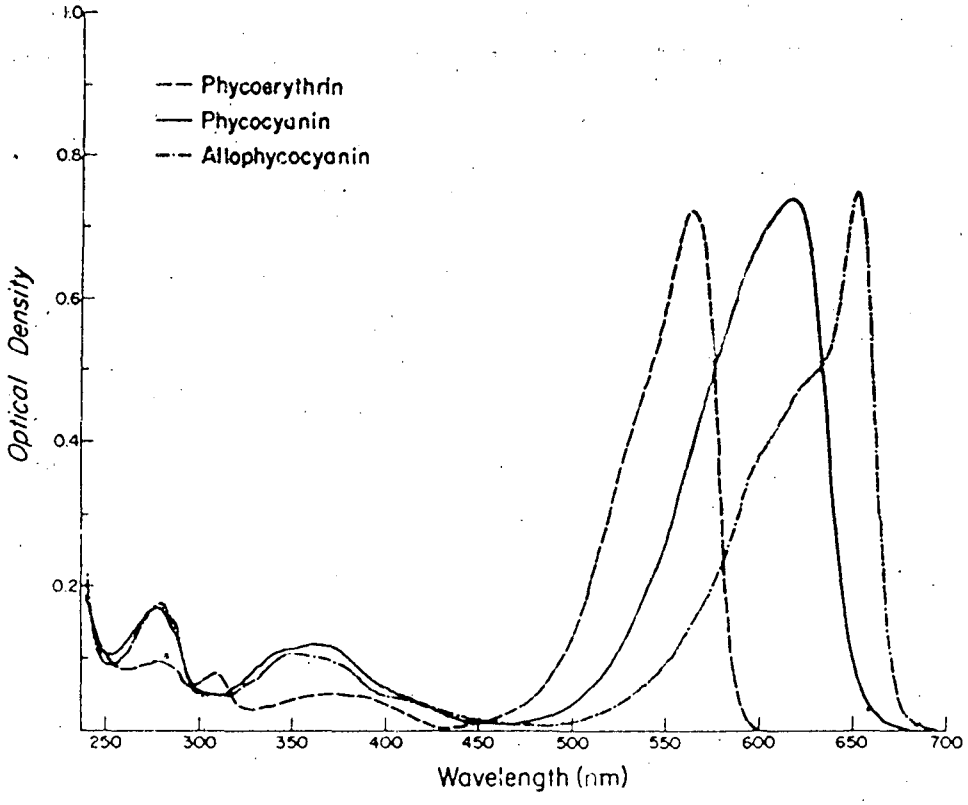

Fig. 6 Absorption spectra of purified billiproteins of *F. Diplosiphon* in 0.1 M sodium phosphate (P_H 7.0).
(From Bennett and Bogorad 1971).

PHYCOBILISOME

Phycobilisomes are supramolecular pigment aggregates that serve as primary light gathering antennae in red algae and in cyanobacteria. These aggregates, composed of phycobiliproteins, greatly extend the range in which light is absorbed and are particularly important under light limiting conditions (Larkum and Weyrauch , 1977). In fact, organisms can adapt to light limiting conditions by increasing total phycobiliprotein content (Ramus et al. 1976) or by specifically producing phycobiliprotein type capable of absorbing the prevalent available wavelengths. Functionally, PBsomes can be considered to be analogues to the light harvesting complexes containing Chl a and Chl b in green plants. Both these are light gathering aggregates which function in passing the trapped energy to the photosynthetic reaction centers in the photosynthetic membrane. However, they differ significantly in their structure and location. PBsomes are directly attached to photosynthetic membrane,but are not major membrane constituents, as are the chlorophyll light harvesting complexes. The light energy absorbed by PBsomes is mainly transferred to PS II. Their transfer to PSI occurs mainly through a spillover from Chl a to PSI Chla antenna (Ley and Butler, 1977). In addition, PBsomes also contain uncolored polypeptides which account for 10-15% of phycobilisomes protein. These colourless polypeptides are alleged to be involved in

complementary chromatic adaptation and hydrophobic attachment to maintain the molecular architecture and its bioenergetic flow in the phycobilisomes.

COMPOSITION

Phycobiliproteins are the primary constituents of PBsomes; they account for bulk of the total proteins (Tandeau and Cohen, 1977; Yamakana et al. 1980). Each phycobiliprotein is made up of polypeptides and usually occurring in equal amounts. α polypeptides are generally smaller than β polypeptides. These phycobiliproteins exist as oligomers with the trimer $(\alpha\beta)_3$ usually being the smallest stable aggregate. The next highest aggregate and basic building block of PBsome is a hexamer. α and β are dissimilar polypeptide chains of approximately 160-180 residues. It is interesting that amino acid sequence of phycobiliproteins is highly conserved (Gantt, 1977; Glazer, 1977; Frank et al. 1978).

The ordered interaction of biliproteins to form PBsomes is mediated by a group of polypeptides named linker polypeptides (Lundell et al. 1981). These uncoloured polypeptides are present in PBsome of cyanobacteria and range in molecular weight from 10 to 95 KDa. They play a significant role in correctly arranging phycobiliprotein hexamers in the PBsome and in conferring specific spectral characteristic to the hexamers.

Whereas biliproteins are all acidic and hydrophilic (Glazer, 1981) the linker polypeptides are basic and very hydrophobic (Lundell et al., 1981). Some properties of the quantitatively major biliproteins are summarized in Table 1.

MORPHOLOGY AND STRUCTURE

PBsomes are structures somewhat larger than ribosomes and vary in shape and size with the species. Most of the Cyanobacteria and red algae have PBsome that are broad, thin and thus said to be disc, or hemidiscoidal, in shape. PBsomes are judged to be intact by two criteria: a characteristic uniformity of size and shape and, more importantly, by their fluorescence emission (Gantt et al., 1979). The characteristic high fluorescence emission from APC, the terminal pigment in the transfer chain, occurs only when PBsomes are intact and energetically well coupled. (Gantt et al. 1976).

In fact PBsomes consist of two morphologically distinct substructures. There is a core consisting of either two or three cylindrical objects, 115 A^{o} in diameter and approximately 120 A^{o} high. The core (Fig.7) cylinders are made up of a stack of four discs 30 A^{o} thick (Zuber et al., 1986). Six rods, each composed of several stacked discs 60 A^{o} thick, and 120 A^{o} in diameter, radiate in a hemidiscoidal array from the sides of three cylinders. APC 670 lies inside core region and PC 620 lies further away in rod region and PEC lies on the periphery.

TABLE - 1 Structural and spectroscopic properties of phycobiliproteins.

Biliprotein	Molecular weight (KD)	Subunit	Chromophore			Visible absorption maxima (nm)	Fluorescence maxima(nm)
			α	β	γ		
Allophycocyanin B	(89)	$(\alpha\beta)_3$	1PCB	1PCB		671,618	680
Allophycocyanin	(104)	$(\alpha\beta)_3$	1-PCB	1PCB		650	660
C-Phycocyanin	(224)	$(\alpha\beta)_6$	1PCB	1PCB		620	637
Phycoerythrin	(104)	$(\alpha\beta)_5$	1-PBV	2PCB		568,600	607
C-Phycoerythrin	(226)	$(\alpha\beta)_6$	2PEB	4PEB		565	577
R-Phycoerythrin	-	$(\alpha\beta)_6$	2PEB	3PEB	1-PEB	567,538,498	578
				1-PUB	1-PUB		

Chromophore types: PCB: Phycocyanobilin; PEB: Phycoerythrobilin; PUB: Phycourobilin; PBV: Phycobiliviolin.

(from Glazer, 1981).

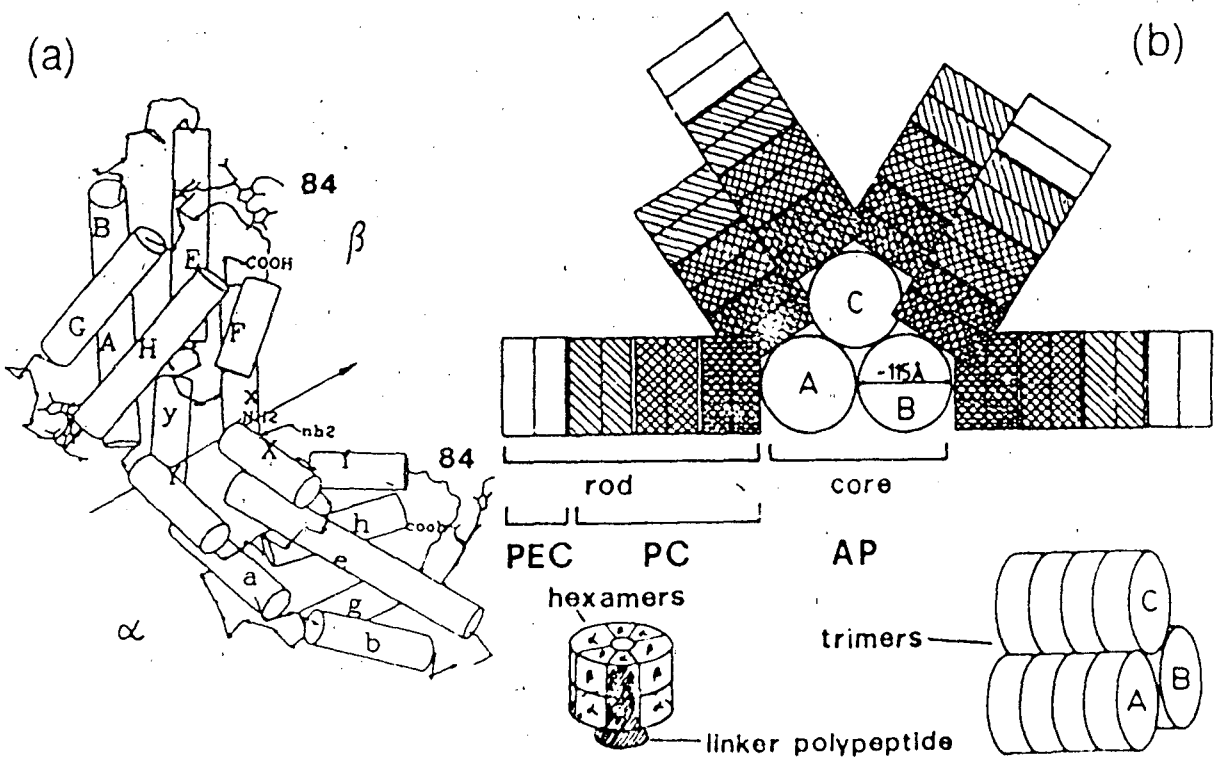

Fig. 7 (a) Three dimensional structure and arrangement of the α -helices (X,Y,A,B,E,F,G,H,) in the (a -β) monomer of C -Phycocyanin from *Mastigo cladus laminosus* (b) Structure and organisation of the phycobillisome of M. laminosus and arrangement of the phycocyanin (PC) and phycoerythrin (PE) hexamers in the rod region and of allophycocyanin (APC) trimers in the core region. The linker polypetides are located in the Central Cavity of the heamers.

(From Zuber 1986)

ENERGY TRANSFER

Phycobiliprotein aggregates are particularly suited for efficient energy transfer. Energy transfer in phycobilisomes is expected to progress from PE to PC to APC and eventually to chlorophyll (Fig. 8).

The PBsomes are the ultimate aggregate and exist as one coherent unit. PBsome can be excited through any of their component pigments with the result that energy is transferred to APC and PS II. Energy migration by inductive resonance has been assumed to be the mechanism of transfer. It is based on the photophysiological properties of the phycobiliproteins (Grabowski and Gantt 1978), particularly on their ovrelapping PE, PC, APC absorption and emission spectra and sufficiently long life times to make the tranfer possible. Energy absorbed by Phycobiliproteins can be transferred to chlorophyll with a high degree of efficiency, 80 to 90%.

CYANOBACTERIAL ELECTRON TRANFER

Cyanobacteria, like higher plants, have photosystems linked in series; they photolyse water and generate oxygen. Chorophyll *a* is the sole type of chlorophyll present. It is the principal light-harvesting pigment of both photosystems. It is present as a membrane bound chlorophyll-protein complex. The accessory pigments are of two types: Phycobiliproteins and carotenoids.

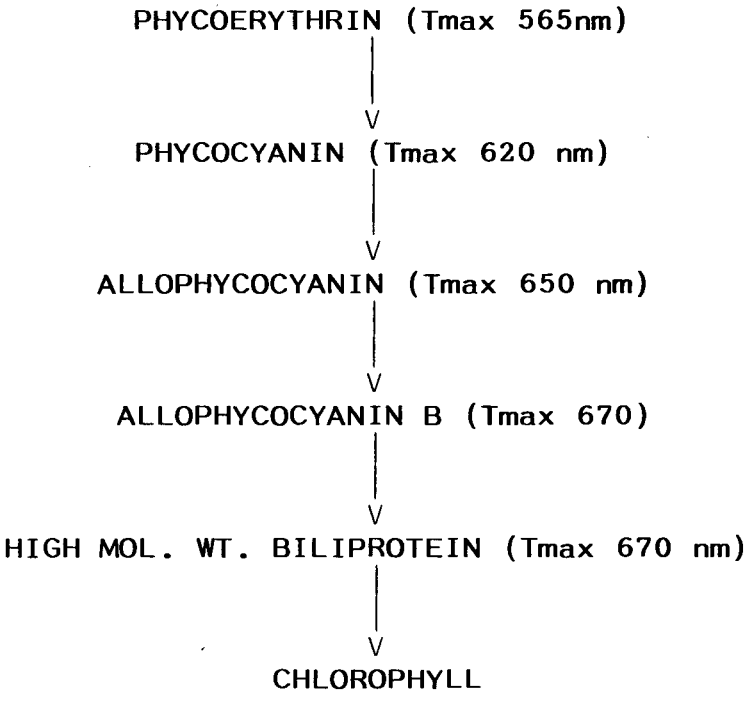

Fig 8. Energy Transfer sequence assumed to occur in Phycobilisomes when they are attached to thylakoids.

The phycobiliproteins are water soluble proteins attached to the outside of the thylakoid membranes that harvests light energy which is transferred mainly to the PS II reaction center. Various carotenoids are located in the thylakoid membranes of cyanobacteria, harvesting light energy which is then transferred to the PS II reaction center. They serve to protect against photooxidative damage. According to Sandmann et al. (1984) there is an inter-action between respiratory and photosynthetic electron transport in cyanobacteria.

HEAT EFFECT

Temperature is considered to be the most important ecological factor that determines the natural distribution and agronomic productivity of plants. The plants are often subjected to wide seasonal variation in temperature regime and even to diurnal fluctuations in temperature. Along with high light intensity and/or water deficit condition, the elevated temperature is capable of causing damage to plant growth leading to reduced crop productivity. The sensitivity of photosynthesis to elevated temperatures is well known (Berry and Bjorkman, 1980; Quinn and Williams, 1985; Yordanov et al., 1986; Mohanty et al. 1987; Mohanty and Mohanty, 1988).

NON-STOMATAL INHIBITION OF PHOTOSYNTHESIS

There have been several attempts to understand the elevated temperature-induced loss of photosynthetic function. The

importance of such studies was realized with the observation that complete inhibition of photosynthesis occurs before any inhibition of dark respiration or other symptoms of high temperature injury could be detected in leaf tissue (Bjorkman, 1975). The heat inhibition of net photosynthesis is not due to stomatal closure nor it is because of an increased resistance to intercellular carbondioxide transport (Bjorkman, 1975; Bjorkman et al., 1976). Heat induced loss of semi-permeability of the plasma membrane, and subcellular membranes occurs at a temperature significantly higher than that causing inhibition of photosynthesis (Berry et al. 1975). All these observations exclude factors other than chloroplast as the site of heat inactivation of photosynthesis. Furthermore, the heat induced loss of CO_2 fixation, O_2 evolution, and chlorophyll a flourescence changes, observed in intact leaves, were comparable with the loss observed in the function of chloroplasts isolated from heat treated leaves (Berry and Bjorkman, 1980). It is thus clear that heat inactivation of photosynthesis primarily reflects a direct effect of heat on chloroplast function in leaves.

TEMPERATURE DEPENDENT CHANGES IN PHOTOELECTRON TRANSPORT

In the photosynthetic system (i.e. chloroplasts or thylakoid membranes) a correlation can be seen between the transition of the phase of membrane lipids and that of photosynthetic

activities. Holton et al. (1964) suggested that the transition of the phase of lipid may occur at room temperature and that the fatty acid content changes depend upon the growth temperatures: higher the growth temperature, higher the content of saturated fatty acids.

The molecular order of the lipid matrix of the thylakoid membrane regulates various photosynthetic processes (Quinn and Willium, 1978). Murata and coworkers, working mainly with Anacystis have demonstrated a correlation between the temperatures at which discontinuities occur in the Arrhenius plots (Murata et al. 1975) for photosynthetic processes including O_2 evolution, P-700 reduction and Hill activity (Ono and Murata, 1979) as well as with the temperature dependence of physical parameters such as Chl a fluorescence (Murata et al., 1975; Murata and Fork, 1975), delayed light emission (Jursinic and Govindjee, 1977, Mohanty and Mohanty, 1988) and electrochromic band shifts (Cno and Murata, 1977). The break points in the Arrhenious plot for the state shift as well as for the cytochrome reduction in thermophilic blue green algae, Synechococcus lividus were obtained by temperature dependent Chl a fluorescence techniques (Nolan and Smillie, 1976, 1977) signifying the influence of growth temperature on membrane linked functions of the blue green algae.

SENSITIVITY OF THYLAKOID ACTIVITY TO HIGH TEMPERATURE

Heat induced inhibition of thykaloid membrane photofunction and/or activity of carbon cycle enzymes, may account for the observed loss of photosynthesis. The CO_2 fixng enzymes are shown to be more heat tolerant than the whole leaf photosynthesis (Tieszen and Sigurdson, 1973; Bjorkman et al., 1976). The thylakoids are shown to be more heat sensitive than other subcellular membranes (Thebud and Santarius, 1982)

HIGH TEMPERATURE DECREASES PS II ACTIVITY

In comparison to thylakoid photofunction, the activity of carbon cycle enzymes is observed to be more heat stable (Berry and Bjorkman 1980). This suggests that heat induced loss of net photosynthesis mostly originates from the heat induced inactivation of thylakoid photofunction. The studies in both *in vivo* i.e. chloroplasts isolated from heat treated leaves (Berry et al. 1975; Bjorkman et al. 1976, 1978; Fork et al. 1985; Mohanty et al. 1987, Mohanty and Mohanty, 1988) and *in vitro* i.e. heating of isolated chloroplasts (Katoh and San-Pietro, 1967: Armond et al. 1978; Gounaris et al. 1983: Sabat et al. 1986),have revealed the heat sensitivity of PS II driven O_2 evolution activity and non cyclic photophosphorylation.

AFFECT OF Mn

Heat induced loss of the capacity for O_2 evolution is attributed to the release of manganese associated with OEC (Miller and Cox, 1984; Nash et al. 1985). Heating is also reported to cause Cl^- depletion from thylakoid (Krishnan and Mohanty, 1984; Nash et al. 1985; Critchley and Chopra 1988). The extrinsic polypeptides (32, 24 and kDa) of OEC (Volger and Santrius, 1981; Nash et al., 1985) are also known to be released partially due to heat treatment.

HIGH TEMPERATURE ENHANCES PS I ACTIVITY

In comparison to PS II catalysed electron transport activity, the PS I mediated electron transport was observed to be heat stable (Berry and Bjorkman, 1980). Recently it has been established that heat induces a significant enhancement in PS I photochemistry (Quinn and Williams, 1985). The heat effect on the PS I photochemistry, measured by polarographic or ESR studies, was observed to be greatly stimulated (Armond et al., 1978; Weis, 1983, 1984, 1985; Sane et al. 1984; Sabat et al., 1986; Thomas et al. 1986; Velitchkova et al., 1988; Sabat and Mohanty, 1989). The extent of stimulation of heat induced PS I electron activity is found to be donor specific. Maximum stimulation is attended by reduced DCPIP as exogenous electron donor than by reduced-TMPD or DAD (Sabat and Mohanty, 1989).

HIGH TEMPERATURE EFFECTS ON CHLOROPHYLL FLUORESCENCE YIELD AND EMISSION

Temperature affects the Chl a fluorescence yield and emission both in chloroplasts (Goedheer, 1970) as well as in algal system (Singhal et al. 1981). When PS II dependent O_2 evolution is inactivated by heat treatment, Chl a fluorescence of PS II decreased, there is an increase in PS I (Dowton and Berry 1982). Thus, studies on temperature dependent changes of photosynthetic parameters provide valuable insight into the nature of electron transport carriers and mechanisms alongwith energy transport and establishes a correlationship between structure and function of the membranes.

RECOVERY OF PHOTOFUNCTION FROM HEAT DAMAGE

The possible recovery of the thylakoid photofunction from heat damage depends on the severity of heat stress, and on the intrinsic genetic ability of plant species to withstand the heat damage. Bauer and Senser, (1979) in ivy leaves, Yordanov et al. (1975) in spinach leaves, weis et al. (1986) in liver wort thalli and Mohanty et al. (1987) in wheat have studied the nature of thylakoid photofunctions after the heat stress. It is reported that the recovery of photosystem photochemistry from heat damage may take place in days (Bauer and Senser, 1979; Weis et al., 1986) or in a time range of hours depending on the severity of

heat stress. Thus the observed alterations in *in vitro* situation is expected to be physiological one.

AFFECT OF HIGH TEMPERATURE ON THE FORMATION AND ACTIVITY OF PHOTOSYNTHETIC APPARATUS

Elevated temperature inhibits Chl accumulation (Onowueme and Lawanson, 1973; Feierabend and Mikus, 1977; Smillie, et al. 1978), and development of 70 S plastid ribosome. High temperature alters chloroplast development by affecting biosynthesis of Chl and formation of normal grana, and even development of PS II photoactivity (Fierabend and Mikus, 1977: Smillie et al. 1978). Onowueme and Lawanson (1973) have studied heat effect in etiolated seedlings during subsequent greening, and found retarding effect of high temperature on Chl accumulation (Mohanty et al. 1987; Mohanty and Mohanty, 1988).

EFFECT OF TEMPERATURE ON BILIPROCTEINS AND PHYCOBILISOMES

The amount of biliprotein, the major light harvisting pigment protein complex in cyanobacterial cells, is affected by a variety of parameters. The most abundantly affected pigment content in blue green alage was found to be phycocyanin. Its concentration fluctuates with temperature (Halldal et al. 1957; Ono and Murata, 1979; Singhal et al. 1981) with CO_2 concentration (Eley, 1971) with light intensity (Halldal, 1958,

1970) and mostly with wavelength of light (Ghosh & Govindjee, 1966; Jones & Myers, 1965; Myers and Kratz, 1955, Oquist, 1974).

Myers and Kratz (1955) first demonstrated the change in pigmentation of both chlorophyll a and for the primary accessory pigment PC, with no significant change in the chlorophyll phycocyanin ratio. A temperature of 40-45^{o} supported good growth when the cells were grown on agar (Halldal et al. 1957). Halldal (1958) found that in Anacystis, in contrast to Anabaena, high temperature (40-45^{o}C) and high illuminances (6 to 8k) favoured the production of phycocyanin, while this pigment was less abundant at both, lower temperatures and illuminance.

Eley (1971) showed that ample CO_2 to cope with the capacity of growth is an essential requirement for cultures at both high radiances and high temperatures.

Goedheer (1976) reported that only at high radiance flux densities (50-100 Wm^{-2}) colour and spectra depended strongly on high temperature. He further proved that only in a limited range of environmental conditons growth is fast and the spectral properties are similar to those observed when the cells are grown at lower irradiance. At low radiant flux densities(0.1 to 1 Wm^{-2}) the colour of the culture was found to be always blue green (Goedheer, 1976). The ratio between PC and Chl absorption was 1.2 to 1.4. No marked differences in PC/Chl ratio were detected

between cells grown at 20 or 37°C. Cells at high temperature did not show difference in PC/Chl ratio though their growth was very slow (Goedheer, 1976).

In Synechococcus 6301, high levels of phycocyanin are obtained under conditions of high temperature, light intensity and CO_2 concentration (Eley 1971; Goedheer, 1976; Oquist, 1974). Such fluctuations are brought about by two mechanisms: change in number of phycobilisome and change in their composition (Lau, et al., 1977; Yamanaka and Glazer, 1980). Conditions favouring increasing PC synthesis resulted in the production of phycobilisomes with longer rod structure but unaltered core and with reversible effects (Yamanaka and Glazer, 1980)

Phycobiliprotein content, as measured spectrophotometrically, is indeed affected by (i) light quality (Bennett and Bogoarad 1973, Bogorad 1975); (ii) light quantity (Jones and Myers, 1965) and (iii) the availability of CO_2, phosphate and nitrate (Allen & Smith, 1969). However,spetrophotometric determinations reflect only intact (chromophore-containing) PC content and do not differentiate changes effected at the level of apoprotein synthesis from those effected at the levels of protein turnover or chromophore attachment.

In other sets of experiments, where affect of short period of heat treatment (preheating) was noticed, it was found that

absorption spectra was stable upto a preheating (10 min), temperature of 55°C in Anacystis (Singhal et al., 1981). However, preheating at 60°C caused a lose of PC absorbance. No shift of blue band was observed. In Anacystis and Porphyridium (Singhal et al.. 1981), preheating caused a lose of absorption, energy transfer from the remaining phycobilins to chl a of pigment system II.

Usually proteins are denatured at 45°C but preheating at 45° C for 10-16 min. did not exhibit any alternation in the absorption characteristics. Thus it seems that membrane bound pigment protein complexes are more stable to heatnig and require higher temperature to show considerable effects.

Fluorescence emission spectra measured at room temperature show that the effect of environmental conditions, though marked in the absorption spectra, is weak in fluorescence spectra.

Although many of the temperature induced photosynthesis processes have been studied in isolated, broken or intact chloroplasts, only a few studies have been carried out with cynobacterial photosynthetic membranes. BGA (cyanobacterial) cells and their photosynthetic membranes constitute interesting materials for the study of photoelectron transport and attendent energy linked processes of photosynthesis. The present study aims at making comparative studies on the effect of various

temperatures on growth parameters of BGA. BGA can be adapted and acclimatised to a variety of growth and nutritional conditions. Present work aims at studying BGA cells grown at different elevated ($40^{o}C$) temperatures that are known to alter physiochemical properties of pigments. The objectives of the present work in particular is to characterise the effect of various temperatures on PC, proteins and PBsomes.

EXPERIMENTAL MATERIALS AND METHODS

Phormidium foveolarum is a trichomatous, filmentous, non nitrogen fixing cyanobacterium. Filament is thin, dark, green, constricted at the cross walls 1.5 uM broad and without attenuated ends. Cells are quardate or somewhat shorter than broad, 0.8 to 1.8 um long with rounded end wall.

The mother culture was prepared after testing for its purity by microscopic examination.

Source of Supply of Slants

Slants of Phormidium fovelarum were obtained from Department of Biological Sciences, Rani Durgawati Vishwvidhyalay, Jabalpur (Madhya Pradesh), India. Sterilised slants were brought to Jawaharlal Nehru University in light conditions and immediately transferred to liquid culture.

Culture conditions

The cells were tranferred from an agar slant to liquid broth. The composition of the liquid broth, used in the present study to culture Phormidium, is given below:

Macronutrients	Quantity g/l
K_2HPO_4	4.0
$MgSO_4.7H_2O$	7.5
$CaCl_2.2H_2O$	3.6
Citric acid	0.6
EDTA	0.1
Na_2CO_3	2.0
$NaNO_3$	150.0
Ferric ammonium citrate	0.6

A_5 solution, 1 ml/l solution(stock)

Micronutriets	Quantity g/l
H_3BO_3	2.86
$MnCl_24H_2O$	1.81
$ZnSO_47H_2O$	0.222
$Na_2MoO_4.2H_2O$	0.079
$Co(NO)_3.6H_2O$	0.0494

CULTURE VESSELS AND STERILIZATION

Superior corning glass 500 ml conical flasks were used for the growth and maintenance of cultures. All solutions were prepared separately, autoclaved at 15 lb/inch^2 pressure for 15 min. The pipettes used for the transfer of cultures, were also autoclaved in a pipette container by covering them with tin foil. All above solutions were mixed after allowing the autoclaved

solutions to reach room temperature under sterile conditions in laminar flow.

Inoculation and maintenance of culture

Inoculation of cells into sterile medium was done at room temperature under sterile conditions in laminar flow.

Stock cultures were maintained in a culture room continuously illuminated by fluorescent tubes (18 Wm^{-2}) at $27\pm1^{o}C$.

Stock cultures at higher temperature i.e. $35^{o}C$ and $40^{o}C$ were maintained in algae growth chamber specially built for this purpose.

All the culture flasks were continuously bubbled with air by aspirators with the circulation of tap water. The cultures were maintained in a bacteria free state by regularly transferring the exponentially growing cultures to fresh sterile medium. Cultures were microscopically examined from time to time to check contamination.

ALGAE GROWTH CHAMBER

Algae growth chamber was fabricated by Atlantis India, with automatic temperature control and regulated airation.

ADAPTATION TO HIGH TEMPERATURE

Cultures were grown at higher temperatures, upto 40°C, by increasing the temperature of algae growth chamber gradually. An enhancement of 2°C was made in one instance.

EXTRACTION AND ESTIMATION OF CHLOROPHYLL a

The Phormidium cells were collected on a Whatman No.1 filter paper placed on a sinctered glass filter which was connected with a vaccum oil pump. The harvested cells were tranferred to harvesting buffer consisting of 7.5% PEG-4000, 1 mM $CC1_2.2H_2O$ and 20 mM HEPES-NaOH (pH 7.5). After transferring to harvesting buffer the cell suspension was centrifuged at 9000 g for 10 min at 10°C. The cells were resuspended in 2 ml of reaction buffer consisting of 25 mM HEPES-NaOH (pH 7.5), 20 mM NACl, 50 ul of cells were pipetted into a clean test tube and 2.95 ml of 100% methanol was added and kept a 55°C for 10 min. The extract was taken and centrifuged by using a table top Remi centrifuge for 10 min at 5,000 rpm. The supernatant was taken for Chl a estimation. The optimal density of Chl a was measured at 665 nm with methanol as blank, using Shimadzu UV-260 dual beam spectrophotometer. Chl a amount was calculated using the extinction coefficient $E_{665} = 74.5\ m^{-1}\ cm^{-1}$ (Mackinney, 1941). Triplicate sets were always maintained.

ASSAYS OF ELECTRON TRANSPORT IN INTACT CELLS

Assays of electron transport activities were carried out using the thermostated 27 $\pm$ 1^{o}C glass cuvette, fitted within a clark-type O_2 electrode (Yellow Spring Instrument Co., USA).

Mid log phase (8 d) culture was harvested by vaccum filtration and washed once with harvested buffer, centrifuged at 9000 g for 10 min. The pellets were resuspended in minimum volume of the reaction buffer. Three ml of reaction mixture consisted of 25 mM HEPES-NaOH (pH 7.5) 20 mM NaCl and intact cells equivalent to 12 to 15 ug Chl *a*. The cells were illuminated with white saturating light (480 Wm^{-2}) at the surface of the vessel obtained from a projector. To maintain the temperature (25^{o}C) of the reaction mixture constant during the illumination, thermostat D water bath (MLW), the assay mixture was continuously stirred during measurement. The changes in the oxygen concentration were recorded by using a LKB Bromma 2210 Stripchart recorder.

PHOTOSYSTEM II ASSAY

Parabenzoquinone (pBQ) mediated electron transport of PS II (H_2O -> pBQ was used with intact cells. Being a lipophilic compound pBQ enters into the intact cells and accepts electrons at plastoquinone (PQ) position (Trebst, 1974). The reaction mixture contained reaction buffer (same as used in cell

harvesting) 0.5 mM $_{p}$BQ and the intact cells equivalent to 12 to 15 ug Chl a.

ESTIMATION OF PHYCOCYANIN

Five ml of the cell suspension was drawn after thoroughly shaking the flask and the cells were collected by centrifuging at 5000 rpm for 10 min. at room temperature. The pellets were washed with 5 ml of saline. The washed pellets were resuspended in five ml. of saline and sonicated for 5 min., with half min. interval, at 12 PS I using MSE sonicator. The broken fragments were centrifuged at 5000 rpm for 15 min. at room temperature. The supernatant was taken for phycocyanin (PC) estimation. The optical density was measured at 624 nm, with saline as blank, using Shimadzu UV 260 dual beam spectrophotometer. Phycocyanin amount was calculated by using the estinction coefficient, E_{622} = 7.3 ml^{-1} cm^{-1} (Gantt et al., 1979).

ISOLATION OF PHYCOBILISOMES

Phycobilisomes were isolated according to Gantt et al (1979). Cells were harvested by vaccum filtration through a Buchner funnel using a Whatman No.1 filter paper and wahed twice in 10 mM potassium-phosphate buffer pH 8.0. Cells, 0.8 ugm by wet weight, were suspended in 10 ml of 750 mM KPO_4 buffer, pH 7.0. Cells were disrupted by sonication for five min., with half min. interval, and incubated with 2% Triton X-1000 for 40 minutes at

$20^{o}C$ to release phycobilisomes from thylakoid membranes. The homogenate was centrifuged at 35,000 g for 30 minutes at $20^{o}C$ in a Sorvall RC-5 centrifuge.

The supernatant were layered on to sucrose step gradient consisting of 1.9, 0.8 and 1.5 ml of 2.0, 1.0, 0.5, 0.25 M sucrose solution respectively, all in 750 mM potassium phosphate buffer (pH 7.0).

Centrifugation was done in Beckman preparative Ultra-centrifuge with SW 50.1 rotor at 140,000 g for four hours at $20^{o}c$. The phycobilisomes recovered as deep blue band were used for spectroscopic measurements. The phycobilisomes were diluted appropriatory in 750 mM phosphate buffer, pH 7.0 and scanned in a wavelength range of 600-700 nm.

ESTIMATION OF TOTAL PROTEIN CONTENT

The total cell protein content was determined with folin-ciocalteu reagent according to Lowry et al. (1951). The reagents used were as follows:

1. 2% Sodium carbonate in 0.1 N NaO_2.
2. 0.5% Copper sulphate in 1% potassium sodium tartrate.
3. To 50 ml of region 1 ml of reagent of 2 is added.
4. Dilute folin-ciocalteu reagent (1:1 vol/vol)

Five ml of cell suspension was drawn after thoroughly shaking the flask and the cells were collected by centrifugation, at 50-00 rpm for 10 min at room temperature. The pellets were washed with 5 ml of saline.

The washed pellets were suspended in 5 ml of 10% TCA and were allowed to stay at 20^{o}C for overnight then they were centrifuged at 9000 g for 15 min. The pellet was suspended in 2 ml of 1 N NaOH and were thoroughly mixed. To 50 ul of test solution 0.95 ml of 1N NaOH was added. To this 1 ml of protein solution 5 ml of alkaline copper sulphate solution was added and allowed to stand for 10 min. at room temperature. To this solution 0.5 ml of diluted folin ciocalteau reagent was added rapidly and thoroughly mixed and allowed to stand for 30 min for full colour development. The optical density was measured at 750 nm in Shimadzu UV-260 spectrophotometer against a reagent blank. In blank 1 ml of distilled water was used instead of protein solution. A set of standard BSA solution (10-100 ug.) was taken for preparation of standard curve and this was used to estimate the amount of unknown protein in the sample.

ABSORPTION SPECTRA OF INTACT CELLS

After harvesting the cells, the pellets were suspended in 3 ml of reaction buffer. The reaction mixture contained reaction buffer 25 mM HEPES-NaOH (pH 7.5) and intact cells equivalent to 10-15 ug Chl. The reaction mixture in the vial was stirred

continuously in dark for different time intervals (2 to 10 min). This cell suspension was taken for scanning the absorption spectra from 400 nm to 750 nm. The absorption spectra of intact cell suspension were taken by using Shimadzu 260 a double beam spectrophotometer. At 750 nm the absorption of cell suspension was adjusted to give approximately the same absorbance reading.

EMISSION SPECTRA OF INTACT CELLS

Sample preparation was done in the same way as described above. The reaction mixture contained reaction buffer and the intact cells equivalent to 12 to 15 ug Chl *a*. The reaction mixture was stirred continuously in dark for five min.

In intact cells the phycocyaninwas excited at 545 nm to take the emission spectra of intact cells. The emission spectra were scanned from 600 nm to 750 nm in Shimadzu (RF-540) Spectro-fluorophotometer.

CHEMICALS

HEPES, methyl viologen, parabenzoquinone, Triton-X were obtained from Sigma Chemicals Company, USA. All chemicals used were of analytical grade and were used without further purification.

RESULTS

In the present study, we have investigated the effect of temperature on the growth parameters of Phormidium fovelarum. In one set, cells were grown at higher temperature to observe heat effect (Part I) and in the other, heat treatment at different temperatures for various time periods was given after seven days of normal growth (Part II). Following heat treatment (Part II) the cultures were allowed to grow at normal temperature ($27^{o}C$).

PART I

GROWTH CHARACTERISTICS

For this study sterilised flasks after proper inoculation were kept at higher temperature $35^{o}C$ and $40^{o}C$) in algal water bath along with flasks at room temperature ($27^{o}C$). Both treated and control cells were provided with identical light intensity (18 Wm^{-2}). Fig. 9 shows the growth curves of control and treated cells monitored for 21 days at prescribed physical and chemical conditions. The scattering characteristic, which otherwise represents the relative growth of cells, was considered as growth measuring parameter of the cells. The scattering was measured at 550 nm. No appreciable change on lag phase was seen at higher temperature (Fig.9). The results indicated that at elevated growth temperature cells grew significantly. Cells at $35^{o}C$

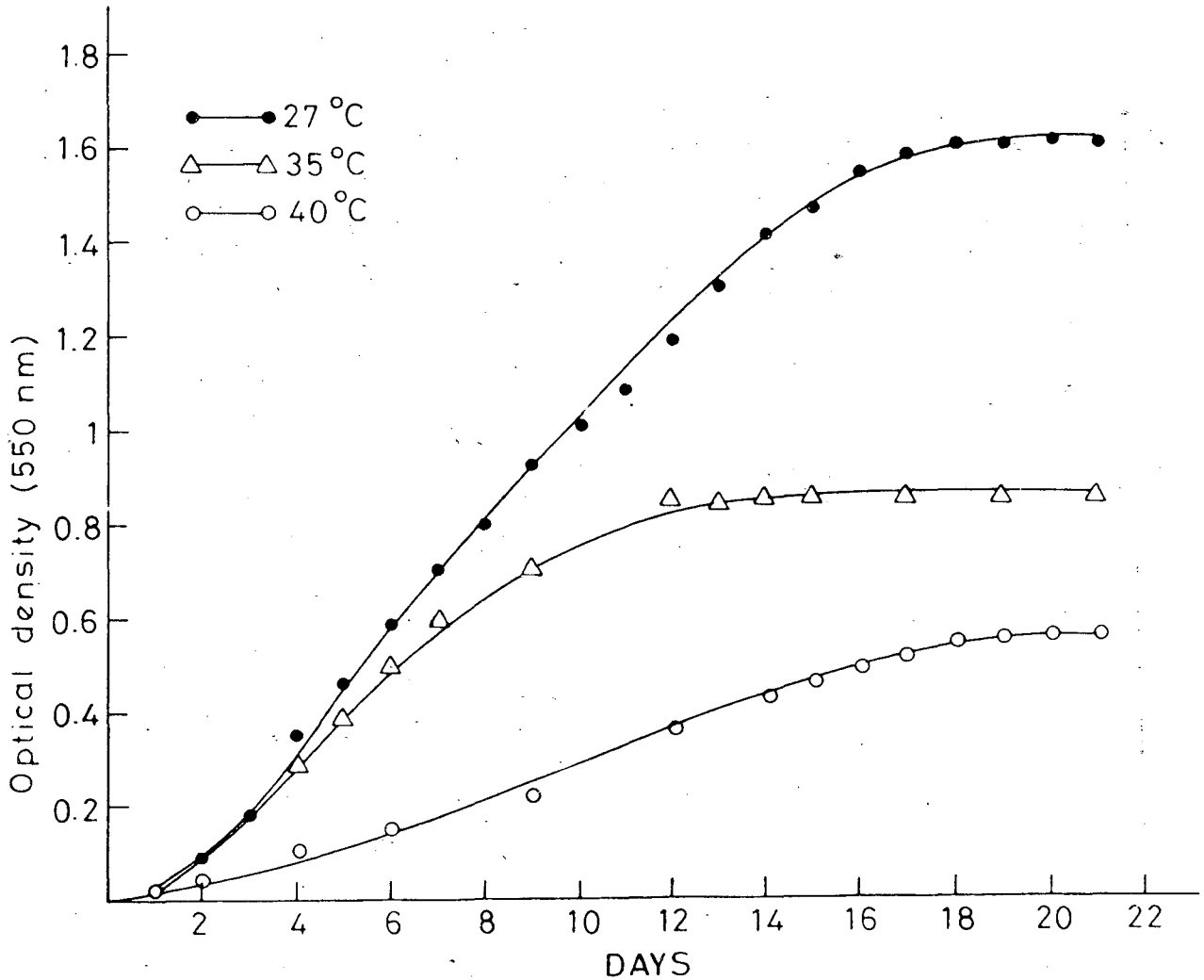

Fig. 9. Growth pattern of *Phormidium fovelarum* at different temperatures. Growth was expressed as an optical density at 550 nm and measured for a period of 21 days at a time interval of 24 hours.

although show a similar growth rate during early lag phase, however cease to grow much early than the control.

Unlike the 35°C grown cells, the cells at 40°C always maintained a low growth profile. Further, log phase declined from 11 days in control to six days at 35°C and to three days at 40°C. Cells did not recover after temperature treatment although there was not much difference during initial days of the growth.

Total protein content and dry weight of cell biomass has direct relation with cell growth. In Fig.10 and Fig.11, the log phase, which is 11 days in control cells, decreases to 4 days for cells grown at 35°C. Cells grown at 40°C shows very short log phase but long stationary phase.

Chlorophyll was extracted and measured as per procedure given in material and methods. Interestingly, similar to the growth parameters, there was also a decrease in chlorophyll a content (Fig. 12) at high temperature. Log phase, which was 14 days in cells grown at normal temperature, decreased to 3 days for cells grown at 35°C. At 40°C rate of chlorophyll synthesis becomes very slow.

Phycocyanin concentration was also affected as a result of heat treatment (Fig.13). Phycocyanin concentration shows same pattern as chlorophyll content. A loss of about 50% phycocyanin concentraion occurs when cells were gown at 35°C. This loss further increased to 67% at 40°C.

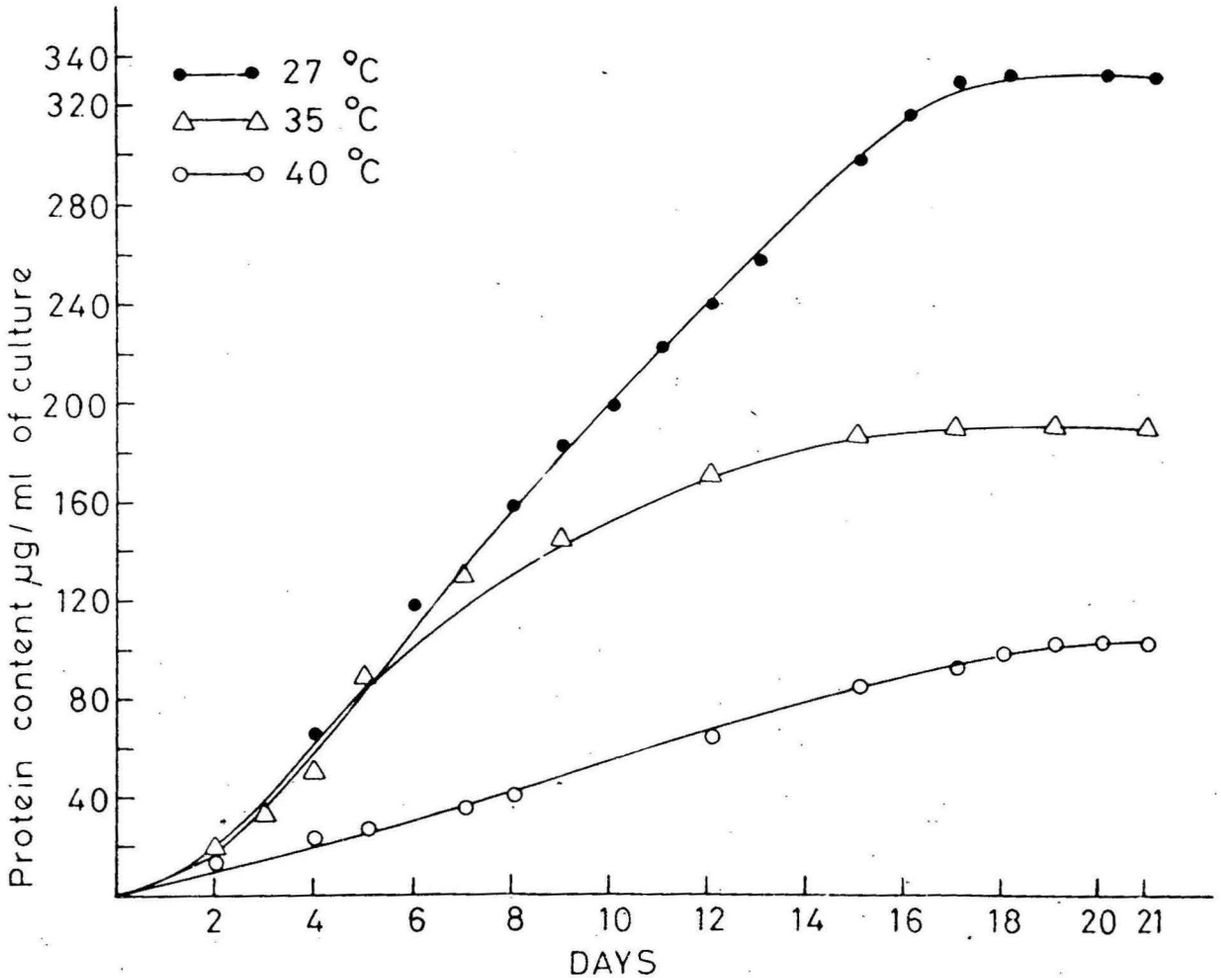

Fig. 10. Effect of temperature on the total cell protein of *Phormidium fovelarum*.

The total protein content was estimated by Lowry's method (Lowry et.al 1951) in control and treated cells.

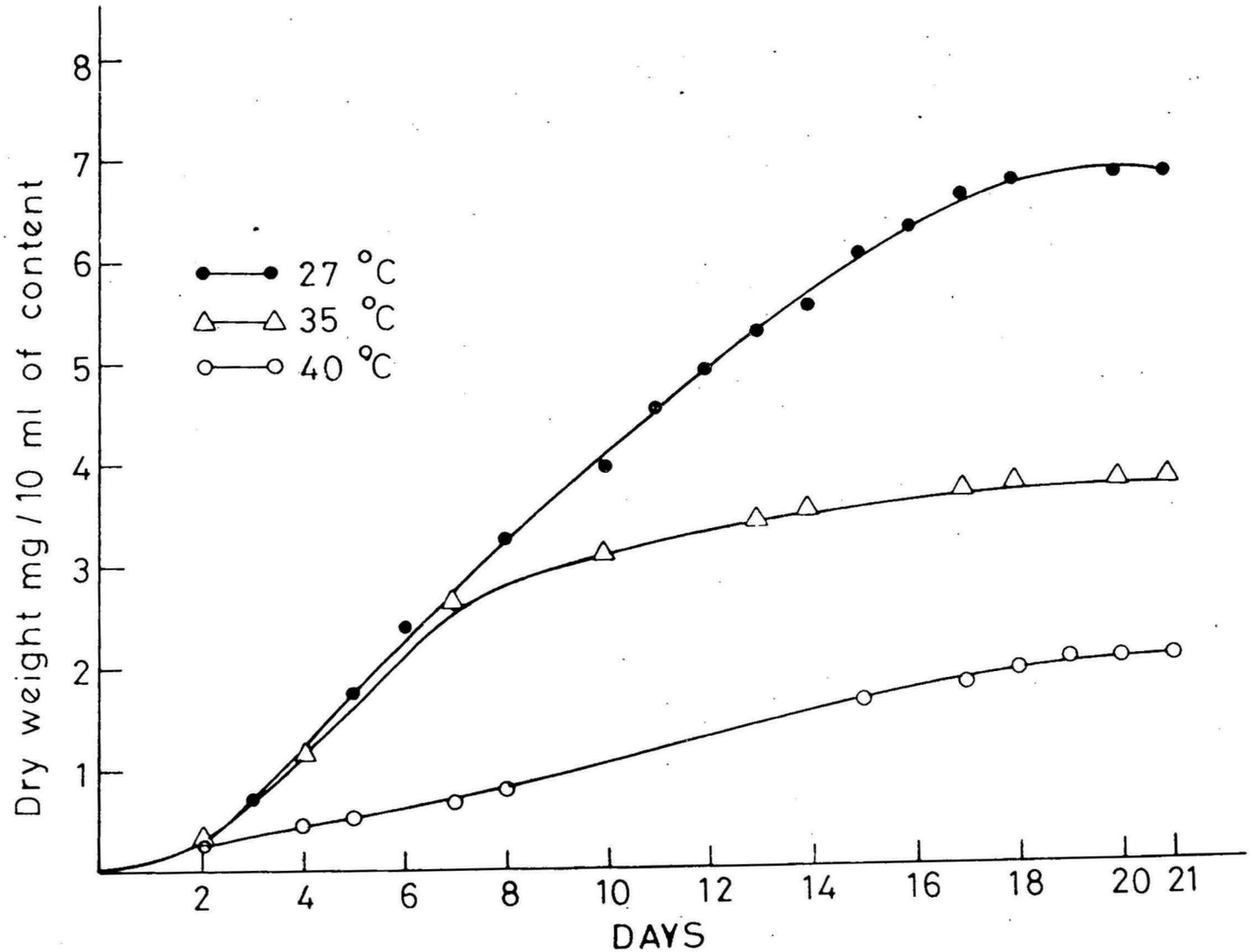

Fig. 11. Effect of temperature on dry weight of cells.
Equal volumes of cell suspensions were harvested, dried, and weighed at regular intervals.

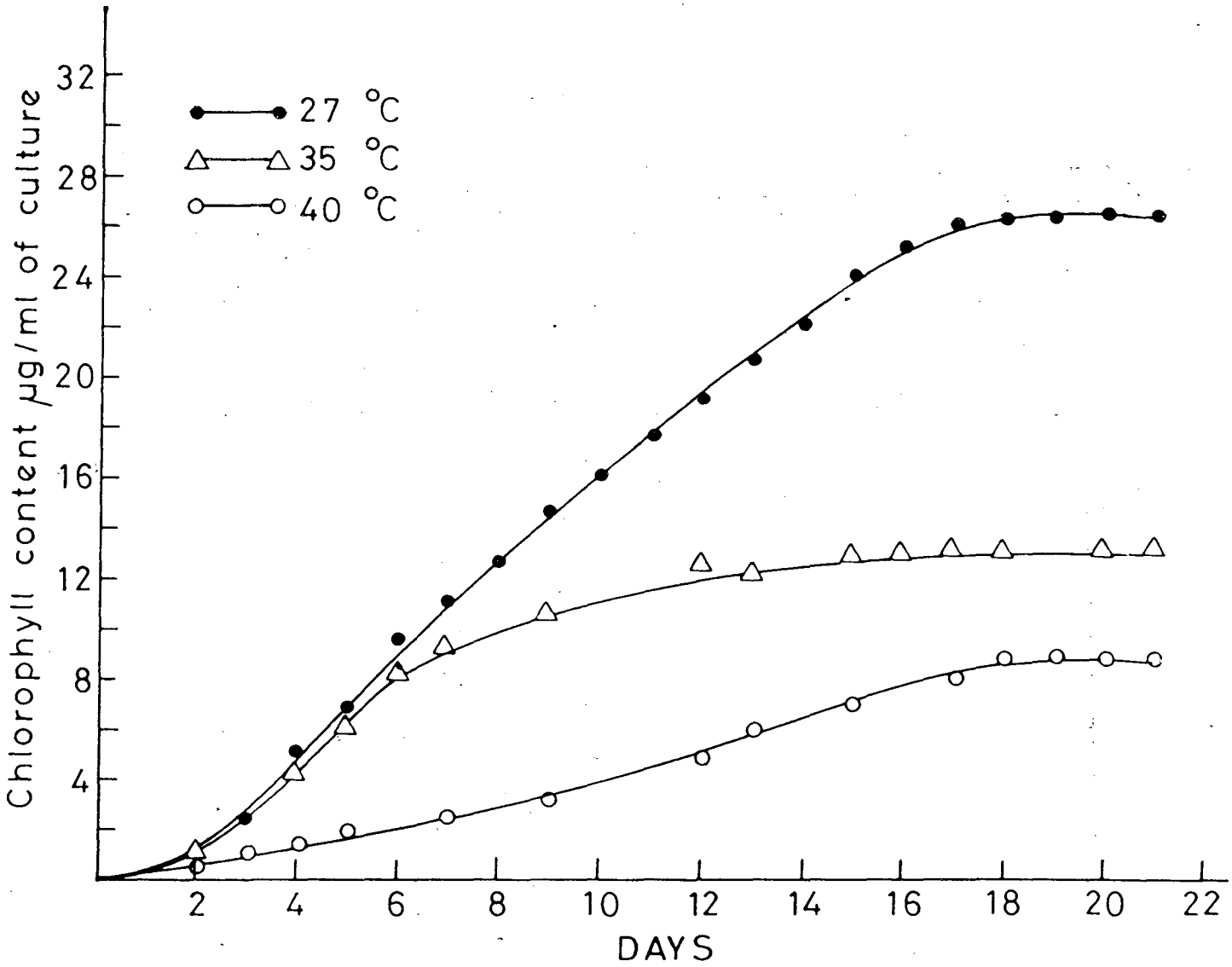

Fig. 12. Effect of temperature on Chlorophyll a contents. Cells were harvested by centrifugation, washed in HEPES - NaOH (p_H 7.5) buffer and later suspended in methanol. The chlorophyll a was quantified as described in Materials and Methods.

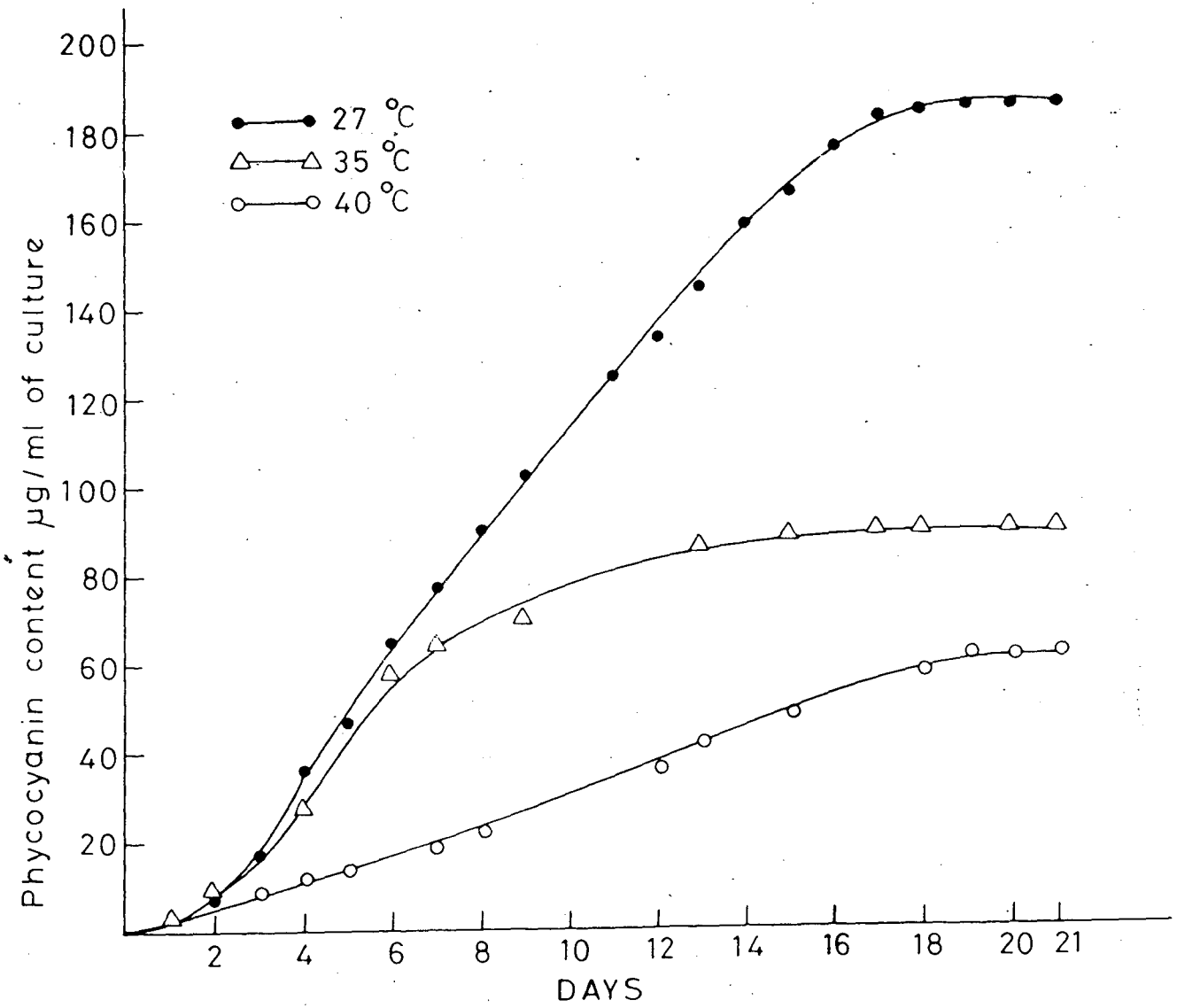

Fig.13 Effect of temperature on phycocyanin levels.

Cells were harvested by centrifugation suspended in saline, sonicated and cell free extracts were used for the phycocyanin estimation as described in Materials and Methods.

SPECTROSCOPIC STUDIES

Fig. 14 shows the absorption spectra of intact cells grown at normal temperature (27°C) as well as for cells grown at 35°C and 40°C. The figure shows absorption maxima at 440, 633 and 680 nm, representing chlorophyll a (440 nm, 680 nm) phycocyanin (633 nm) absorption respectively. A difference in the absorption pattern is clearly observed in the scan. The low content of phycocyanin and chlorophyll in the cells grown at 40°C was marked by very small peak at 633 nm and 680 nm respectively. Peak at 633 nm for the cells at 40°C clearly indicates that phycocyanin was much affected than chlorophyll, when cells were grown at higher temperatures.

CHLOROPHYLLS

The absorption of the chlorophyll a extracted from the control and from the cells grown at 35°C and 40°C are depicted in Fig.15. No significant change in the absorption pattern of chlorophyll a was noticed, though chlorophyll content was found less in the cells grown at 35°C and 40°C. The chlorophyll absorption maxima at 665 nm in the methanol extract are clearly marked.

PHYCOCYANIN

The absorption of the phycocyanin extracted from the control cells and from cells grown at 35°C and 40°C, are given in Fig.16.

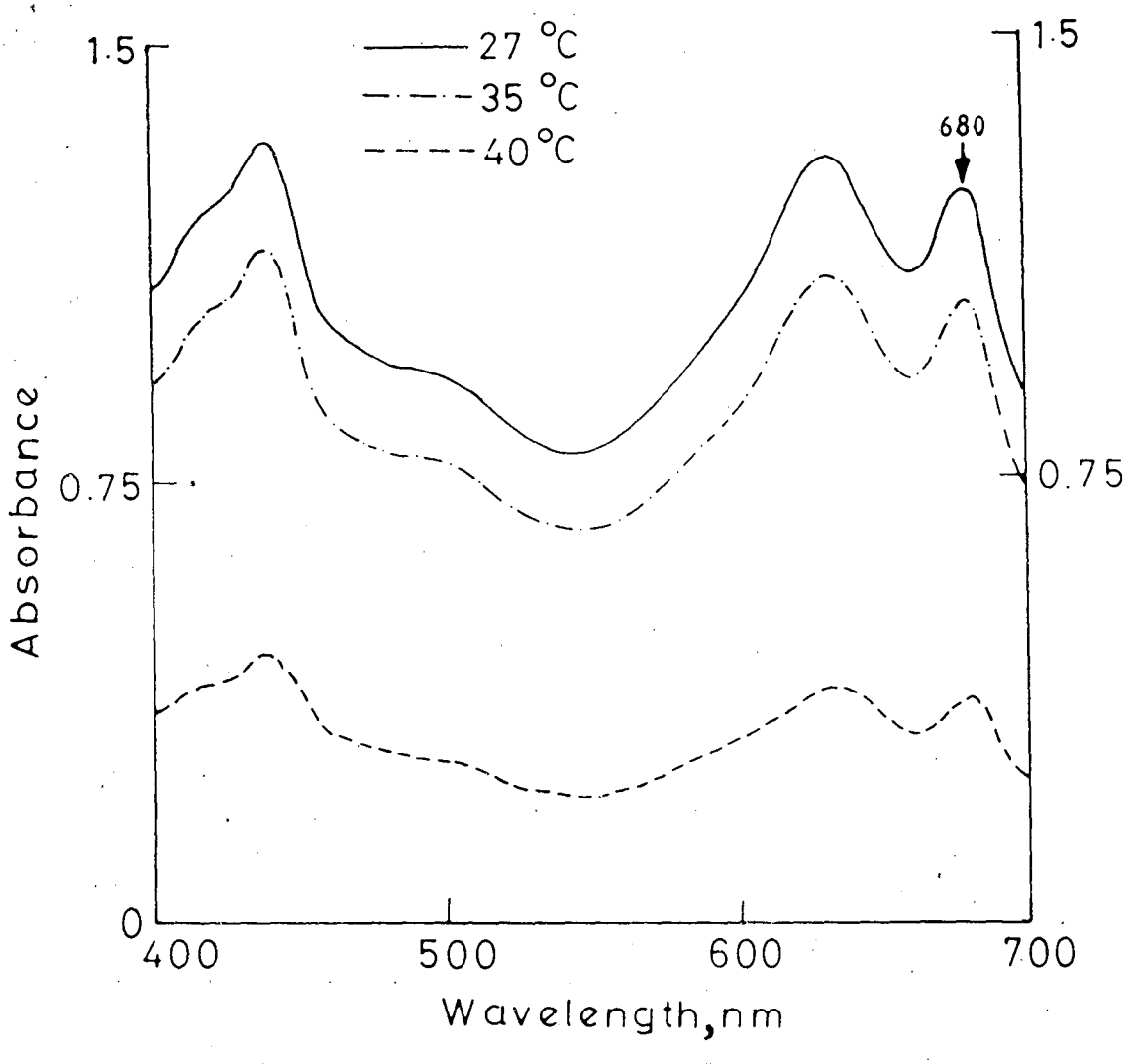

Fig. 14. Absorption spectra of intact cells at different temperatures.

Cells were harvested, suspended in HEPES - NaOH buffer (P_H 7.5). Absorption spectra was monitored using UV - 260 Shimadzu model spectrophotometer.

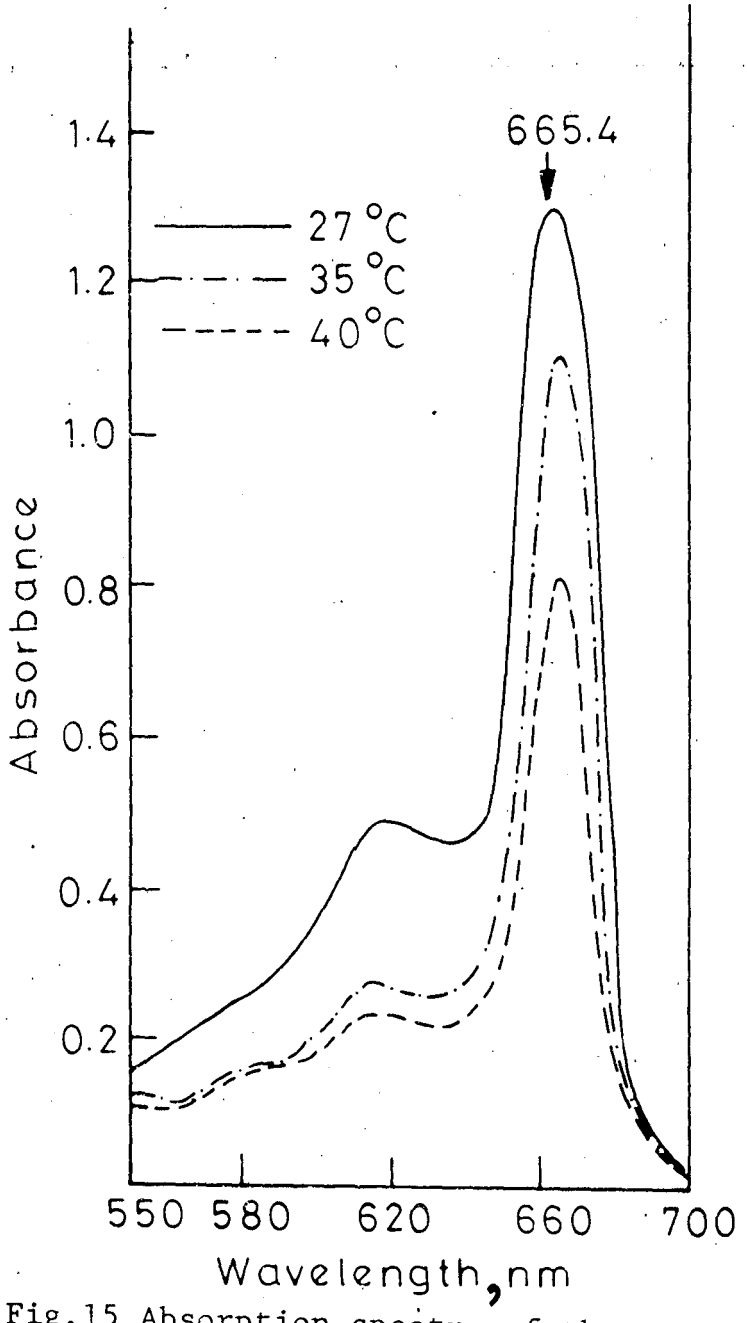

Fig.15 Absorption spectra of the methanol extracts from the cells grown at different temperatures.

Details were given in the Materials and Methods.

Here also no significant change in the absorption pattern of phgycocyanin was observed. Phycocyanin concentration was found to be less in cells grown at 35°C and 40°C as compared tocontrol. The phycocyanin extract was obtained as explained in Material and Methods. It shows absorption maxima at 624 nm in all cases. A small hump at 680 nm was discernible indicating the presence of trace amount of chlorophyll a which was not removed beyond the extent of 95% under the given procedure.

PHYCOBILISOMES

Phycobilisomes were isolated as per the methodology described in Material and Methods. Phycobilisome absorption spectra is depicted in Fig.17. For normal cells, as well as for cells grown at 35°C and 40°C, no significant change was observed. The amount of total phycobilisomes was less in heat treated cells as compared to normal cells. The phycobilisomes absorption maxima at 627 nm are clearly marked.

Fig. 18 depicts the fluorescence emission spectra of phycobilisomes extracted from cells. Isolation of phycobilisome has been described in Material and Methods. Phycobilisomes equivalent to 60 ug protein were taken in 3 ml cuvette and excited at 545 nm. The emission was recorded from 610 to 760 nm. The excitation slit as well as emission silt was 2.5 nm each. 2 nm shift in red region was noticed for PBsomes isolated from

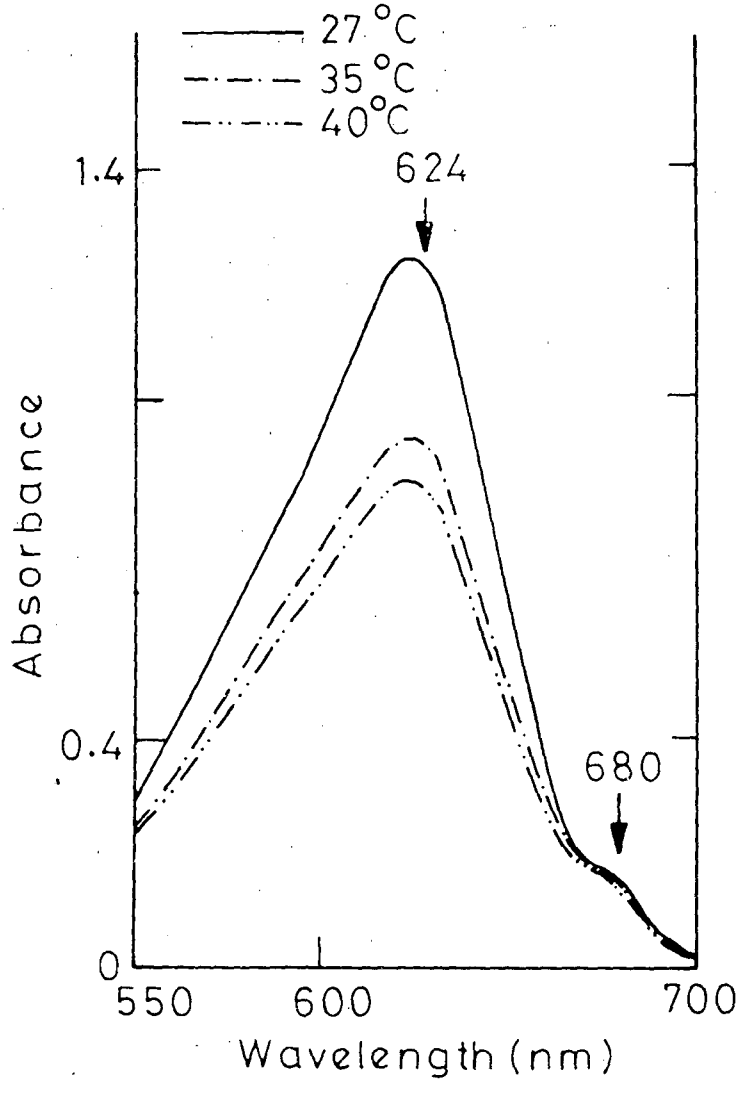

Fig. 16. Absorption spectra of phycocyanin from the cells grown at different temperatures.

Details were given in the Material and Methods.

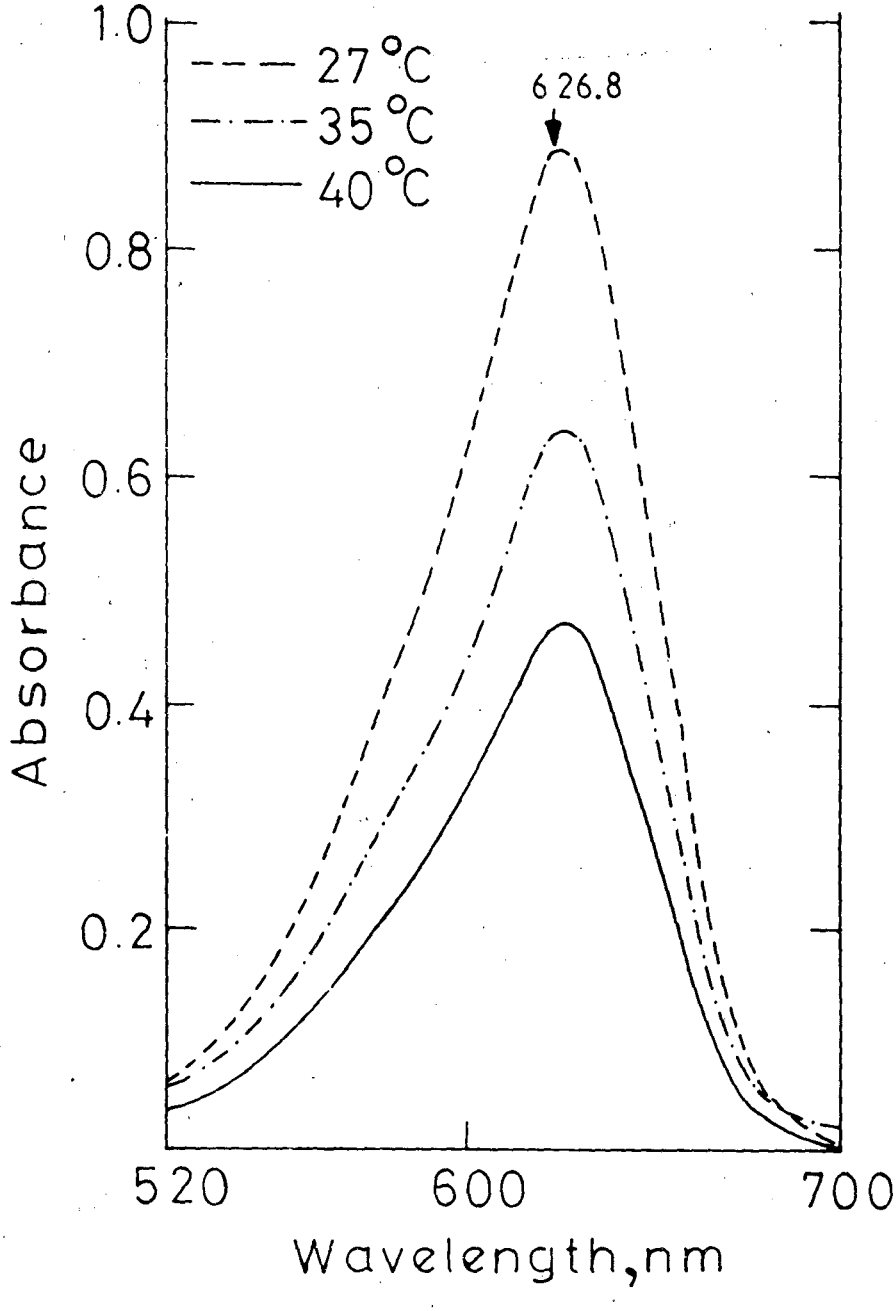

Fig., 17. Absorption spectra for phycobilisomes as monitored in the visible range.

Phycobilisomes were isolated according to Gantt et. al. (1974) and scanned as given in the Material and Methods.

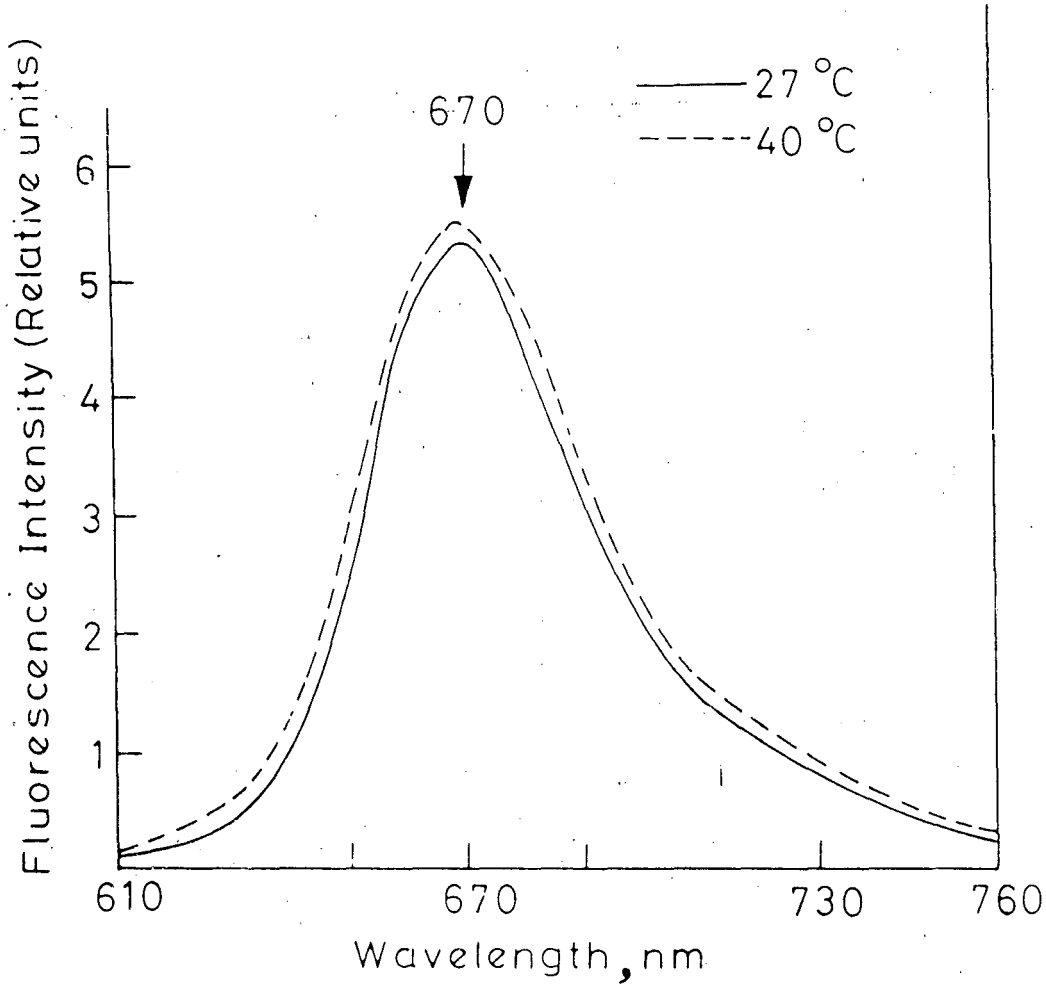

Fig.18 Fluorescence emission spectra of phycobilisomes of *Phormidium*.

Phycobilisomes equivalent to 60 μg protein were excited at 545nm. The emission was recorded from 610 to 760 nm. The excitation and emission slit was 2.5nm each.

cells grown at 40°C. Small increase in fluorescence intensity was also noticed for phycobilisomes from 40°C grown cells.

PS II MEDIATED ELECTRON TRANSPORT

Effect of heat was investigated on PS II catalysed P-BQ supported Hill reaction in intact cells of phormidium. P-BQ is a lipophilic artificial electron acceptor, known to accept electron from PQ pool. Normal cells harvested, in the mid Log phase of the culture, showed a high rate of PS II dependent O_2 evolution (350 u moles O_2 mg $chl^{-1}h^{-1}$) Rate of O_2 evolution was suppressed for the cells grown at 40°C. For the cells grown at 40°C rate of O_2 evolution was found to be 300 u moles O_2 mg $chl^{-1}h^{-1}$. A decrease of 10 % was noticed in rate of oxygen evolution when cells were grown at 40°C.

PART II

In this part cells were grown for six days at normal temperature (27°C). On 7th day cells were given heat treatment at differenet temperatures for different periods of time. After specific temperature and time period, cells were grown again at normal temperature for 24 days and their growth pattern was studied.

Fig. 19 shows the optical density measured at 550 nm for normal cells as well as for treated cells (35°C, 4 hr, 5 hr).

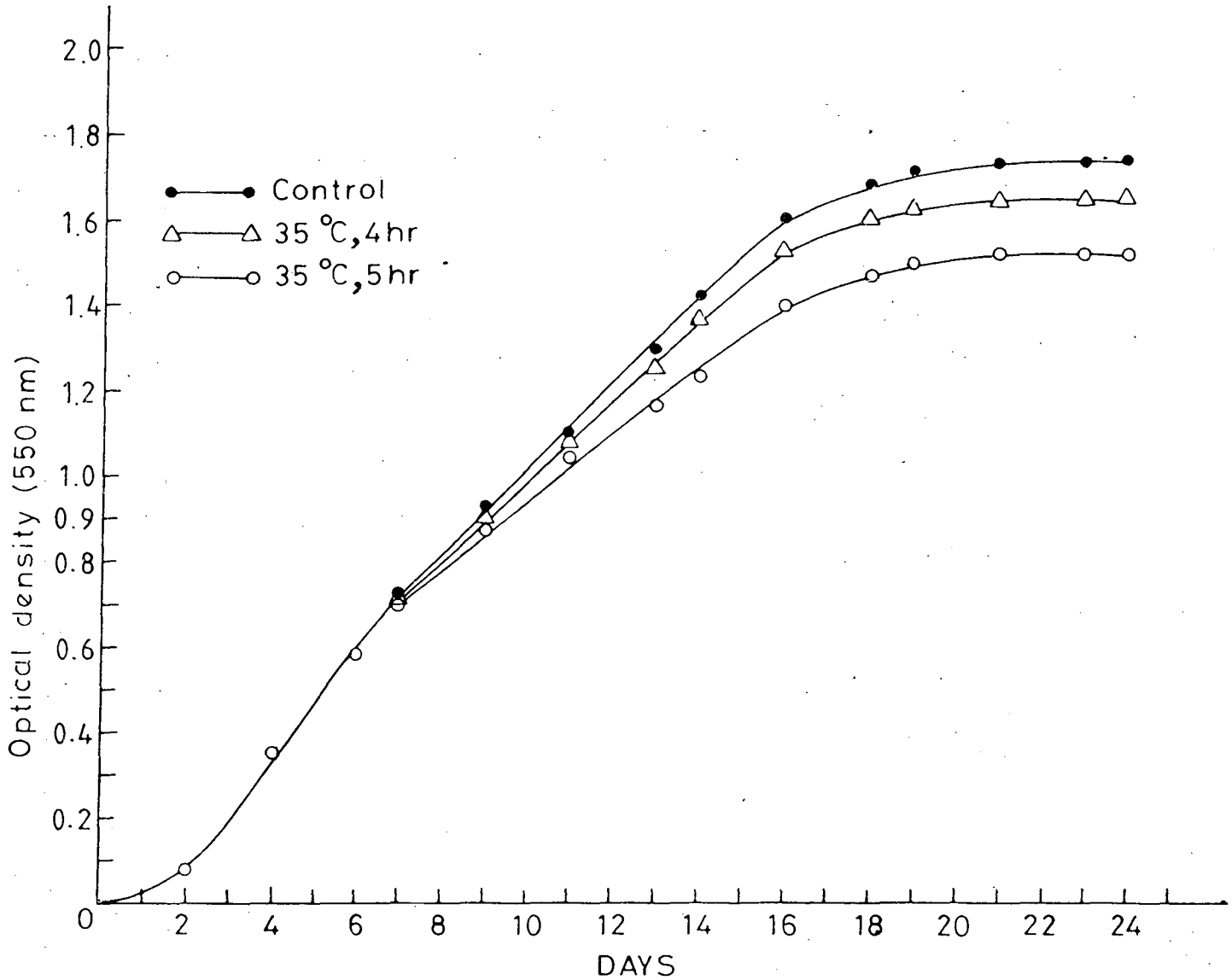

Fig. 19. Growth pattern of *Phormidium fovelarum* after heat treatment on 7th day at 35° C for a time period of 4 hrs and 5 hrs.

On 7th day culture flasks were taken, specific treatment given and kept again at 27° C for futher growth.

Minute effect of heat was observed on the growth of cells when treatment was given at 35°C for four and five hours. Fig 20 and 21 shows that there is slight decline in total protein content and dry weight biomass in the treated cells. Decline also becomes apparent only on 5th and 6th days after treatment. Approximately 11% decrease takes place in total protein content and cell biomass when cells were treated at 35°C for 5 hr. Methanol extract of chlorophyll (Fig.22) and phycocyanin extract (Fig.23) also shows a similar decline. Chlorophyll decrease is found to be 10% when 4 hours treatement is given which increased to approx. 15% at 5 hours treatmet (Fig. 23).

Fig. 24 shows the growth of cells at normal temperature and treated ones at 40°C for 4 hours and 5 hours. It shows that heat effect becomes apparent if treatment is given at 40°C for four and five hrs. Growth of treated cells starts declining from first day of treatment. Recovery never takes place in any of the cases of treated cells (Fig.25 and 26). The decline in treated cells for total protein and dry weight is gradual i.e. deviation between control and 4 hours treated cells and between 4 hours and 5 hours treated cells is aproximately the same. Protein content decreases by 13% at 5 hours treatment. Dry weight biomass decreases by 18% after 5 hours treatment. Methanol extract of chlorophyll (Fig.27) and phycocyanin extract (Fig.28) show much deviation after treatment. Chlorophyll concentration decreases

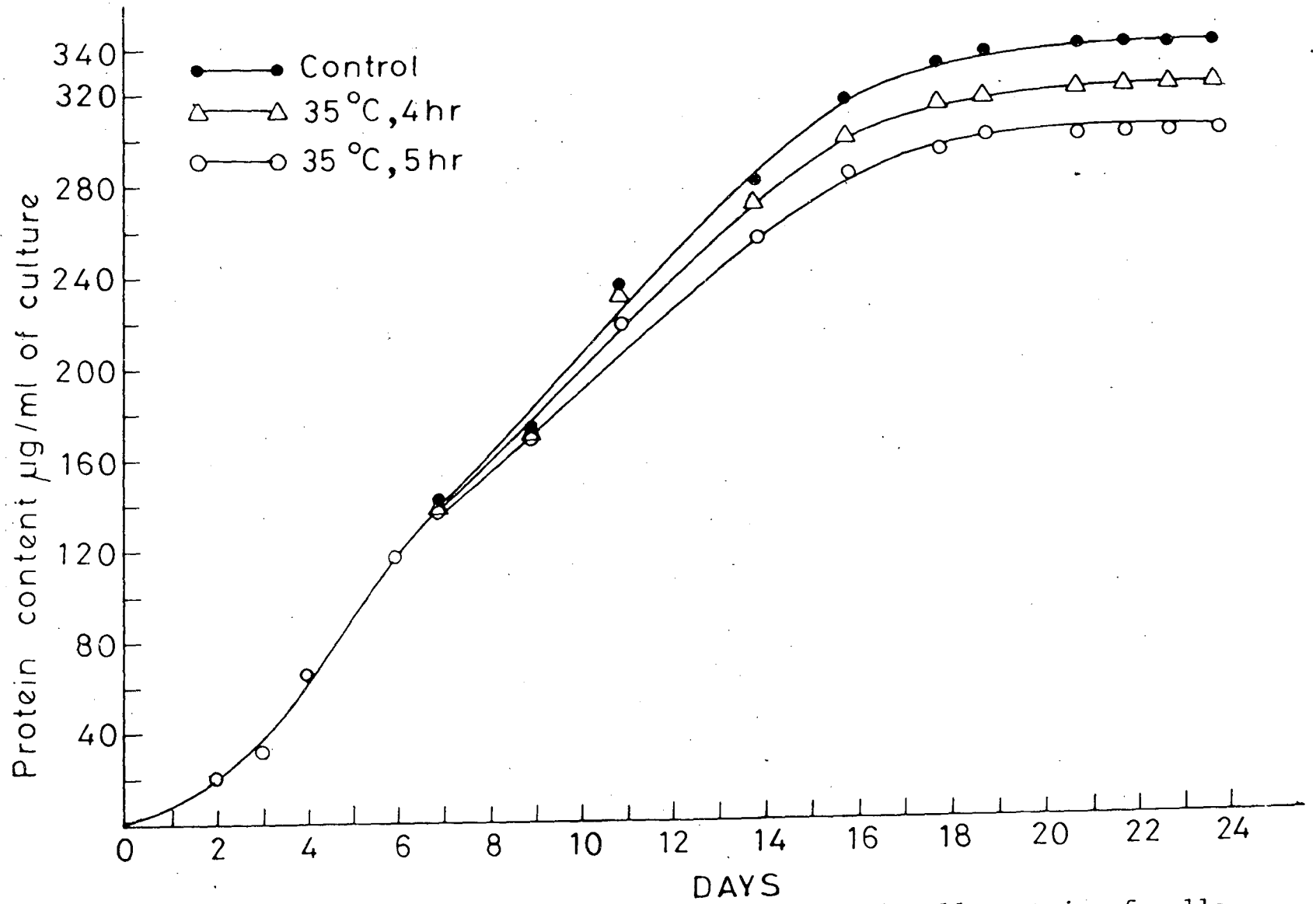

Fig.20 Effect of heat treatment (as in Fig. 19) on total cell protein of cells. Total protein content was measured by Lowry's method.

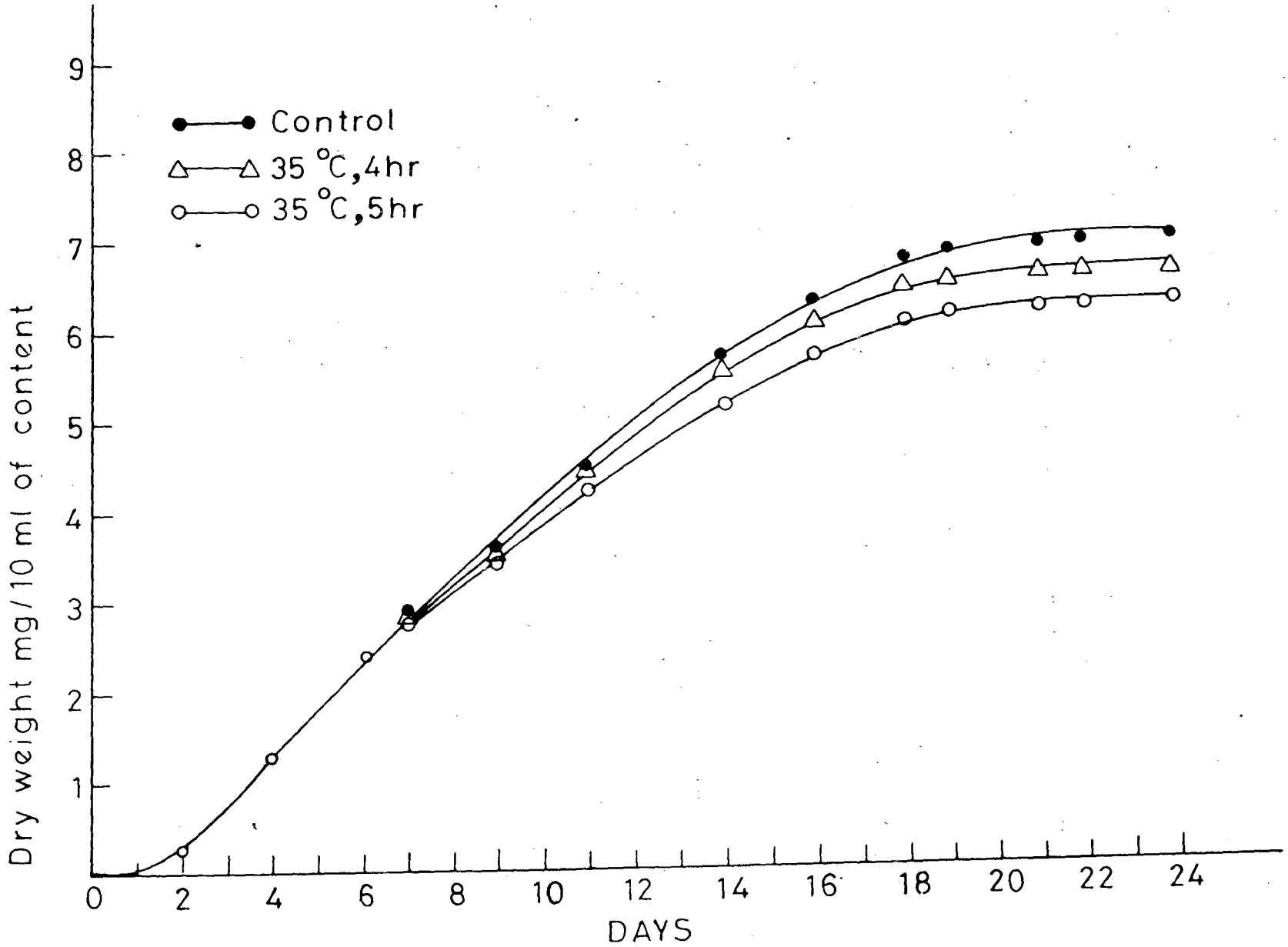

Fig. 21. Effect of heat treatment (as given in Fig. 19) on dry weight of cells.

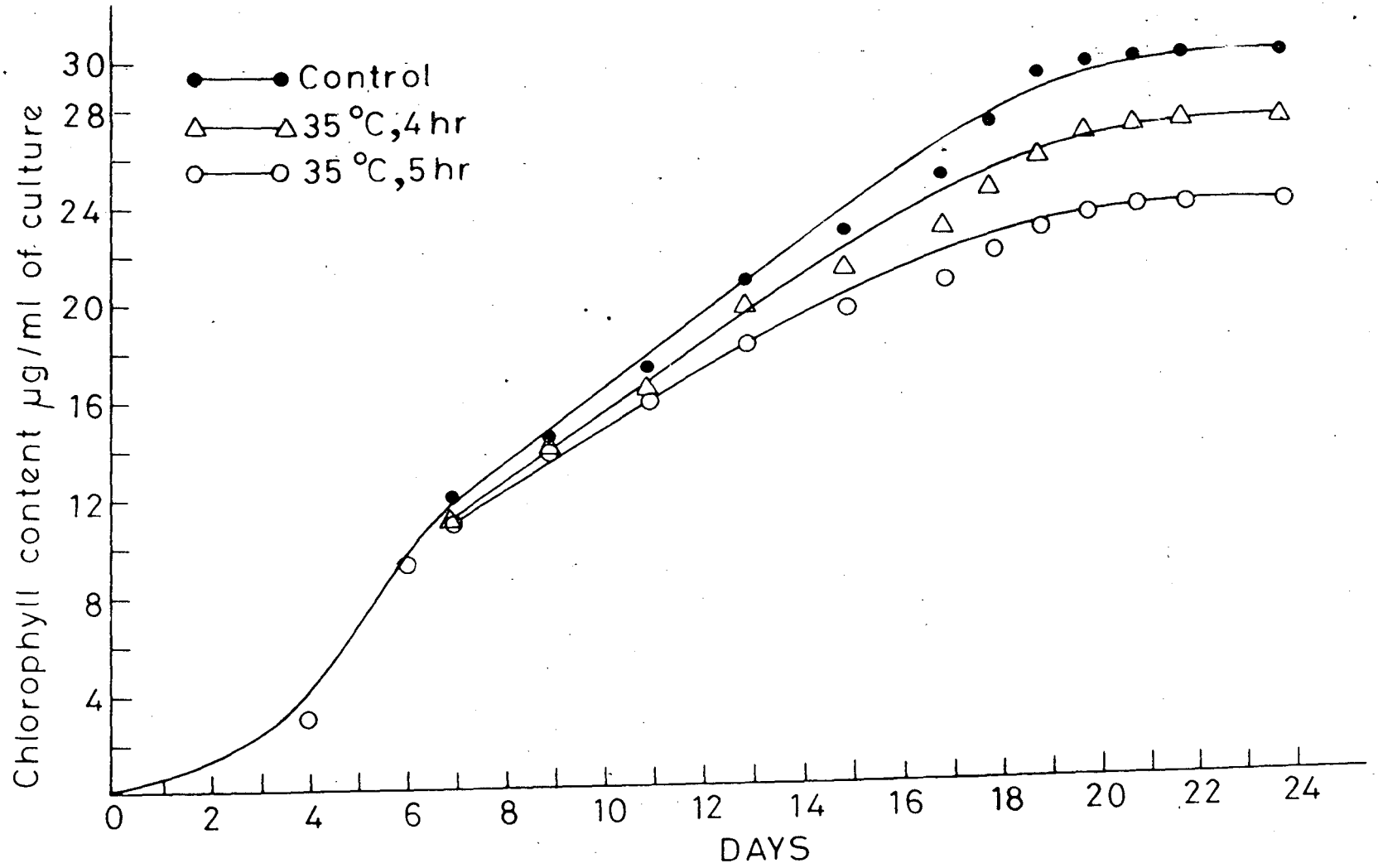

Fig. 22. Effect of heat treatment (as given in Fig. 19) on chlorophyll a contents.

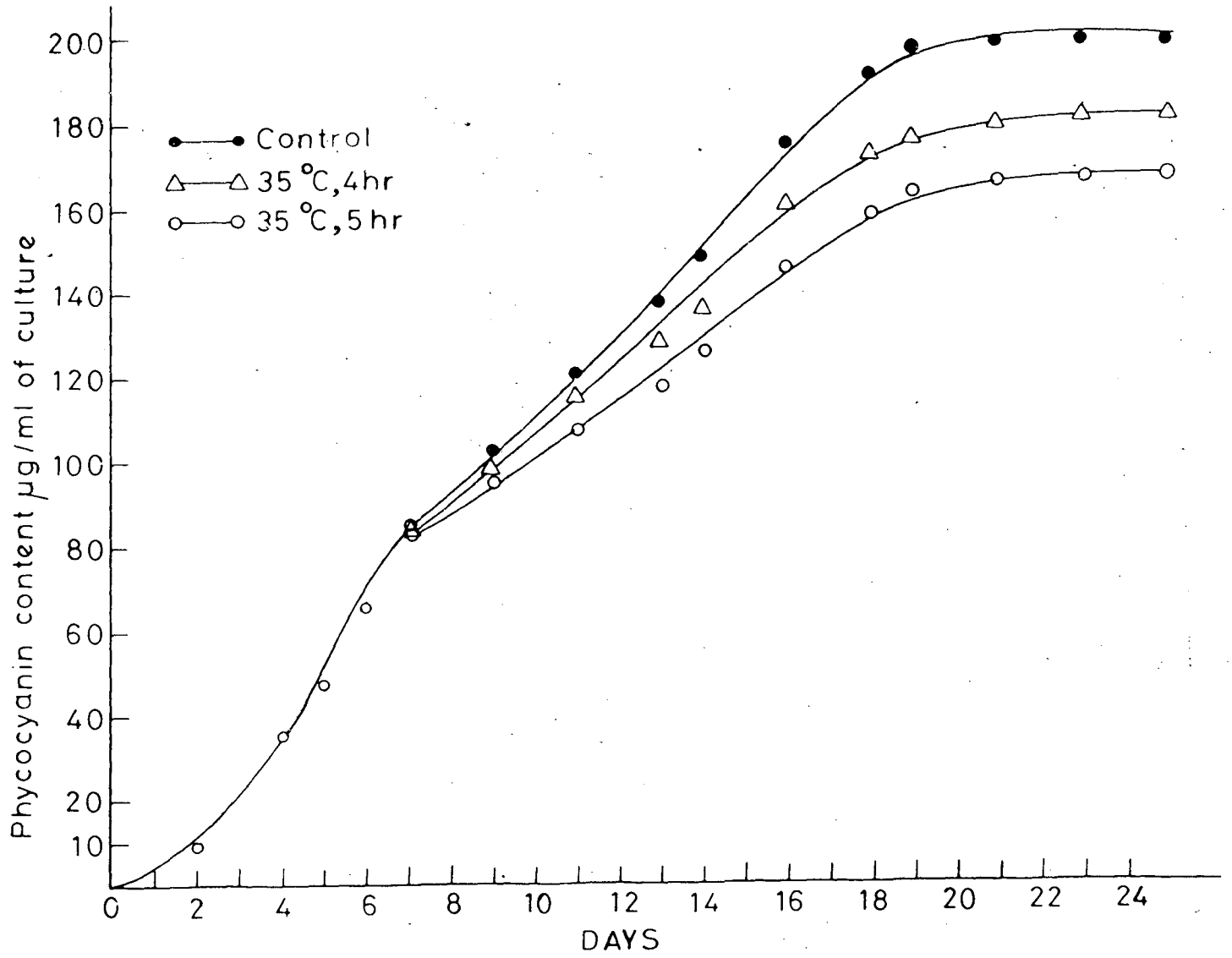

Fig. 23. Effect of heat treatment (as given in Fig. 19) on phycocyanin levels.

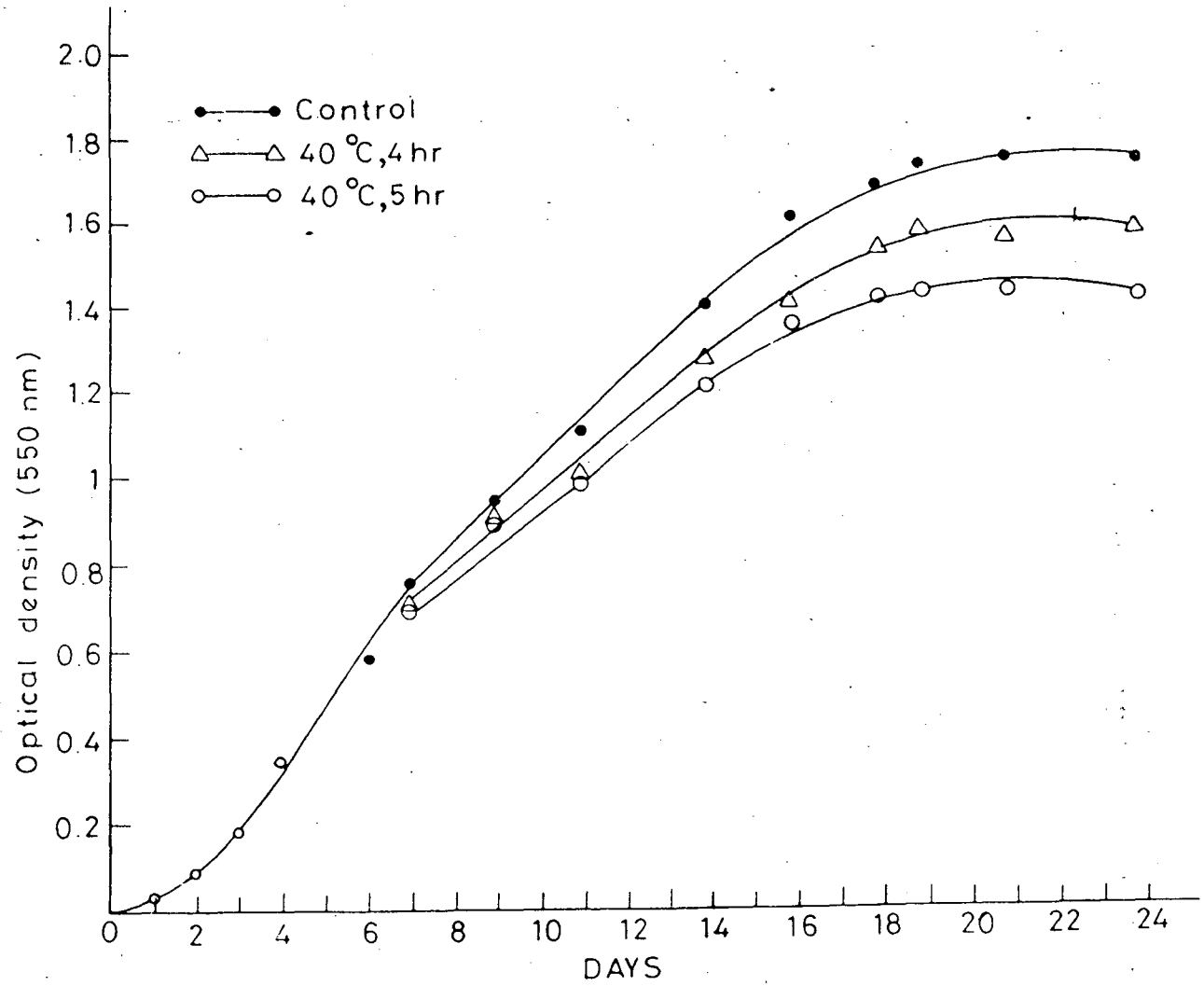

Fig.24 Growth pattern of *Phormidium fovelarum* after heat treatment on 7th day, at 40°C for a time period of 4 hrs and 5 hrs:

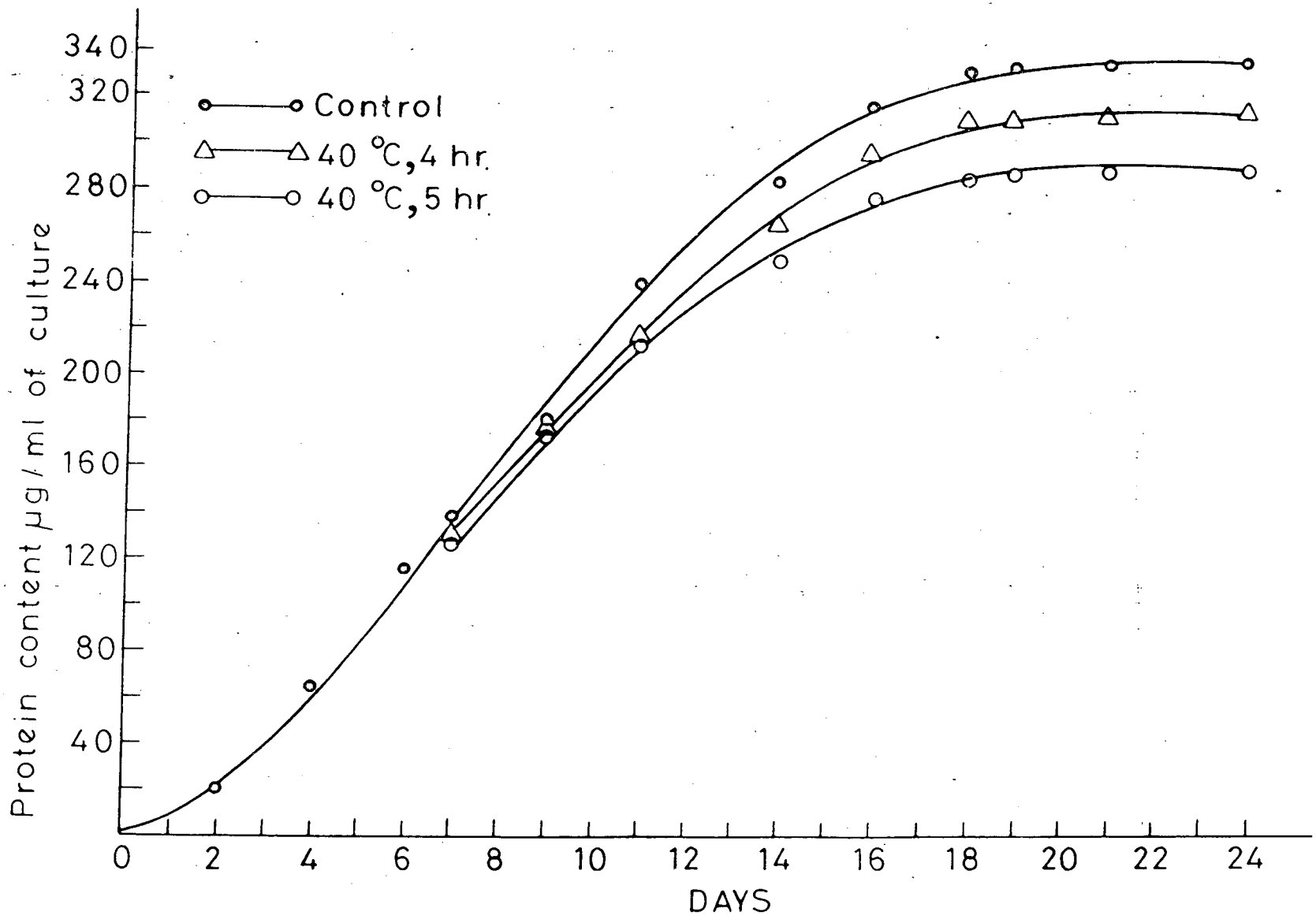

Fig.25 Effect of heat treatment (as given Fig.24) on total cell protein of cells.

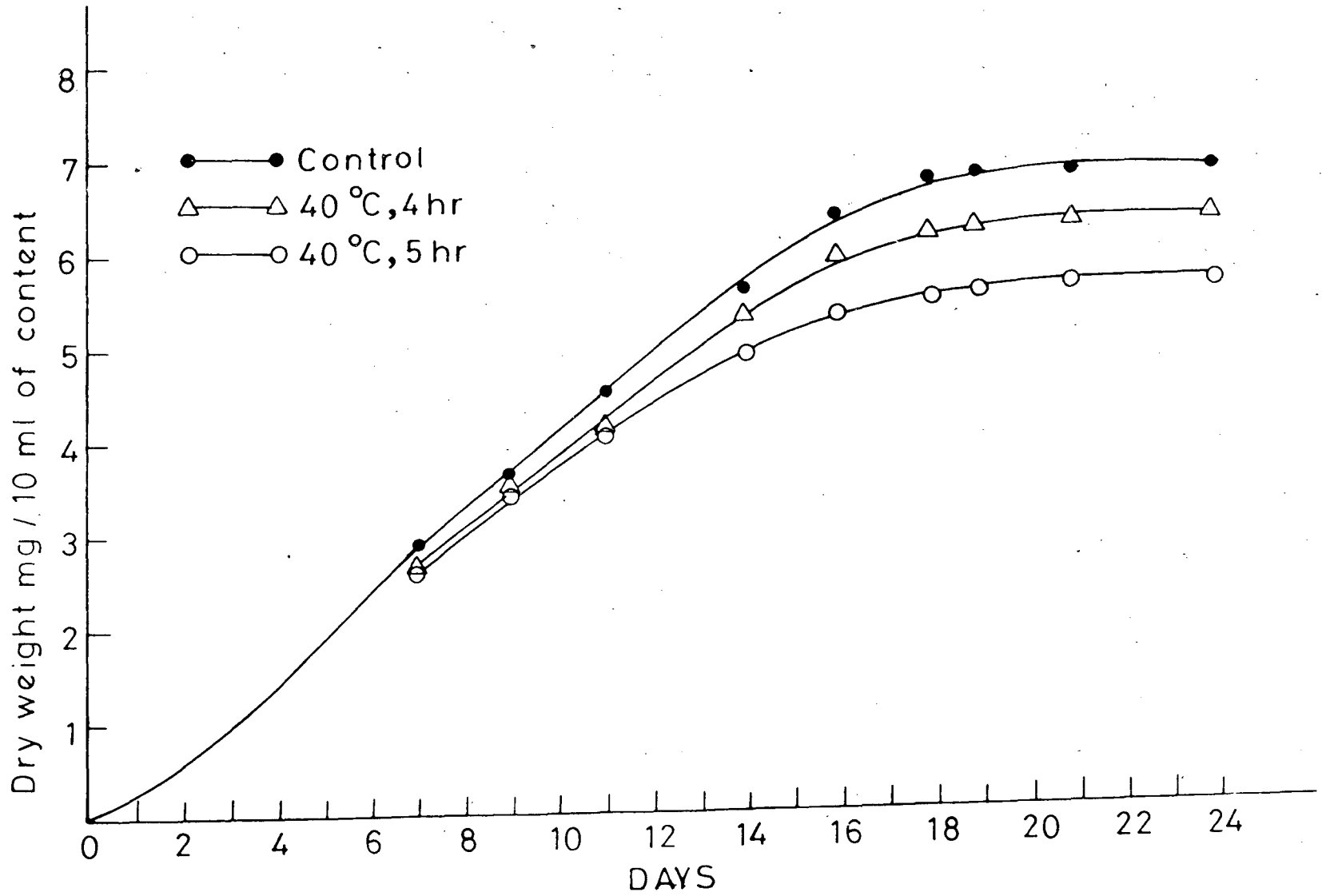

Fig. 26. Effect of heat reatment (as given in Fig. 24) on dry weight of cells.

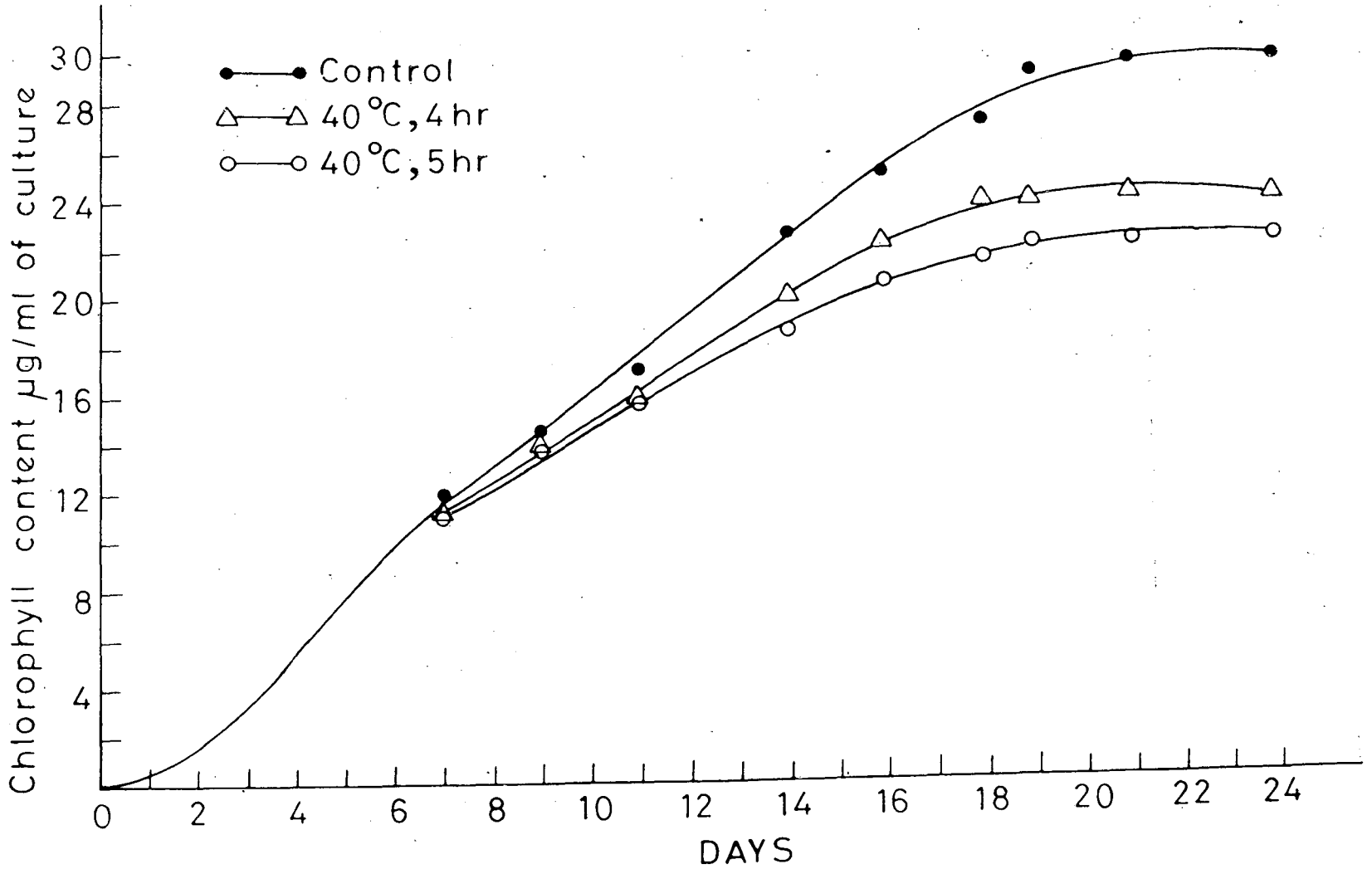

Fig. 27. Effect of heat treatment (as given in Fig. 24) on chlorophyll a contents.

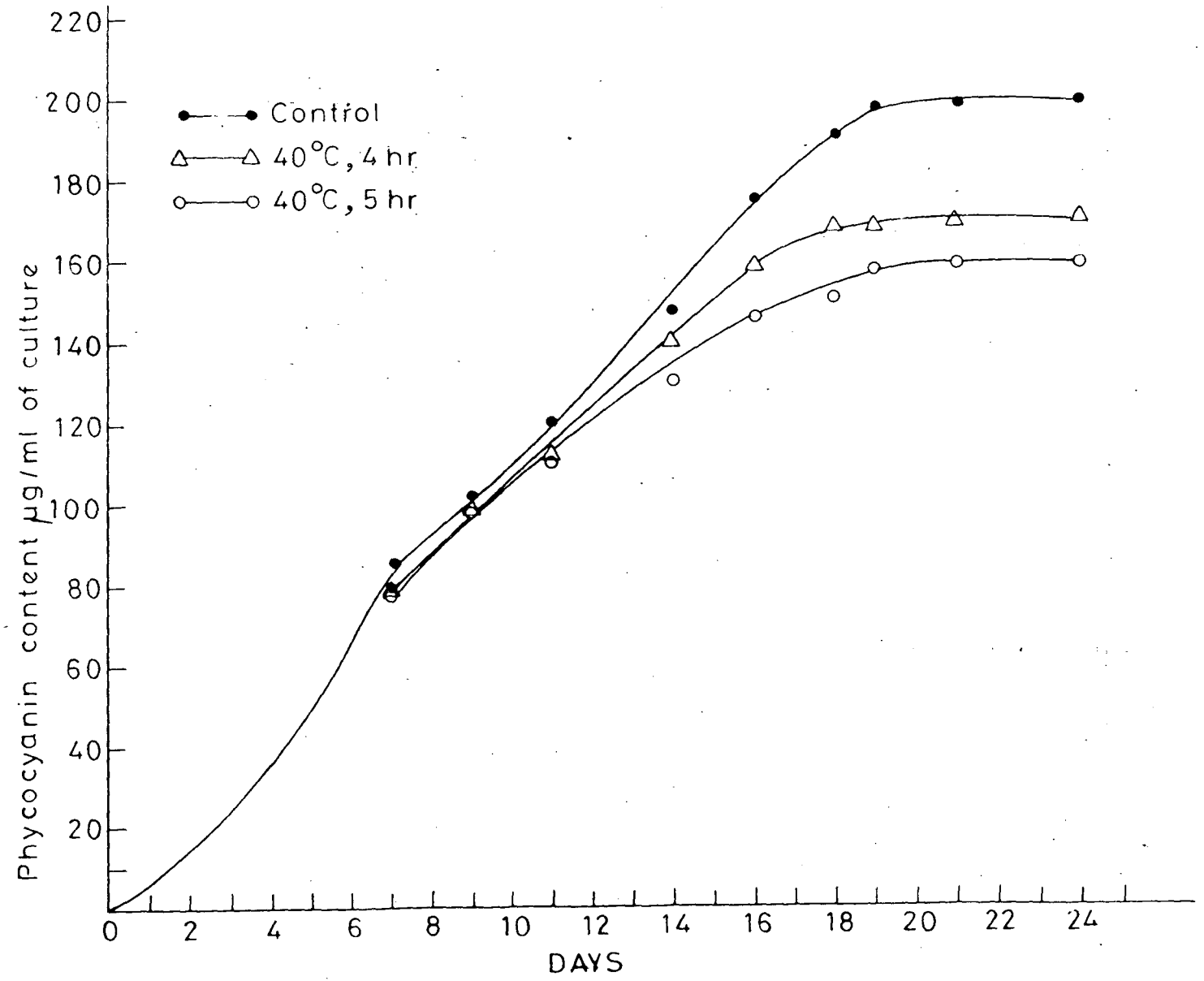

Fig.28 Effect of heat treatment (as given in Fig.24) on phycocyanin levels.

by 20% after 4 hours treatment which is increased to 26% if 5 hours heat treatment is given (Fig. 27). Decline in chlorophyll concentration is abrupt after 4 hours treatment. Phycocyanin concentration decreases by 15% if 4 hours treatment is given, which further increased to 20% after 5 hours treatment (Fig.28).

Figs. 29-31 show remarkable heat effect on the growth of cells treated at 45^{o}C for 4 and 5 hours. Heat treatment of 5 hours at 45^{o}C becomes injurious to cells whereas 4 hours treatment too shows considerable variation with control. Fig. 29 shows optical density (growth pattern) of normal and treated cells. Here growth of cells (4 hours treatment) declines in the beginning but later it recovers on 13th day after treatment. Cells treated for 5 hours show slow growth in the beginning, continuing to do so upto 9th day of treatment. On the 10th day after treatment it starts recovering but does not recover like normal cell growth. Figs. 30 and 31 for total protein and dry weight cell biomass also confirm this growth pattern. Figs. 32 and 33 show methanol extract of chlorophyll and phycocyanin content. At 45^{o}C for 5 hours, their content declines initially but recovers on 9th day of treatment up to some extent. For 5 hours treatment, phycocyanin gets a major set back from control cells and then starts recovery. Recovery does not reach more than 67% of normal phycocyanin content.

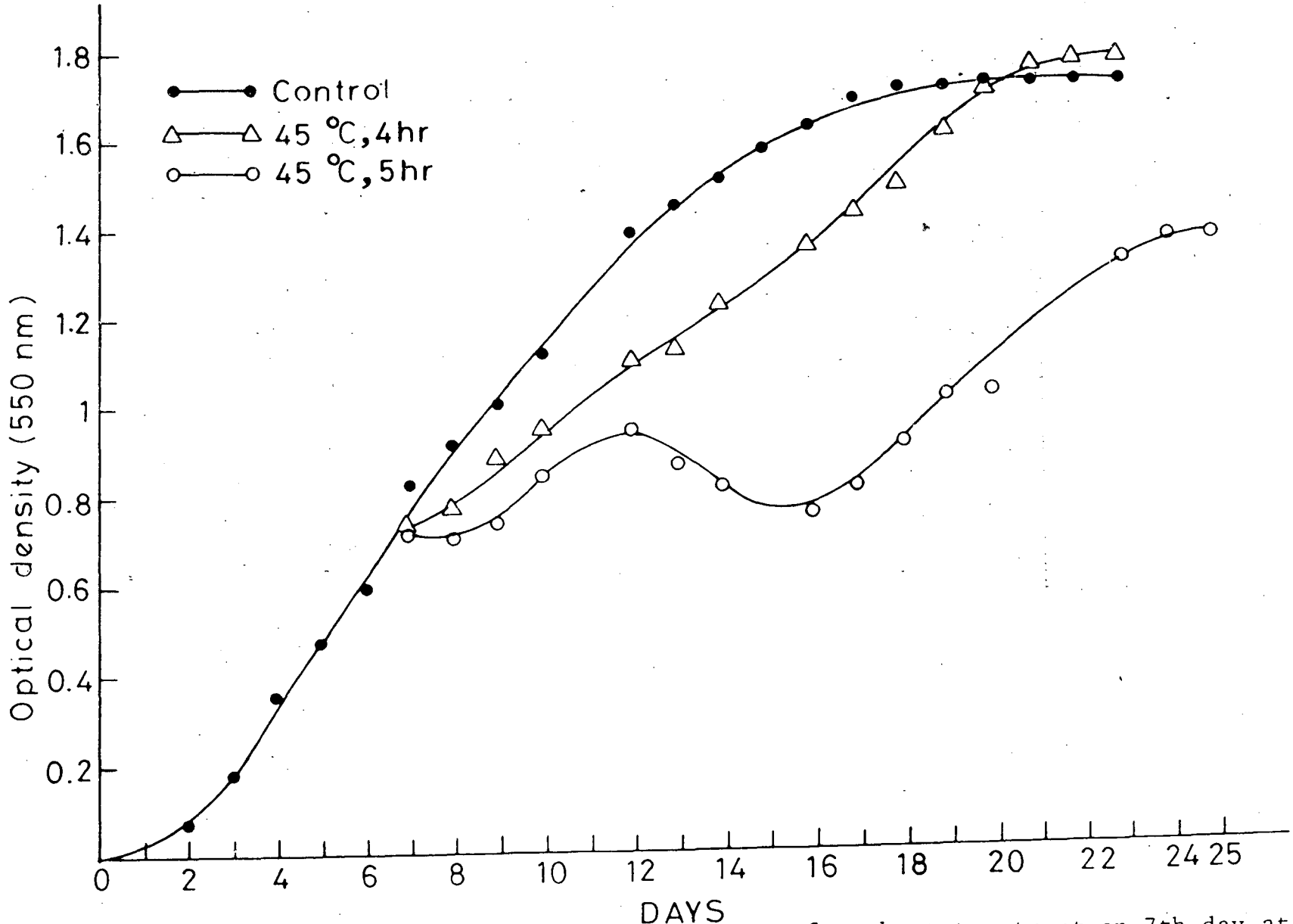

Fig. 29 Growth pattern of *Phormidium fovelarum* after heat treatment on 7th day at 45° C for a time period of 4 hrs and 5 hrs.

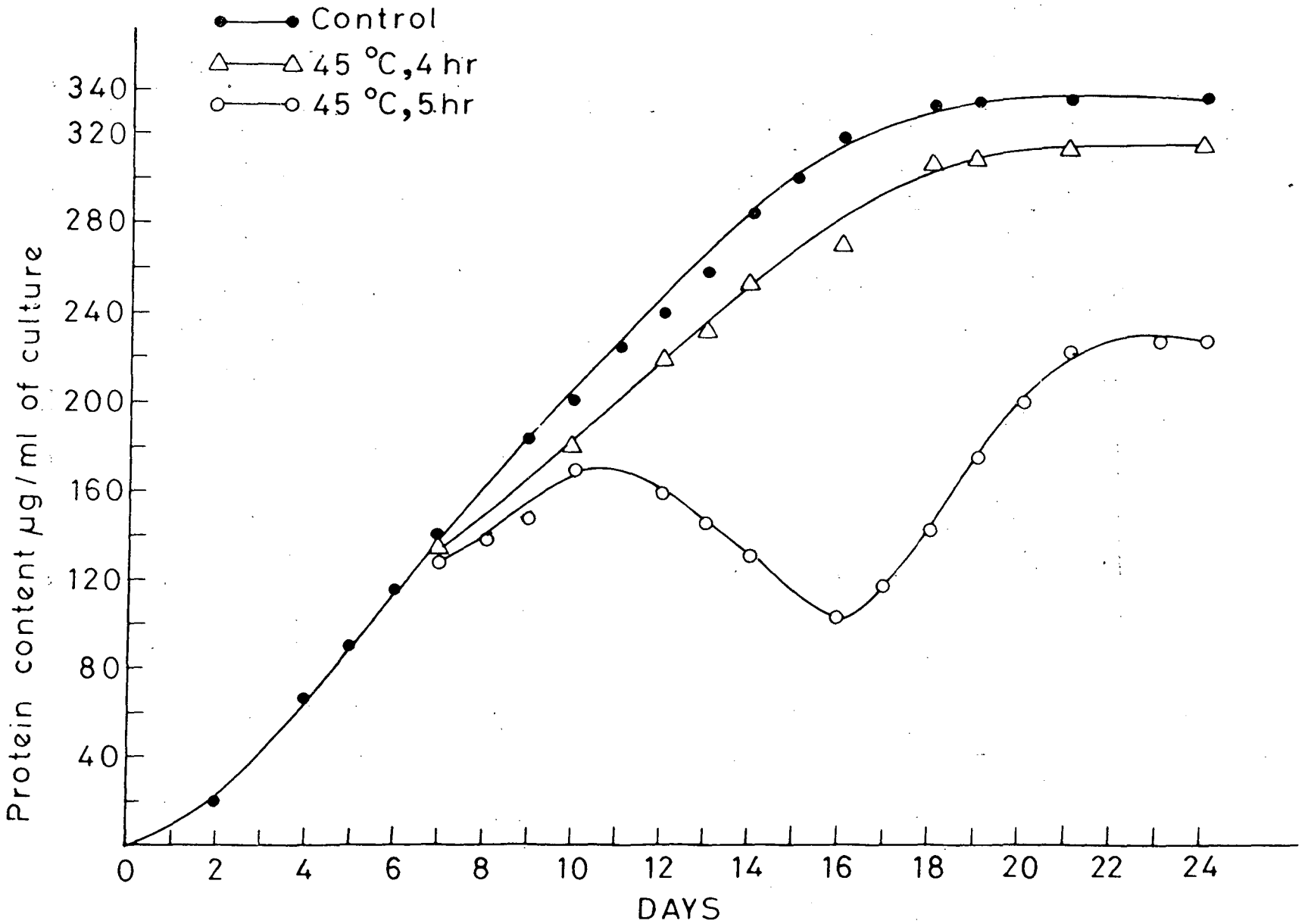

Fig.30 Effect of heat treatment (as given in Fig.29) on total cell protein cells.

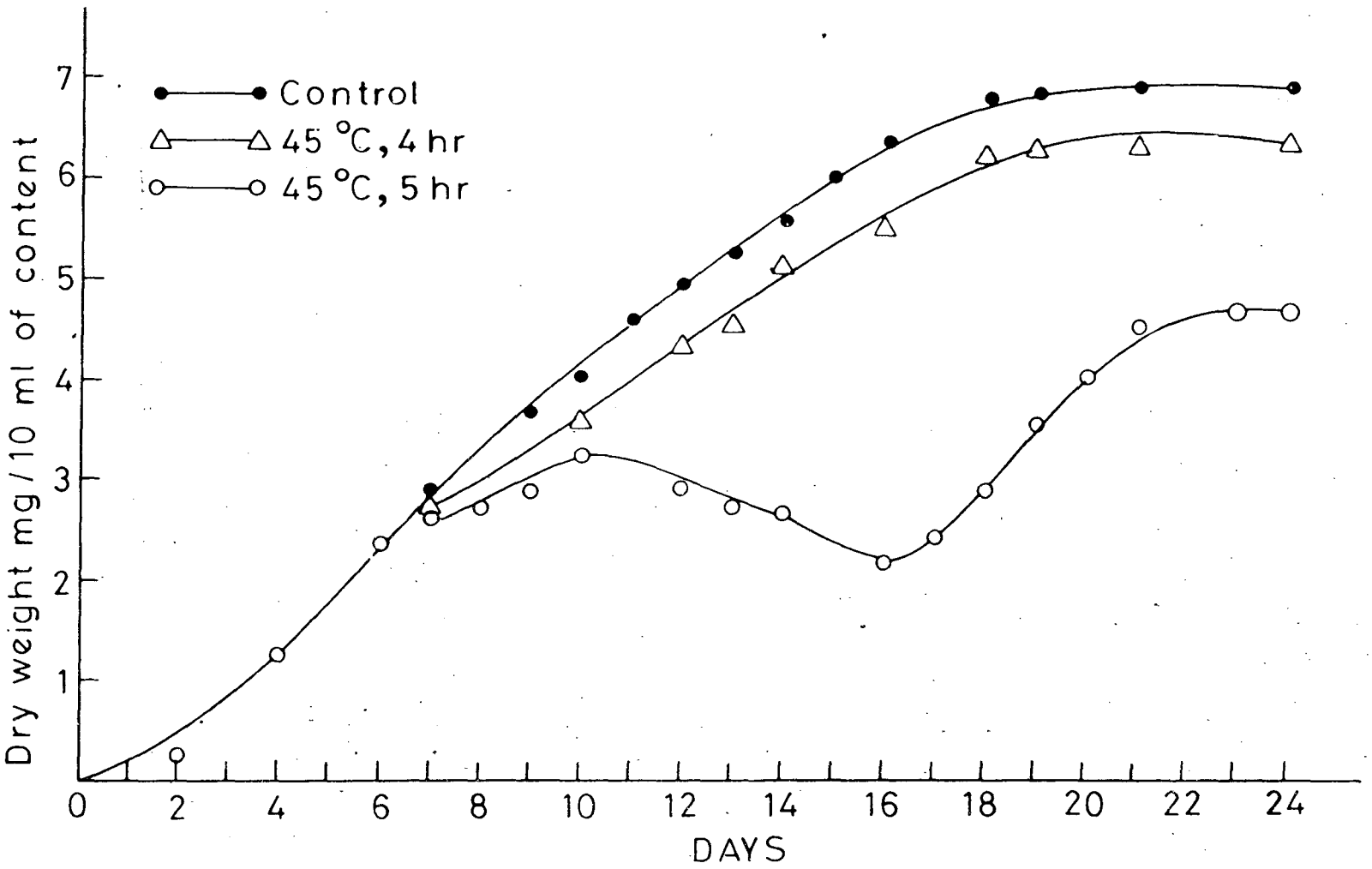

Fig. 31. Effect of heat treatment (as given in Fig. 29) on dry weight of cells.

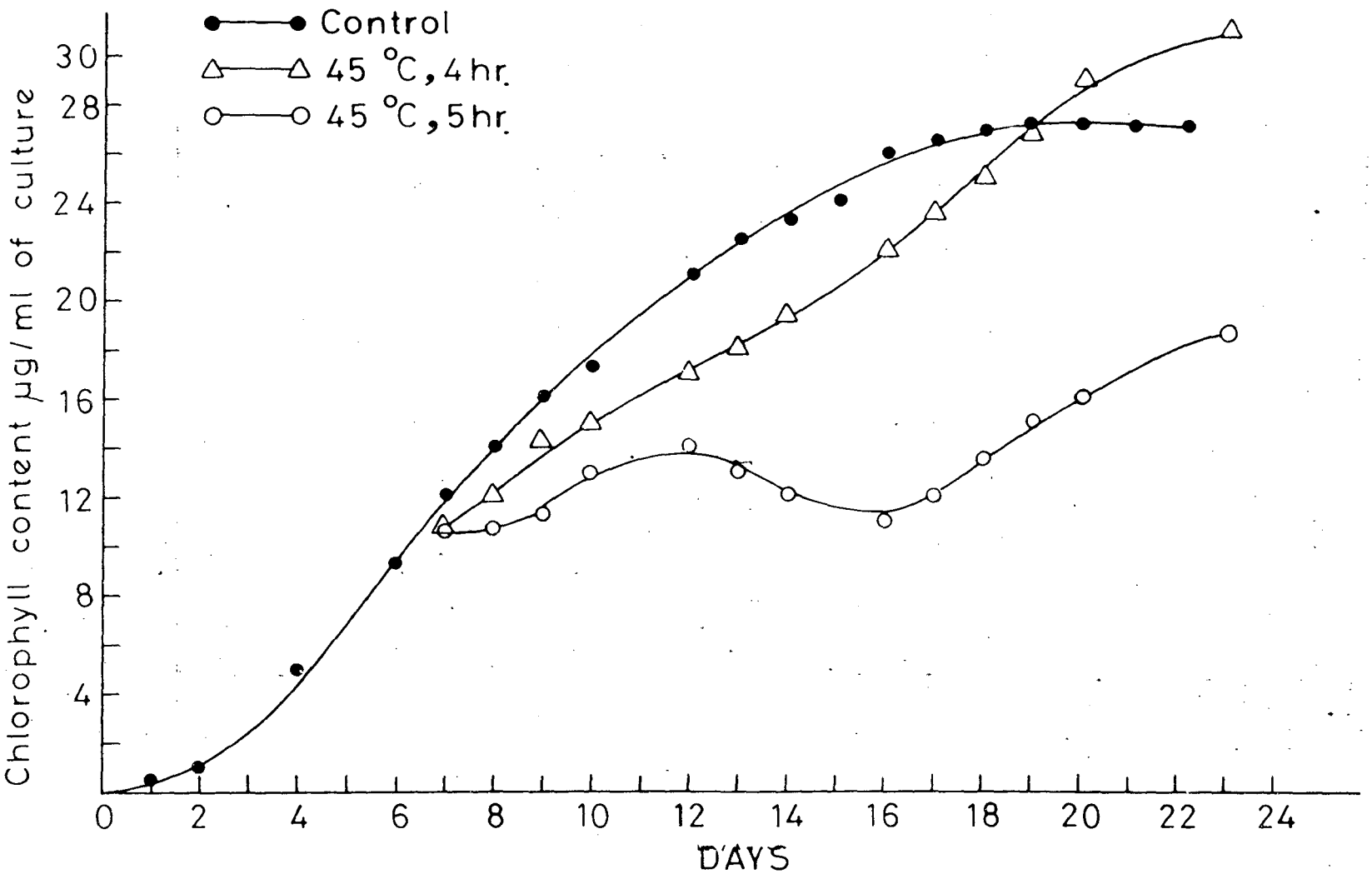

Fig. 32. Effect of heat treatment (as given in Fig. 29) chlorophyll a contents.

Fig. 34 shows the room temperature ($25^{o}C$) fluorescence emission spectra of intact normal cells as well as of treated one. Treatment of $45^{o}C$ for 5 hours was given and spectra was taken immediately. Fig. 34A represents the intensity of fluorescence of cells excited at 440 nm. No remarkable change in the position of peaks was found although intensity of fluorescence changes for both the peaks i.e. ratio of 682 nm/660 decreases to 0.93 in treated samples from 1.26 in control sample. Fig. 34B represents the fluoroescence intensity when cells were excited at 545 nm. Here again negligible change in the position of 658 nm peak was observed. Intensity of fluorescence gets increased by 2 units in treated cell compond to control.

Fig. 35 shows the fluorescence spectra of normal and treated cells after 24 hours of treatment. Treatment was given at $45^{o}C$ for 5 hours. After treatment cells were kept at normal temperature ($27^{o}C$) for 24 hours. Fig. 35A shows fluorescence intensity when cells were excited at 440 nm. No change was found in position of peaks. Ratio of 682/660 which is 1.25 for control cells comes to 1.4 for treated one. Fig. 35B shows the fluorescence intensity when cells were excited at 545 nm. Again no remarkable change was observed in position of 658 nm peaks, though intensity of 658 nm peak decreased by 2 units.

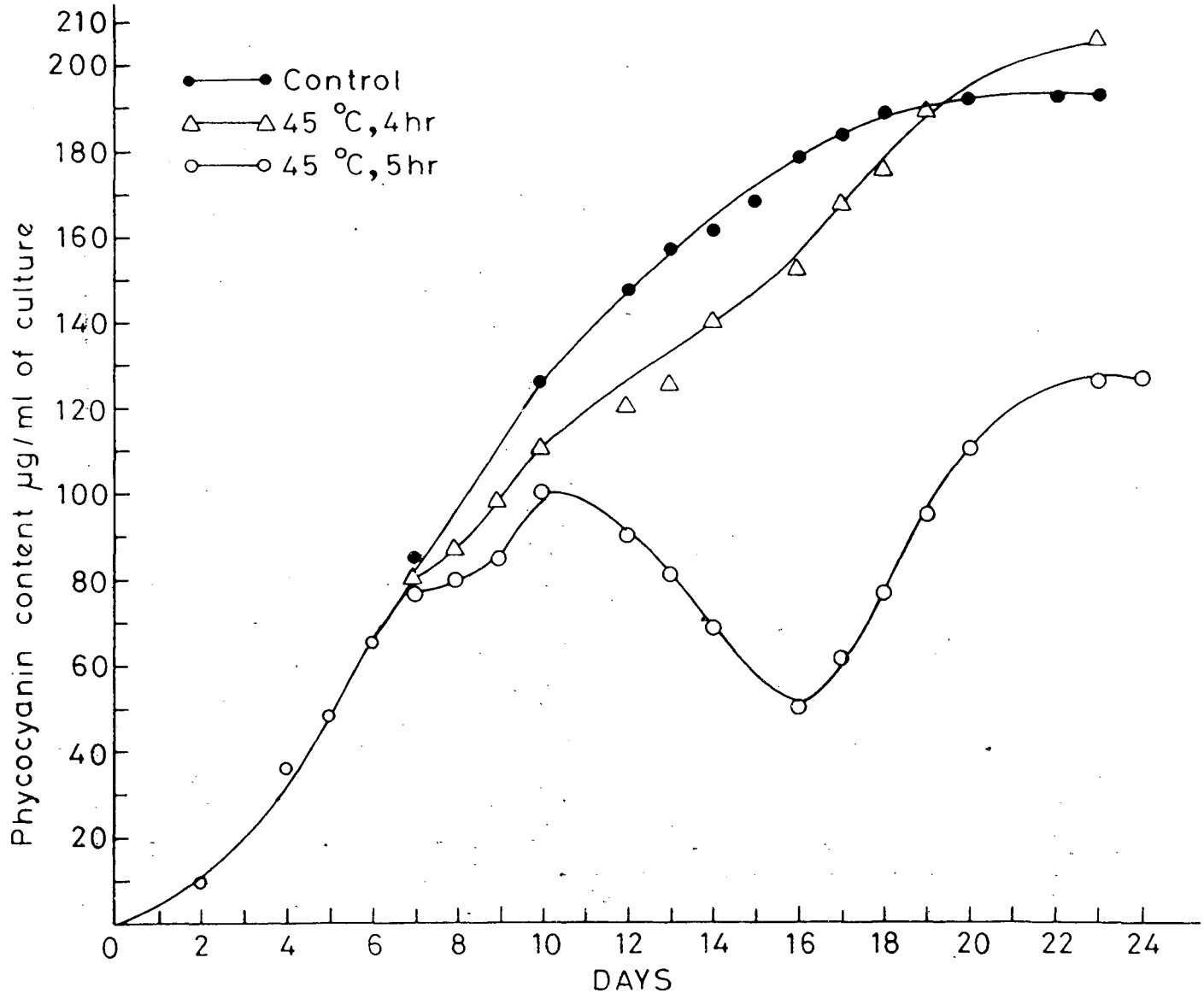

Fig.33 Effect of heat treatment (as given in Fig.29) on phycocyanin levels.

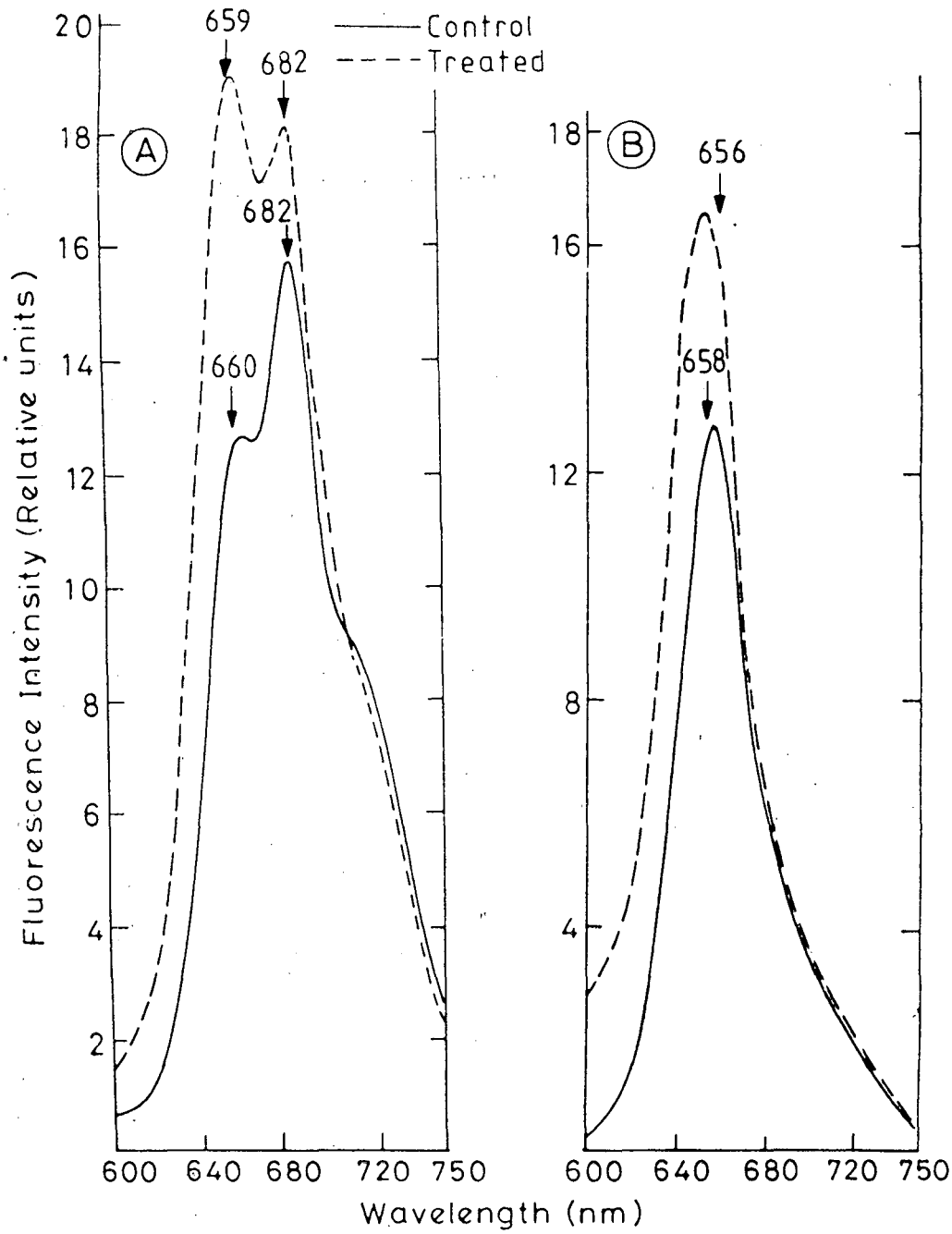

Fig. 34. Room temperature (25°C), fluorescence emission spectra of intact phormidium cells.
Solid line indicates the fluorescence characteristic of control cells and dotted line represents the fluorescene emission of temperature treated (45°C, 5hr) cells. The spectra was recorded immediately after temperature treatment. The cells equivalent to 3µg chl ml^{-1} were excited at 440 nm light (a) and also at 545 nm light (B). The emmision was recorded from 600 nm to 750 nm .The excitation and emission slit was 15 nm each.

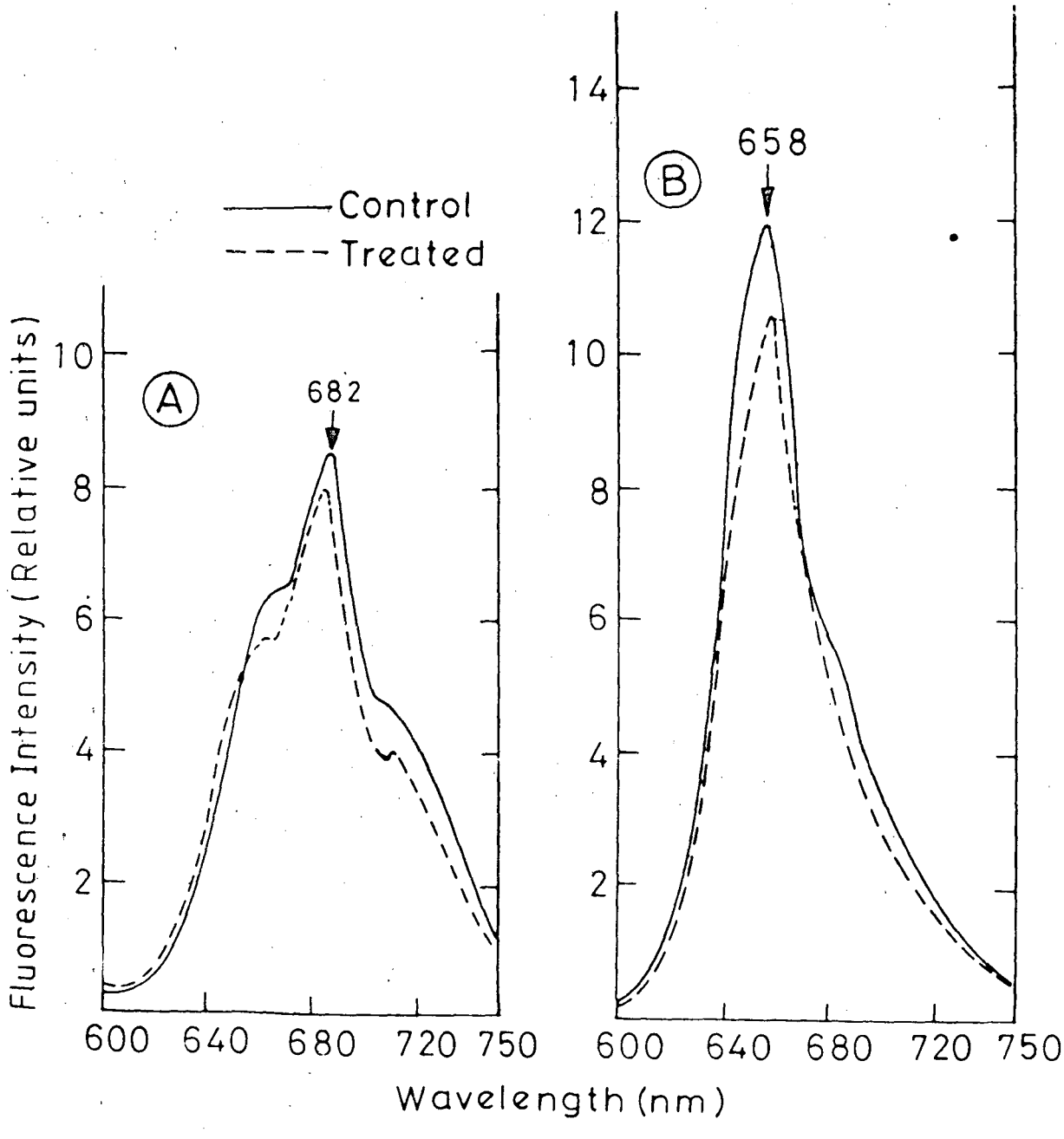

Fig. 35. Florescence emission characteristic of control and temperature treated (45°C, 5 hr) Phormidium cells excited at 440 nm (A) and 545 nm (B). The emission was recorded after 24 hr to temperature treatment. Other details of measurements were same as in Fig. 34.

DISCUSSION

Cyanobacteria possess not only the photosynthetic pigments, Chl *a* but also phycobiliprotein complexes contributing characteristic colour to the organism. The phycobiliproteins play a major role as light harvesting pigments in cyanobacteria and red algae. A large fraction of the light energy, used to drive photosynthesis and virtually all that channeled to photosystems II, is absorbed by these chromoproteins. All members of both groups synthesize phycocyanin and allophycocyanin. The overlapping visible absorption bands of these two pigments lie in the red region extending from approximately 600 to 660 nm. Cellular light absorption in this spectral region is mainly attributable to phycocyanin, since allophycocyanin is usually a minor pigment.

Till now many of the temperature induced photosynthetic processes have been sudied in isolated, intact or broken chloroplasts, while only a few have been carried out with cyanobacterial photosynthetic membranes. Since the composition of the pigment system of the BGA depends to a large extent on environmental conditions (light, CO_2 concentration, temperature etc.), the aim of the present work was to investigate the growth of blue green algal cells at different elevated (35^oC, 40^oC) temperatures that are known to alter the

physiochemical properties of the pigments. Further more, in this work we have also examined the effects of preheating algal cells on their growth pattern as well as pigment concentrations. In particular, the objective of the present investigation was to characterise the effect of elevated temperatures ($35^{o}C$, $40^{o}C$) on PC, protein and PBsomes content and also on the light absorption characteristics of the major photosynthetic pigments of the algal cells which are known to be temperature sensitive.

Cells grown at high temperatures ($35^{o}C$, $40^{o}C$) showed a decrease in growth. Growth rate was monitored by measuring the scattering phenomenon at 550 nm (Fig. 9). Elevated temperature adaption resulted in decline of Cell growth. This decline is also accompanied by decrease in the amount of total cell proteins as well as the dry weight of cells (Fig. 10, 11). Chlorophyll and phycocyanin content showed a decrease with elevated temperature adaptation (Fig. 12, 13). Contrary to the observation of Eley (1971) in Synechoccus, our results indicate that elevated temperature adaptation not only impairs the growth rate of cells but also effects the overall cellular synthesis of the organism. A significant decline in total protein and consequent lowering of dry mass of the organism, is indicative of the fact that elevated temperature adaptation probably effects the cellular constituents at their synthesis level. The significant decline in the absorption at 550 nm in temperature

adapted cells seems to be the consequence of slow rate of cell division as compared to control. To overcome the temperature effect, as shown by Eley (1971) and Goedher (1976), a higher flux density of light as well as abundant amount of carbondioxide is needed during the elevated temperature adaptation. In all probability the failure of Phormidium to withstand the temperature shock may have been due to the absence of higher illumination as well as of enough carbondioxide. As mentioned in the above cited paper the organisms were grown at air level of CO_2 (330 ppm) and comparatively low level of illumination (16 Wm^{-2}).

Heat tolerant cells can lead to heat injury only if protein synthesizing enzymes are thermostable (Measured by the temperature at which 50% of their activity is lost). Contrary to earlier reports (Alexendrov, 1964) that thermotolerance is due to thermostability of proteins which is determined genetically (Alexandrov et al. 1961) it is not found to be a phylogenetic character of a species. It has been repeatedly reported in case of higher plants as well as lower organisms that hardening can be induced by heat shock (Alexandrov et al 1961). As reported by Alexandrov (1970) heat injury in our experiments is also found to be a balance between destructive (degradation of protein) and constructive (repair) effects. At $35^{o}C$ repair is more whereas at $40^{o}C$ it is overcome by destructive effects. Decline in

concentration of Chl. and decrease in rate of O_2 evolution supports the hypothesis of Alexandrov (1964) who postulated photosynthesis suppression at high temperatures that induce hardening. However heat tolerance of BGA takes place through out the temperature range for growth though not favouring formation of factors that stabilize all the different proteins. Contrary to this, decrease in protein concentration testifies the established fact of Feldman (1966) that stabilisation of specific protein molecules takes place during heat hardening. Cell hardening has also been indicated in the case of cucumber and wheat (Feldman et al. 1966). Bogen (1948) also proved that this stabilisation is due to folding of proteins. Results also tally with the concept that as against increase in thermostability of enzymes, its activity decrease with adaptation to high temperature (Christopherson 1963, Feldman 1968).

Absorption spectra of chlorophyll and phycocyanin pigments in vivo (intact cells fig 14) and in vitro (pigment extract Fig 15-16) did not indicate any significant alterations in their absorption characteristics. However, a significant decrease in the concentrations of chlorophyll and phycocyanin was noticed. (Fig. 15, 16). Temperature induced decline in phycocyanin content is revealed by the peak intensity (Fig. 14) which was more as compared to chlorophyll at relatively high temperature adaplation (40°C). Similar to Goedheer (1976), no

marked differences in phycocyanin/chlorophyll ratio were detected between cells grown at 27°C or 35°C. But contrary to their observation, ratio between phycocyanin and chlorophyll absorption measured at 633 and 680 nm, showed a marked decrease from 1.18 to 1.06 between cells grown at 27°C and 40°C. Similar to *in vivo* situation, the isolated pigments like chlorophyll and phycocyanin from control and temperature adapted algal cells, though not showing any alteration in their absorption characteristic their *per se* amount of pigments was much less in temperature adapted cells as compared to control.

Since phycocyanin is the major constituent of the light harvesting pigment system of cyanobacteria, and represents about 80% of the phycobilisomes proteins, we further studied the absorption characteristics of isolated intact phycobilisomes, from control and high temperature grown cells. No change in the absorption characteristics of phycobilisomes isolated from control and treated cells, was noticed. However, a marked decrease in the absorption intensity (Fig. 17) at 627 nm was evident. Since at room temperature the major peak at 627 nm originates from phycocyanin, it could be visualised that the decline in the peak is principally due to a decline in the content of this pigment. Stable peak of phycobilisomes absorption at 627 nm indicates that their composition and intactness has not been altered though their concentration decreases at high temperatures (35°C, 40°C).

Further, no significant structural alteration in phycobilisomes was noticed during analysis of room temperature fluorescence emission spectra (Fig. 18, with comparable amount of phycobilisomes measured by their protein content). This comparison indicates that temperature adaptation does not effect the structural composition of the phycobilisomes.

Fig. 19 shows that heat treatment inhibit cell growth even if normal conditions are provided to treated cells after temperature treatment. Although cells recover after the temperature treatment ($35^{o}C$, 4 hr, 5 hr) but some inhibitory effect of heat persists for ever. Fig. 20 and 21 show that after the treatment, once the inhibition starts affecting protein synthesis and biomass production, it does not get recovered in later stages. The failure for restoration of comparable cell growth, as that of control, even in short term temperature treated samples, may be a consequence of the reduced growth rate of cells due to temperature induced inhibition of cell metabolism.

Decrease in chlorophyll and PC concentration in treated cells is more pronounced than total protein or biomass inhibition. This fact indicates that some progenitor of pigment synthesis gets affected during heat treatment and is reflected during later stages of life cycle. Effect of $40^{o}C$ treatment, whether for 4 hr. or 5 hr., proves that this effect operates

immediately after the treatment. Although, it is not significant immediately, it goes on increasing during further growth. Heat effect on total protein and biomass is more pronounced at 40°C than at 35°C. This clearly points to the fact that 40°C treatment causes more damage during treatment, and has more persisting consequences. Chlorophyll content and PC concentration are much effected at 40°C for 4 hr. Inhibitory effect is clearly noticed in 4 hr treatment; further inhibition by 5 hr treatment takes place very slowly. This observation points to the fact that damage to chlorophyll and PC synthesis takes place during 4 hr treatment. Some crucial pigment protein seems to be severely effected that reveals its effect in the long run.

Results shows that temperature induced injury is an elastic (reversible) one. As reported by Hilberg (1900) growth of plants is inhibited at temperatures that are not immediately fatal. In higher plants (Bean, Pea) growth was inhibited at 45°C (Hilberg, 1900). Even fungus spores show effect of heat strain too high for growth. A 1-2 min exposure of a leaf of Nicotiana to 40°C produced a reversible sublethal heat weakening (Engelbrecht and Mothes 1960, 1964). In our case we can point out that heat strain at 35°C and 40°C for 4 hr or 5 hr is injurious although it does not stop the growth of organisms. The reason for this decline in growth is supposed to be due to the production of biochemical lesions. As reported by Kurtz (1958), if accumulation of an intermediate substance necessary for growth is

inhibited at high temperatures, growth inhibition may occur. In Neurospora crassa this type of injury is due to decline in conc. of adenine at high temperatures (Galston and Hands 1949). Langridge and Griffing (1959) have supported this concept in the case of some races of Arabidopsis thaliana. Similarly starr and Parks (1962) found that sterol synthesis by yeast was inhibited increasingly above 30°C.

Lepeschkin (1935) reported a kind of metabolic injury by breakdown of protoplasmic protein. Heat injury for short period had no effect on the total time needed to produce killing of Spirogyra. From this long period he also concluded that protein denaturation is reversible physically and chemically. Same conclusion was arrived at by Allen (1950) in case of bacteria. Hirsch (1954) reported similar observations in case of Neurospora crassa. According to these authors heat inhibition occurs when the speed of resynthesis of an indispensable component is unable to compensate for its degradation.

This inhibition may be due to uncoupling between oxidation and phosphorylation at high temperatures as reported in Corn (Kurkova and Andreeva, 1966). The decreased phosphorylation would certainly lead to decreased synthesis of protein as well as other substances. Similar observations have been made for Chlorella (Semenko et al., 1969). Contrary to this, the incorporation of amino acids into protein was seen to decrease

when Physarum was subjected to heat shock at 40°C for a period of 10 to 30 minutes (Schiebel et al. 1969).

In all the cases discussed above i.e. in higher plants, fungi, algae and bacteria inhibition of growth takes place due to any one or more of the reasons when temperature treatment was given between 30°C to 45°C for 2 min to 2 hrs. But in cyanobacteria same inhibition occurs only after 4 or 5 hr treatment. This clearly indicates that cyanobacteria are more heat tolerant than other organisms.

Heat treatment of 45°C for 4 hr leads to severe damage to cells. Decline in growth, protein content and dry weight biomass starts from the first day of treatment. Heat treatment of 4 hr at 40°C makes a decline initially but later on it recovers and approaches almost to the level of control cells. Cells that were given treatment of 5 hr at 45°C depicts very slow growth, then they start declining for 5 days (11th to 16th day). On 17th day they start recovering but do not reach level at par with the control cells. Same pattern is observed for total protein and biomass. Chlorophyll and PC inhibition is also similar to that of initial growth pattern of treated cells (45°C, 5 hr).

Heat injury at high temperature (45°C, 5 hr) is supposed to be direct one i.e severe and irreversible. As reported by Bolehradek (1935) this is induced by brief exposures to heat stress and appears either during heating or later. This killing

is due to protein denaturation (Bolehradek, 1935). During heat treatment a metabolic injury could result if a specific disturbance in the normal process occurs at high temperature leading to the formation of a toxic substance. This toxic substance in later stages causes inhibition of growth of those cells that had recovered from temperature effect earlier. Petinov and Molotkovsky (1957) suggest that heat injury is due to the toxic effect of NH_3 produced at high temperature. In cyanobacteria duration of this heat exposure is found to be very high to show direct killing effect in comparison to other organisms like higher plants and algae. Temperature treated cells show increasing intensity of fluorescence emission. Although the fluorescence intensity increases both for phycocyanin and chlorophyll, the degree of this increase was higher for phycocyanin. The situation was similar when cells were excited at 545 nm. This may be a consequence of uncoupling of phycobillsome from chlorophyll which in turn may effect the photochemistry of the membrane. However this transient change is found to be reversible (Fig 35) after 24 hr. of heat treatment ($45^{o}C$, 5 hr). The relative change in the alteration persist but to a much lower extent.

On the basis of these observations we may conclude that whatever the change due to damage to phycobilisomes and chlorophyll, taking place during heat treatment, it is recovered during subsequent period of recovery to a large extent.

CONCLUSION AND SUMMARY

Cyanobacteria possess photosynthetic pigments both Chl*a* and phycobiliprotein complexes. The phycobiliproteins play a major role as light harvesting pigments in cyanobacteria and red algae. Absorption bands of these two pigments lie in the region extending from 600 to 660 nm. Although many of the temperature induced photosynthetic processes have been studied in isolated chloroplasts, only a few have been carried out with cyanobacteria. The aim of the present work was to investigate the growth of blue green algal cells at elevated temperatures ($35^{o}C$, $40^{o}C$). Further, the effects of preheating algal cells on their growth pattern has also been examined. In particular, we have made an attempt to investigate the effect of elevated temperatures on PC, protein and PBsomes content and light absorption characteristics of photosynthetic pigments of algal cells.

Cells grown at high temperatures ($35^{o}C$, $40^{o}C$) showed a decrease in growth which is also accompanied by low level of total protein and dry weight of cells. Results indicate that elevated temperature adaptation not only impairs the growth rate of cells but also affects the overall cellular synthesis of the organism. The decline in the absorption at 550 nm may be a

consequence of slow rate of cell division. The failure of Phormidium to withstand temperature shock may be due to the absence of higher illumination as well as due to lack of enough carbondioxide. A significant decrease in the concentration of chlorophyll and PC was noticed. Their ratio (PC/Chl) measured at 633 and 680 nm show a marked decrease from 1.18 to 1.06 between cells grown at 27°C and 40°C. No change in the absorption characteristics of phycobilisomes isolated from control and treated cells was noticed. Since at room temperature the major peak at 627 nm originates from phycocyanin, it could be visualised that the decline in the peak is principally due to the decline in the content of this pigment. No significant change in fluorescence emission spectra of phycobilisomes indicate that structural identity of phycobilisomes has not been effected by treatment temperatures.

Preheating (35°C, 4 hr, 5 hr) algal cells show that heat treatment inhibit cell growth. Once the inhibition starts for protein synthesis and biomass production, it does not get recovered in later stages. Decrease in pigments concentration is more pronounced than total protein or biomass production. 40°C treatment manifests its effect immediately after the treatment (4 hr or 5 hr) which goes on increasing during further growth. Heat treatment (45°C, 4 hr) causes a severe damage to cells indicated by decline in growth, protein content and dry weight of biomass.

Later, it recovers and approaches almost equal to control cells. Cells that were given treatment of 5 hr at 45^{o}C depicts slow growth, declining for 5 days and then recovery, though they do not reach at par to control. Fluorescence emission intensity is more for treated cells when spectra was taken immediately after treatment and cells were excited both at 440 and 545 nm. This may be a consequence of uncoupling of energy transfer from PC to chlorophyll. However, this transient change is found to be reversible after 24 hrs of treatment at 45^{o}C, 5 hr.

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