The rich variety of colors displayed by nature has presented a challenge to both the chemist and the biologist. Enthusiasm regarding their synthesis in different living systems and their various functions have provided new insights into the intricacies as well as the beauties of nature.

Carotenoids are one of the most widespread and important pigments in living organisms. Their study has received considerable attention from biological scientists in recent years. Carotenoids are synthesized by some bacteria, algae, fungi and some higher plants. Animals, however, depend on their diet for the supply of carotenoids and subsequently transform them into characteristic pigments e.g. non aromatic carotenes and non aromatic xanthophylls in sponges. Considerable amount of fascinating work has been done on their chemistry and biosynthesis.

The term carotenoid refers to a group of unsaponifiable fat soluble pigments, grouped under terpenoids and are synthesized from isoprene units.

\[ \text{Isoprene Unit.} \]

The family of carotenoids include 'carotenes' which are hydrocarbons (e.g. lycopene, \(\beta\)-carotene etc.)
and Xanthophylls which contain oxygen in various functional forms as one or more alcohol groups and carbonyl groups (e.g., cryptoxanthin, lutein, zeaxanthin, etc.). The number of naturally occurring derivatives is considerable but most are derivatives of either \( \beta \)-carotene, \( \alpha \)-carotene, \( \gamma \)-carotene or lycopene (Fig. 1).

Most carotenoids are brightly colored due to the presence of a chromophore group, consisting mainly of a chain of conjugated double bonds in the molecule. The majority of carotenoids are tetra-terpenes, formed by the joining together of eight isoprene units. The linkage of these units is in the normal head-to-tail manner, except at the centre of the molecule where the order is reversed so that the \( C_{40} \) skeleton viewed as a whole, is symmetrical.

In the last 10-15 years, the widespread adoption of spectroscopic techniques, notably NMR and mass spectrometry has revolutionized structural studies in this field. As a result many new carotenoids have been recognized and the absolute configuration of natural carotenoids has been elucidated, about half of which are believed to be chiral. About 300 carotenoids are known, most of them being xanthophylls and the number of carotenes relatively small.
Fig. 1: Structures of some common carotenoids.
Although the occurrence of carotenoids has been reported for well over a century, the study of their chemistry may be said to have started only in the late twenties in this century, structures of some of the more readily available representatives were determined, largely through the classical work of Karrer and other important studies were undertaken by Kuhn, Zechmeister, Heilbron and their respective collaborators. Most carotenoids were given trivial names but recently, a systematic nomenclature recommended by IUPAC has been published (1). The chemistry of some naturally occurring carotenoids has been described by Straub (2) (Fig. 2).

After the structural details of carotenoids were known, the tracing of their biosynthetic pathway was easier. Several reviews have appeared on the biosynthetic and regulatory aspects of carotenoids in various organisms by Goodwin (3,4), Porter and Anderson (5), Britton (6), Liaason Jensen and Andrews (7) and McDermott et al (8).

Distribution of carotenoids in nature:

Carotenoids are widely distributed in nature. Their presence has been reported in higher plants, fungi, algae, bacteria, some insects, birds and in marine animals.
Fig. 2: Structures of some novel carotenoids.
Higher Plantss: Major carotenoids like β-carotene, lutein, violaxanthin and neoxanthin are reported in the leaves and some fruits of green plants (4). β-carotene is often accompanied by α-carotene. Cryptoxanthin and zeaxanthin are occasionally found as minor components of the Xanthophyll fraction. Phytoene and phytofluene are found in trace amounts. In the leaves of laticiferous plants of genera Euphorbia, besides all the major carotenoids, epoxide carotenoids and α-cryptoxanthin have also been identified (5).

In the seedlings, β-carotene, lutein, violaxanthin and zeaxanthin have been reported along with the chlorophylls (10-12).

Large amount of carotenoids, relatively species specific, are produced in some fruits e.g. rubixanthin in rose hips and capsanthin and capsorubin in red peppers (13-15). Tomatoes accumulate acyclic carotenoids like lycopene during ripening (16). 1,2-epoxy-carotenoids (epoxides of β & γ-carotene) have also been reported in tomatoes (17,18). Carotene or its derivatives are rapidly synthesized during ripening in some fruits e.g. red palm, yellow maize. A similar phenomenon was observed in oranges (19,20), pumpkins (21), mangoes (22-24) and various citrus fruits (25).
Higher carotenoid concentration has been reported in peels of ripened banana (26) and apple (27, 28). The colorless polyenes phytene and phytofluene are often present in carotenogenic fruits.

Carotenoids of flowers are characterized by highly oxidized xanthophylls especially the 5-6-epoxides such as auroxanthin and flavoxanthin. Some flowers contain species-specific pigments, for example echscholtzianth in Echscholtzia canadensis. Ornamental flowers like Marigold (Tagetes erecta) contain different carotenoids and their esterified derivatives in the petals (29).

A few roots contain significant amounts of carotenoids and among those that do, carotenes generally predominate, e.g., carrots and sweet potatoes; while in certain species large amounts of xanthophylls are reported e.g., yellow flushed potatoes and wild carrots. The presence of lycopene, phytene, phytofluene and norstokesene have also been reported in carrots (30, 31).

8) Algae: The major carotenoid pigments found in algae are β-carotene, lutein, violaxanthin, neoxanthin and small amount of α-carotene. Thus the carotenoid distribution in algae is very similar to green leaves of higher plants. The presence of β-carotene has been
reported in green algae *Bryopsis corticulans* (32), in diatoms and blue green algae (33), *Syrodinium galatheanum* contains diatoxanthin, diadinoxanthin, neoxanthin and three alkali labile unidentified carotenoids (34). Variations in the carotenoid pigments at different periods of the cell cycle of *Chlamydomonas reinhardtii* have been reported (35).

C) **Fungi**: In the case of fungi, the carotenoids are well distributed in the order *Mucorales* (36). The important pigments being present are the carotenoids, though minor components like zeaxanthin and cryptoxanthin have been detected occasionally. Mainly alicyclic carotenes like β-carotene, α-carotene, γ-carotene, δ-carotene, Σ-carotene etc., have been detected in fungi. Fungal carotenoids are frequently acidic. In heterothallic *Mucorales*, organisms like *Phycocystis baleaeanus* and *Alkalesus trispora*, the major pigment is β-carotene, though single cultures of these fungi are carotenogenic, mating produces a 15 to 20 fold increase in carotene content (37). This is attributed to the production of a sex hormone trisporic acid (71) (38). In *Neurospora crassa* lycopene has been detected along with alicyclic carotenes (39). In *N. crassa* carotenoids are localized in lipid globules and in the membranes of endoplasmic reticulum (40). In
the mycelium of G. trispora two pools of $\beta$-carotene have been detected (41). One of them is associated with the cell wall and heavy mitochondria and the other with the fat inclusions (42). Similarly two pools of $\beta$-carotene have also been reported in chloroplast (43).

The presence of different carotenoids have also been reported in Verticillium agaricinum (44) and in Laccinum genus (45).

D) Bacteria: In recent years much work has been done on the bacterial carotenoids. Many photosynthetic and non-photosynthetic bacteria are known to synthesize carotenoids. The photosynthetic bacteria of the family Athiorhodaceae are characterized by the presence of acyclic carotenoids, normally containing tertiary - hydroxyl and methoxyl groups at C-1 and C-1' and often with double bonds at carbon 3 and 4. Spirilloxanthin is normally present in photosynthetic bacteria (46). A carotenoprotein from the chromatophores of Rhodospirillum rubrum has been characterized (47). In Rhodopseudomonas spheroides and R. gelatinosa, spheroidene and hydroxyphorobolene have been detected (48-50). R. capsulata also contain desmethyl spheroidenone (51). Chlorobium limicola contains chlorobactene as the major carotenoid together with $\gamma$-carotene and variety of other carotenoids (52).

In nitrogen fixing bacterium Azotospirillum brasilense, nitrogen fixation and carotenoid biosynthesis simultaneously
progressed under conditions of less than 0.5 mM NH₄Cl
countentration in the growth medium (53). 2'-3' trans
dihydroxy -2-nor-β-carotene -3-4 dione and other
carotenoids have been detected from Rhizobium lupini (54).

Photo induced synthesis of carotenoids has been
found in non-photosynthetic bacterium Gravisibacterium
curangum (55). Flavobacterium dehydrogenase showed the
presence of a \( \alpha_{50} \) carotenoid Decaprenoxanthin (56).
β-\( \alpha\)-\( \beta \)-zeacarotene has also been reported in
flavobacterium species (57).

In Mycobacterium kansasii acyclic and cyclic
carotenoids such as lycopene, \( \alpha \)-carotene and \( \beta \)-carotene
are known to be present (58). Many strains of Staphy-
lococcus aureus produce distinctive orange or yellow
pigments. The major xanthophyll responsible for orange
color being staphyloxanthin (59).

In gliding myxobacteria, the pigments are mainly
carotenoid glycosides (60,61). In Myxococcus fulvus
myxobacton is known to be present (62,63). Anaerobic
spirochaetes have shown the presence of torulene (64).
In Halobacterium, bacterioruberin and carinaxanthin
have been reported (65,66). Bis-anhydro bacterioruberin
of Corynebacterium poinsettiae (67) is an example of
higher carotenoids.
C) **Animals:** In animals, sometimes carotenoids are bound to a protein and form a carotenoprotein complex. These complexes occur widely among invertebrates. The known example is *crustacyanin* from lobster *Homarus aequalis*. Carotenoprotein is also found in the skin of starfish *Asterias rubens* (68). Sponges are filter feeding animals and their carotenoids are derived either from their diet or from symbionts. Several sponges are capable of aromatizing carotenoids of dietary origin (69). The sponge carotenoids are grouped as non-aromatic carotenes, non-aromatic xanthophylles, ocyl carotenes and aromatic oxygenated carotenoids. Carotenoids have also been separated from crab *Portunus puber*, prawn *Macrobrachium birmanicum chaores*, and fish like *Heteropneustes fossilis* as well as in copepod - *Diaptomus novadensis* (70). Recently carotenoid pigments have been isolated from the ladybird beetle *Coccinella septempunctata* (71). The pigments of the ladybird beetle seem to be of microbial origin rather than plant origin. In other insects like *Papilio* species, the pigments are of plant origin (72).

**Functions of carotenoids:**

A large variety of functions have been attributed to carotenoids and have been reviewed by various authors.
(73-77,3). The functions of carotenoids can be broadly divided into two groups, the photofunctions and the non-photofunctions.

1. Photofunctions: Those functions which involve direct or indirect effects of light as mediated by carotenoids are termed photofunctions.

a. Photoprotection: The protective role of carotenoids against irreversible photo reduction was discovered in green plants by Wolf and Witt (70,79). *Rhodopseudomonas sphaeroides* and *Sarcina lutea* defective in the production of colored carotenoids is photosensitive in the presence of air (80,81). In *Rhodopseudomonas sphaeroides* it has been found that the formation of special carotenoid triplet states via very rapid energy transport from excited chlorophyll and their fast radiation-lose decay is at least one mechanism for protective action of carotenoids to irreversible photo-oxidation of the chlorophylls (82-84). Carotenoids quench triplet states of chlorophyll and serve to protect the photosynthetic apparatus from chlorophyll triplet sensitized photo-dynamic damage. The bacteriochlorophyll of the carotenoidless photosynthesis center isolated from *Rhodospirillum rubrum* was bleached irreversibly when exposed to intense infrared light in the presence of oxygen, then four
purified bacterial carotenoids (sphaeroidene, Sphaeroidenes, spirilloxanthin and Chloroxanthin) were fixed on to them, they conferred protection against this photodynamic bleaching (85). Study with effect of superoxide, singlet oxygen (generated from toluidene blue activated by red light) and hydroxyl radicals (generated from superoxide radicals) on a white mutant strain and wild type Micrococcus luteus have shown that treatment of cells with hydroxyl radicals results in a slight loss of viability in carotenoid containing strain, but a substantial loss of viability in a carotenoidless mutant. It is also reported that carotenoid protects cells not only against singlet oxygen but also from the destructive hydroxyl radical (86). Study with maize have shown that carotenoids with ionone ring at both ends prevent the formation of semicrystalline-chlorophyll and hence stabilize the molecular architecture of thylakoids (87).

It has been reported that patients suffering from light sensitive erythropoietic photoporphyrin developed tolerance to sunlight on treatment with β-carotene supplemented diet (88). Individuals of the copepod Diaptomus havadensis that contain high concentration of carotenoids survive significantly better in natural intensities of visible light than less pigmented copepods (89).
b. Photosynthesis: Carotenoids are essential components of the photosynthetic membrane in micro-organisms and higher plants. They function as accessory light harvesting pigments and protect against chlorophyll sensitized photo-oxidation. Many articles have appeared on the role of carotenoids in photosynthesis (90,91,97). Three main pigment protein complexes have been isolated from chromatophores of purple photosynthetic bacteria: light harvesting complex (LHC), light focusing complex (LFC) and reaction center (RC) (92). The molecules of colored carotenoids stabilize the structure of LHC and LFC in photosynthetic bacteria (93). It has been observed that light absorbed by carotenoids can be transferred with 100% efficiency to chlorophyll of both photosystem I and II (94,95). Carotenoids perform an antenna function by transferring the energy of absorbed light at the singlet excited state level to the chlorophyll system for the execution of photosynthesis (95). Bishop (96) demonstrated that a carotenoid minus mutant of *Scenedesmus obliquus* could not carry out the reactions associated with photosystem II whereas photosystem I remained unaffected.

Carotenoids also have a role to play in phototrophism (97-103) and phototaxis (104).

2) Non-photo functions: The widespread occurrence of carotenoids in the reproductive structure of plants and
animals has been noted since 1950 (105).

In fungi, of order *Mucorales*, &carotene is a precursor of the sex hormone trisporic acid and hence plays an important role in sexual reproduction (106, 107).

In spore coats, pollen grain and in walls of many algae and fungi, a cross linked polymer sporopollenin is present. It is an oxidative polymerized product of carotenoids (108, 109). It probably imparts resistance to the wall against biological and chemical attack (110). The ascospore walls of *Neurospora crassa* and *L. intracellularis* as well as the zygophores of *Mucor mucido* are known to possess sporopollenin (111).

The role of carotenoids in the color polymorphism of two species of the sea anemone genus *Anemonopsis* - *A. granulifera* and *A. cavernata* has been reported. The three color morphs are subjectively classified as red, orange red and orange. This is due to the presence of varying concentrations of three different carotenoids - astaxanthin, 2'-norastaxanthin and 2,2'-bionorastaxanthin. This varying proportions of carotenoids represent genetic differences in the carotenoid bio-transformation pathway. Hence carotenoids serve as markers of genetic differentiation between populations and species (112, 113).
The function of carotenoids in nitrogen fixing bacterium *Azospirillum brasilense* strain cd has been reported recently. In this bacterium, carotenoids protect the nitrogenase enzyme from oxidative inactivation (53).

The distribution and role of carotenoids in leaves of *Nicotiana tabacum* (tobacco plants) during ripening has been elucidated. Carotenoids are the precursors of tobacco flavour constituents.

Applications of carotenoids include their use as food colors, as pigments in poultry feed, in fish feeds, as Vitamin A precursors in animal feeds and as colors for pharmaceutical and cosmetic products. Patients with certain dermatological diseases are treated with carotenoids to alleviate the photosensitivity associated with these conditions. Recently, Vitamin A and its derivatives have been shown to have anti-tumor activity. Experiments with animals have suggested that carotenoid pigments, irrespective of their Vitamin A activity, may have anti-tumor activity for light induced (UV-B) tumors of the skin (114). The protective effect of carotenoids in the animal gastric ulcer formation has also been reported (115). Vitamin A and β-carotene inhibit tumor regrowth after the tumor burden was reduced by radiation or chemotherapy.
they improve the anti-tumor response to radiation and chemotherapy (110). Carotenoids have proved to be helpful in the prevention of skin cancer in occupational workers dealing with tar and phototoxic substances in open fields (117).

Biosynthesis:

The biosynthesis of carotenoids has been extensively studied in various systems and several reviews have appeared on the subject (5, 118-122).

Carotenoid structures are built from C₅ 'isoprene' units (123). The basic C₅ unit, isopentenyl pyrophosphate (IPP) was shown to be the building block for carotenoids (124). IPP can either be formed from acetate (Fig.3) or leucine (Fig.4).

The carotenoid biosynthetic pathway can be divided into four sections: 1) the formation of the universal isoprenoid precursor — IPP via mevalonic acid (MVA) (Fig.3), 2) the conversion of IPP in to the first C₄₀ carotenoid phytoene (Fig.5), 3) the formation of the fully unsaturated acyclic lycopene (Fig.6) and finally, 4) the cyclization of lycopene to cyclic carotenoids like β-carotene (Fig.7).

1. **Formation of IPP**: The utilization of acetate for the synthesis of β-carotene was first demonstrated in
CoASH
2CH₃COSCoA → CH₂COCH₂COSCoA
Acetyl CoA → Acetoacetyl-CoA
+ CH₃COSCoA
2NADPH, H⁺ CoASH
CH₃C(OH)CH₂OH → CH₂C(OH)CH₂COSCoA
CH₂COOH CoASH 2NADP⁺ CH₂COOH
β-Hydroxy-β-methylglutaryl-CoA
Mevalonic Acid
2ATP 2 Steps 2ADP
CO₂, H₂O
CH₃C(OH)CH₂CH₂O−P−P− → CH₃C(OH)CH₂CH₂O−P−P−CH₃
CH₂COOH ATP ADP+P
Methylmalonyl 5-phosphate
Isopentenyl pyrophosphate

Fig. 3: Conversion of acetyl-CoA into isopentenyl pyrophosphate.
Fig. 4: Formation of HMG-CoA from leucine.
Fig. 6: Conversion of isopentenyl pyrophosphate into phytoene.
An alternative pathway from phytoene to lycopene
(For ease of presentation phytoene is represented in its all-trans form):
Fig. 7. Cyclization pathway for formation of β-carotene.
*P. blakoeleanus* (125-127). Condensation of the two molecules of acetate CoA in the presence of an enzyme α-keto thiolase leads to the formation of acetoacetyl CoA. This acetoacetyl CoA condenses with another molecule of acetate CoA to give a C₅ compound β-hydroxy, β-methyl glutaryl CoA (HMG CoA) in the presence of an enzyme HMG CoA synthetase. The CoA moiety of acetoacetyl CoA is retained in the HMG CoA (128,129). HMG CoA can also be formed from aminoacid leucine and valine (130). The pathway is known to be operative in *P. blakoeleanus* (131), green tissue (132), *E. gracilis* (133), *Chlorella pyrenoids* and *B. trispora* (134,135).

HMG CoA undergoes an essentially irreversible two step reduction to give mevalonic acid (MVA). This reaction requires an enzyme HMG CoA reductase and two molecules of NADPH (136-138). The incorporation of HMG CoA and HMG into terpenoids by intact cells and cell free systems has been accomplished (139-141). The incorporation of MVA into carotenoids has been shown in several systems such as *Acidophilus ATCC 4963* (142), *P. blakoeleanus* (130,141,143,144), *Nocor hiomalis* (145), *C. rosea* (146), yeast (147-149), *B. trispora* (134), *E. gracilis* (133), higher plants (150,151), carrot root preparations (143,144), tomatoes (143,152,153) and liver (154).
MVA is converted into MVA-5-phosphate. The enzyme responsible for this phosphorylation is MVA kinase (Mevalonate kinase) (155-157). It is an ATP, Mg\(^{++}\) requiring enzyme. In some systems, the enzyme requires cysteine-hydrochloride or glutathione for activation. It can be inhibited by parachloromercuri benzoate (PCMB), suggesting the involvement of a \(-SH\) group at the active site (158). MVA-5-phosphate is rephosphorylated to form Mevalonate-5-pyro phosphate (MVAPP) with the help of enzyme 5-phospho mevalonate kinase (159). It is also an ATP, Mg\(^{++}\) requiring enzyme. Both these phosphorylating enzymes have been reported in different systems (160-169). MVA kinase has been reported in E. triopora (170), yeast cells (158), liver (171), plant systems (162,156), E. gracilis and Chlamydomonas (172). The cell free extract of carrots and E. triopora can synthesize carotene from acetate and MVA (173,174). The MVA concentration of fruit and vegetables has been reviewed (175). The enzyme responsible for the conversion of MVAPP to IPP is MVA-pyro phosphate anhydro decarboxylase (176). This reaction has been well worked out and is known to occur in several systems (177-180). MVA kinase, 5-phospho MVA kinase and MVA pyrophosphate anhydro decarboxylase from rat liver are known to be inhibited by phenyl and phenolic compounds (181).
IPP is isomerized to dimethyl allyl pyrophosphate (DMAPP) by the enzyme IPP isomerase (149,182,183). The reaction is Mg ++ dependent and sensitive to iodoacetamide (184). DMAPP acts as a starter for the polymerization of isoprene units. It condenses with one molecule of IPP to form a C_{10} unit geranyl-pyrophosphate (GPP). Two enzymes have been recognized for this reaction. One is farnesyl-PP synthetase (185,186). Another is a newly discovered prenyl transferase, which is specific for C_{10} formation. This enzyme has been isolated from Micrococcus lysodeikticus (187) and spinach chloroplasts (188). It can be inhibited by farnesyl-PP (189).

The addition of one IPP molecule to geranyl-PP (GPP) results in the formation of farnesyl-PP (FPP) (185,190-192). This reaction requires enzyme FPP synthetase. Thus it can carry out the synthesis of both C_{10} and C_{15} molecules.

The addition of second IPP unit results in the formation of geranyl geranyl-PP (GGPP) (193,194) (Fig. 5). The enzyme responsible for this reaction is GGPP synthetase. It has been observed that FPP gets incorporated into β-carotene when cell free extracts of
P. blackeadeanuus (195), tomato plastid (196) and carrots are used (196); however, addition of RVA enhances this incorporation indicating that C<sup>20</sup> unit is probably a preferred substrate to the C<sup>15</sup> compound for carotene synthesis. In yeasts, it was demonstrated that FPP gets converted to GGPP by the enzyme GGPP synthetase (193). The presence of the above enzyme has also been shown in carrots (197) and M. lysodeikticus (198). GGPP was shown to be a direct precursor of carotenoids in a Phycomyces cell-free preparation (199), tomato plastid enzyme (193) and bean leaf chloroplasts (200). Salubilized membrane fractions of Micrococcus luteus is able to synthesize a mixture of labelled FPP and GGPP from 2-<sup>14</sup>C RVA. From this extract a protein (M.W. 210,000) has been isolated which is able to synthesize FPP and GGPP. This protein is composed of six polypeptides. In this organism, the enzymes for conversion of RVA to poly isoprenoid-PP form a membrane-bound multienzyme complex (200).

GGPP synthetase activity increases with decrease in FPP synthetase activity suggesting different control mechanisms of sterol and carotene pathways once the C<sup>10</sup> unit is synthesized (201).

Condensation of two GGPP molecules leads to the formation of prlycopersene. This has been demonstrated
in some carrot strains (202) and \textit{N. crassa} (203). However, later investigations failed to demonstrate the presence of prolycopersene (196, 204).

Prephytoene-PP (prolycopersene-PP) is an intermediate between GGPP and Phytoene (Fig. 5) and has been isolated from \textit{N. crassa} preparation (205). In baker's yeast preparation the conversion of GGPP to prephytoene-PP and lycopersene has been demonstrated (205). The enzyme pre phytoene-PP synthetase responsible for this reaction has been isolated from tomato plastid preparations (207). The incorporation of \textsuperscript{14}C GGPP into lycopersene by tomato plastid cell free system has been studied by Barnes et al (200). They have also shown the conversion of \textsuperscript{14}C lycopersene and \textsuperscript{14}C prolycopersene-PP to phytoene and lycopene. Earlier, a mechanism for the formation of phytoene by tail to tail condensation of two GGPP molecules had been reported (209,210).

The formation of phytoene from 2-\textsuperscript{14}C-CAVA using cell free extracts of \textit{Phycomyces} strain C \textsuperscript{115} (\(\beta\)-carotene accumulating) and strain C \textsubscript{9} (lycopene accumulating) has been reported. This reaction was not enhanced by FAD or pyridine nucleotides. Thus, phytoene synthesis does not require cofactors (211). The incorporation of 2-\textsuperscript{14}C-CAVA into phytoene and other more unsaturated carotenoids has been reported in membrane bound fraction of \textit{N. crassa} (212).
3. **Formation of lycopene**— Phytoene undergoes successive losses of two hydrogen atoms to give rise to phytofluene, \( \gamma \)-carotene, neurosporone and lycopene (fig.6). The conversion of phytoene to lycopene has been demonstrated in several systems, e.g. cell-free preparations of *P. blakesleeanus* (213), *Flavobacterium spp* (214), chloroplast preparations (215) and tomato plastids (182). These four successive desaturations are carried out by the dehydrogenase complex, containing four copies of the same enzyme (216). Two inhibitors of the dehydrogenation step have been reported. Diphylleline inhibits dehydrogenation in *Myxococcus fulvus* (217), *Rhodospirillum rubrum* (218), *P. tripora* (219) and *P. blakesleeanus* (220). Another inhibitor is a herbicide sandoz 6706 which causes accumulation of phytoene in wheat seedlings (221) and *M. fulvus* (217). The conversion of \[^{14}C\]ALA into both phytofluene and \( \gamma \)-carotene by *P. blakesleeanus* strain C115 is increased on addition of either FAD or NADP while the formation of lycopene by *P. blakesleeanus* strain C12 is stimulated by both NADP and NAD. NADP also enhances the biosynthesis of phytofluene, \( \gamma \)-carotene and neurosporone. Thus phytoene desaturation requires cofactors (211).

4. **Formation of cyclic carotenoids**— The cyclization of neurosporone or lycopene occurs to give rise to bicyclic
\( \alpha \)-carotene, \( \beta \)-carotene and \( \varepsilon \)-carotene via the monocyclic
\( \lambda \)-zea carotene, \( \beta \)-zeacarotene, \( \gamma \)-carotene and \( \delta \)-carotene.

Two pathways exist for the formation of \( \beta \)-carotene —
one from lycopene and the other from neurosporene.

Kushuaha et al (222) have demonstrated the conversion
of lycopene to \( \gamma \)-, \( \varepsilon \)-, \( \lambda \)- and \( \beta \)-carotene by soluble enzyme
systems from tomato fruit plastids and spinach chloro-
plasts (fig. 8). This reaction probably requires FAD
and NADP. The incorporation of labelled lycopene,
\( \gamma \)-carotene and neurosporene into \( \beta \)-carotene by cell
free extracts of \( \text{P. blakesleeanus} \) has been reported (223).

Studies with mutants of this mold favour the conversion
of neurosporene to \( \beta \)-carotene bypassing lycopene (224).

Two known inhibitors of the cyclization steps, nicotine
and 2-(4-chlorophenylthio)-triethylamine (CPTA) accu-
sulate lycopene in different systems like grape fruits
(225), citrus fruits (226), Cucurbit cotyledons (227),
\( \text{P. blakesleeanus} \) (228), \( \text{G. triapora} \) (229), \( \text{G. fulvus} \) (217)
Mycobacterium marinum (230) and halophiles (231). In
experiments with organisms other than filamentous
fungi, the evidence in favour of lycopene is greater
but the natural occurrence of \( \beta \)-zeacarotene makes it
clear that cyclization can take place at the neurosporene
level (142). Chloroplast system prepared from \( \text{Capsicum}
annuum} \) fruits has shown that neurosporene and lycopene
are cyclized into \( \beta \)-carotene. However, lycopene is the
Fig. 1: Possible cyclisation pathways for the formation of β-carotene. --- → Two or more reactions → Direct reactions.
preferred substrate (232). The $C_{50}$ bacterioruberin is made by the addition of a $C_{5}$ IPP unit to each end of $C_{40}$ lycopene chain followed by introduction of four $-OH$ groups (233).

Not much information is available regarding the biosynthesis of xanthophylls. It is generally accepted that the introduction of oxygen functions into carotenoids occurs at a later stage in the biosynthesis, e.g. xanthanthin is thought to be formed by hydroxylation of $\beta$-carotene. It has been established by using an $18O$ isotope that the oxygen of hydroxy and epoxy substituents in the xanthophylls of *Chloroally* and *Phaseolus lunatus* comes from molecular oxygen rather than from $H_2O$ (234). Dark grown cultures of *Scenedesmus obliquus* contain large amount of $\beta$-carotene. On exposure of this cultures to light, accumulated $\beta$-carotene is converted to xanthanthin and lutein (235). Using deuterium labelling it has been observed in *Rhodopseudomonas vannii* and *Rhodospirillum rubrum* that lycopene is converted into rhodopin and then spirilloxanthin, while in *Rhodopseudomonas* the conversion of neurosporene into the spheroidene and then hydroxyspheroidene has been observed (236).

In *Flavobacterium* cell free preparations the incorporation of labelled mevalonate into xanthanthin was shown (237).
Many reviews have appeared on the bio-synthesis of $C_{45}$ and $C_{50}$ carotenoids and hydroxy as well as methyl carotenoids (120, 121, 239, 239).

Factors affecting carotenogenesis:

1. Nutritional control: In $P$. blakesleeanus it was observed that for carotene production, maltose and glucose were better as compared to xylose and fructose (240). Glucose is the best carbon source for carotenogenesis in $R$. trispora, $P$. blakesleeanus and $R$. oryzae (240). Dextrin and sucrose stimulate carotenogenesis when hydrolysed casein is used as a nitrogen source (241). Glycerol is the most effective single carbon source in Rhodotorula rubra (76), Mycobacterium phlei (242) and $R$. rhodochnorus (243). Gloeocapsa emersonii is normally non-carotenogenic but in the presence of high level of bicarbonate, it synthesizes $\gamma$-carotene (244). Lower concentrations of glycerol stimulate carotene production in an extreme halophile $H$. cutirubrum (245).

Leucine and valine enhance carotene production in $P$. blakesleeanus (131). Asparagine and glycine are equally effective in promoting carotenogenesis in $R$. trispora (73), while leucine and valine stimulate carotenogenesis in suboptimal concentration of glucose (246). Leucine is known to be an early precursor of carotenogenesis in the chloroplasts of higher plants (142, 247). In $R$. trispora, the inhibition of growth and carotene synthesis were
This inhibition is reversed by Kreb's cycle intermediates, the extent of restoration depending on the final pH of the medium (248). Glycine is effectively incorporated into β-carotene by \( \text{P. blekeaeleanus} \) (249). In \( \text{P. blekeaeleanus} \) and \( \text{Sporobolomyces roseus} \), the carotene level depends on the Ca/N ratio in the medium (250, 251). In \( \text{Rhodotorula glutinis} \), Ag\(^{++} \) stimulates carotenogenesis (252). On the usual glucose asparagine medium the initial pH (6.2) drops to 2.6 - 3.0 during growth of \( \text{P. blekeaeleanus} \). If the pH change is prevented by buffering the medium, carotenogenesis is almost completely inhibited although growth is unaffected (248). Similarly, washed mycelia dissimilating glucose will produce β-carotene only in an unbuffered medium (250).

The role of different vitamins and growth factors in various microorganisms has been discussed by Goodwin (73).

The effect of various antibiotics on carotenogenesis has been studied (253, 254). Streptomycin inhibits carotenogenesis in \( \text{E. gracilis} \) and \( \text{P. blekeaeleanus} \). The replacement of asparagine in the culture medium by \( \text{NH}_4\text{NO}_3 \) renders the mold insensitive to streptomycin (253). Antifungal antibiotics like mycostatin, aureofungin and
amphotericin-B are inhibitory to carotenogenesis in

\( \text{A. trispora} \) and \( \text{N. crassa} \). Gracofilvin stimulates
carotene synthesis in \( \text{N. crassa} \) but inhibits in \( \text{A. trispora} \)
(170). In \( \text{N. crassa} \) penicillin and ampicillin inhibit
carotenogenesis but in \( \text{A. trispora} \) these two antibiotics
have a stimulatory effect (170, 254).

2. Effect of Light—Light stimulates carotenoid
synthesis in \( \text{A. blakeadeseanu} \) and \( \text{Penicillium oxysporum} \)
which normally form reasonable amounts of carotenoids
in the dark (255-258). In \( \text{Chlorococcea fritschii} \)
(Cyanobacteria), light affects the quantitative carote-
noid composition and the fixation of carotenoids into
the photosynthetic membrane (259). Photoreceptor system
of mycelial cells of \( \text{N. crassa} \) is known to control
synthesis and dehydrogenation of phytoene and formation
of colored carotenoids. In addition to this, light can
directly control synthesis and dehydrogenation of
phytoene and formation of colored carotenoids. Again,
light can directly control the levels of coenzymes like
\( \text{NAD}^+ \), \( \text{NADP}^+ \) and \( \text{NADH} \) necessary for these reactions (260).
Photo regulated synthesis of membrane bound caroteno-
genic enzymes in \( \text{A. crassa} \) has also been reported (261).
In \( \text{A. blakeadeseanu} \) light inhibits the destruction of
\( \beta \)-carotene \textit{in vivo} (262). Light affects caroteno-
genesis both qualitatively and quantitatively in higher
Higher intensity of light favors zeaxanthin and violoxanthin formation at the expense of \( \beta \)-carotene and lutein in maize seedlings (265), leaves (73), algae (266) and photosynthetic bacteria (267). In *E. gracilis* var. bacillaris grown heterotrophically, complete exclusion of light results in a reduction in the carotene content (266). There is a stimulation of carotene formation by light in *Fusarium aquaeductum* (269), *Mycobacterium* species (270), *N. crassa* (271) and *P. blakesleeanus* (272), but reduced carotene synthesis in *Choanephora cucbitctorum* (273), *Blastocladiella emersonii* (274) and *P. tripora* (275). Light acts as a trigger to carotene formation in *N. crassa* (276), *P. blakesleeanus* (266) and *Fusarium oxyseproum* (277). The ribosomal RNA is implicated in the photo induction of carotenogenesis in *Verticillium alboatrum* (278). The features of chromogenic response and the mechanism of carotenoid synthesis in *Mycobacterium marinum* (279) and *P. kastenii* (280) are known. \( \text{H}_2\text{O}_2 \) (\( 10^{-1} - 10^{-2} \text{M} \)) and PCMB (5x10^{-5}M) stimulate the photo induction of carotenogenesis in *Fusarium aquaeductum* (278).

3. Temperature and \( \text{O}_2 \) requirement: Carotenoid synthesis in *P. blakesleeanus* is qualitatively the same in cultures grown over the temperature range 5°-25°C.
the optimum being 25°C (281). The effects of temperature and O₂ on carotene formation have been reviewed by Goodwin (73). In photosynthetic organisms, strongly aerobic conditions inhibit carotenogenesis (287), while semi-aerobic conditions markedly reduce the carotene content (282). In fungi lowering the incubation temperature lowers carotenogenesis (73). F. griseus produces more carotene at low temperatures (283). In bacteria low temperatures favor carotenogenesis (242, 243).

4. Stimulators: Anderson et al (284) have reported that various acid hydrolysates of wheat, oats, and soyabean products stimulate carotenogenesis. Cieglar et al (285) have used β-ionone and citrus products like citrus pulp, citrus molasses and essential oils from citrus industries to stimulate carotenogenesis in F. tricomprense.

It has been reported earlier that β-ionone, vegetable oils and hydrocarbons stimulate carotenogenesis (285, 286). Later on, it was observed that β-ionone, α-ionone, abscisic acid and Vitamin A bear a structural similarity to triosporic acid, a sex hormone of Fucorales which stimulates carotenogenesis and prove stimulatory to carotene formation (287). Vitamin A, β-ionone and α-ionone are stimulatory to carotene synthesis in
Various TCA cycle intermediates stimulate carotenogenesis (253). Compounds like \( \beta \)-([diethylamino]-propoxy)-benzene, \( \delta \)-([diethylamino]-butoxy)-benzene, 4\( \beta \)-([diethylamino]-ethoxy]-benzaldehyde and diethylamino ethyl anisolate caused a 5 to 12 fold increase in carotene content in grape fruits with an accumulation of lycopene. The mode of action of these components appears to be similar to CPTA (289).

In grape fruits, 2-([diethylamino]ethanol) esters of benzoic, hexanoic, 4-phenyl butyric and cinnamic acid stimulated carotenogenesis (290). Carotene synthesis in citrus fruits is stimulated by variety of tertiary amines and lycopene becomes the major pigment (225). In 8-trispora treatment with amines like triethylamine hydrochloride, tributylamine hydrochloride and 4\( \beta \)-([diethylamino]-ethoxy]-benzaldehyde showed an increase in the accumulation of lycopene (291). In various systems, compounds of the general formula \((C_2H_5)_2N\cdot CH_2\cdot R\) stimulate the production of trans carotenoids whereas compounds of the type \(-CH_2\cdot N\cdot R_1\) regulate the biosynthesis of cis carotenoids (292). Various metal ions like Fe\(^{++}\), Mg\(^{++}\), and Cu\(^{++}\) stimulate carotenogenesis in 8-trispora and Cu\(^{++}\) proved to be the best (293).

5. **Inhibitors:** The mode of action of a range of
herbicides is expressed in two principal ways – firstly by blocking the synthesis of carotenoids in developing tissues and secondly by inducing the destruction of pre-existing carotenoids in mature tissues. In the first case the desaturation reaction between phytanediol and norcanthadiene are the major sites for herbicidal attack. In the second case the herbicides act indirectly through inhibition of chloroplast electron transport and cause a breakdown of a photo protection mechanism that is already operational. This has been seen in green plants and in algae (294-298).

Another compound Di-n-butylphthalate (DBP) is a widely used plasticizer. In green plants it causes chlorophyll deficiency, as well as accumulation of phytene, at the same time carotene and xanthophylls are more or less completely lacking (299).

Regulation of carotene biosynthesis:

1. Genetic regulation: The genetic regulation of the carotenoid pathway has been widely studied using mutants of P. blakesleeanus defective in carotenoid production (103, 257, 380). Three main types of mutants of this mold have been isolated a) those accumulating lycopene, b) those accumulating phytene and c) those accumulating small amounts of β-carotene but no other carotenoids.
Genetic complementation studies revealed that each typo corresponds to mutants in a single gene termed car R, car B, and car A respectively (301). Studies with heterokaryons of phytene accumulating mutants revealed that four copies of the car B gene product associated in an enzyme complex act sequentially in the conversion of phytene to lycopene (302). Phycomyces heterokaryons containing two types of nuclei, one type from a strain failing to produce carotenoids and the other from a strain accumulating lycopene are of many different shades of color, from white to orange according to the proportion of the individual nuclei (303). The observation suggested that perhaps a mixture of carotenoids was due to the operation of dehydrogenase complex containing four copies of the same enzyme. Two copies of an enzyme lyase are known to be present for the cyclization of lycopene (304).

On treatment with CPTA, there was an increase in the carotene content in gene car A mutants. Lycopene and ß-carotene being the major pigments (305). The effect of CPTA was not observed in the presence of cycloheximide, known inhibitor of protein synthesis. This suggested that the blocking of cyclization of lycopene, release a negative control by ß-carotene and that gene car A is involved in this regulatory mechanism. Probably the
effect of car A mutation is an extreme reduction in the basal level of β-carotene required to trigger the negative control. Three independent mutants of P. blakesleeanus resulting in over accumulation of β-carotene are recessive and belong to the same complementation group. The corresponding gene has been named car 3 (306). It is suggested that the car 3 gene product participates in the end product regulation of the pathway. Car A and Car R are probably segments of a single bifunctional gene (307).

By genetic manipulation β-carotene and lycopene super producing strains of P. blakesleeanus have been isolated (308).

Light inhibits the destruction of β-carotene in P. blakesleeanus (262). Two mutants, mad A and mad B, have been isolated which are 'blind' for phototropism, mad B gene product is necessary for the light effect (262).

The carotenoid mutations of Rhodopseudomonas capsulata map in 5 tight clusters which may correspond to separate genes, all situated within a small chromosome segment. This raises the possibility of a coordinated regulation in a 'carotenoid-operon' (309). In this organism, neurosporene to spheroidene conversion steps have been identified by mutation studies. Mutations
in the Crt B or Crt E gene result in the absence of all carotenoids. In one strain U4 of *R. capulata* the light harvesting II (LHII) antenna complex have 3 polypeptides (310). Two uncomplexed polypeptides out of 3, inhibit carotenoid biosynthesis. The genes coding for these polypeptides have been cloned from an R-prime plasmid carrying all the genes for photosynthesis. It has been hypothesized that the R-prime and its cloned derivatives may regulate the expression of the genes for carotenogenesis (311).

*Halobacterium halobium* shows spontaneous mutations in this ability to produce C\textsubscript{40} and C\textsubscript{50} carotenoids. Mutant U\textsubscript{4002} is found to contain C\textsubscript{50} pigment, neurosporene and lycopene but no \( \beta \)-carotene. Mutant\#3 does not produce C\textsubscript{50} pigment but synthesizes lycopene, \( \beta \)-carotene and retinal. Mutant U5002-1 has been found to produce only phytoene and does not synthesize either C\textsubscript{50} or any other C\textsubscript{40} carotene beyond phytoene. This shows a block between the conversion of phytoene to phytofluene while mutant\#3 shows block between the conversion of lycopene to C\textsubscript{50} carotenoids. U4002 shows block in the conversion of lycopene to \( \beta \)-carotene.

2. **Biochemical regulation:** In *B. trispora*, mated cultures produce more carotene as compared to single cultures (38). This is attributed to the production of
A sex hormone, trisporic acid (TA), which derepresses some key enzymes in the carotene biosynthetic pathway (312). Later on it was shown that TA stimulates carotenogenesis by acting on the conversion of mevalonate-S-P\textsubscript{4} to dimethylallyl pyrophosphate (313).

An inhibitor protein (IP) to carotene biosynthesis has been isolated from single cultures of *B. trispora*. It is a heat labile, trypsin sensitive protein having a molecular weight of about 47000 (314). When individual strains are mated or when TA is added to single cultures, IP disappears. In mated cultures IP is replaced by stimulatory proteins (SP) to carotene biosynthesis (314). On mating, a membrane bound neutral serine protease was synthesized which showed IP degradative activity when it incubated with purified IP. There was an overall stimulation of carotene production when the IP-protease interaction mixture was added to the single culture (293).

On mating, there was a conspicuous rise in the content of cAMP (315). In mated cultures carotene synthesis decreased with increase in the glucose concentrations, the effect being reversed by exogenous addition of cyclic AMP. As cAMP addition does not affect carotenogenesis in the single cultures, it has been proposed that the TA synthesis in *B. trispora* is
catabolically repressed (315). TA, like cAMP, could also reverse the glucose effect on carotene production (316).

Photoreceptor system of mycelial cells of \textit{N. crassa} is known to control synthesis and degradation of phytene and formation of colored carotenoids. After illumination, during a long period, cellular concentration of cAMP but not cGMP falls down, the value of its change correlates with the rate of further accumulation of pigments. Mutant cells Cr-1 with a defective adenylate cyclase and low cAMP content demonstrate a 7-10 fold elevated levels of carotenoid pigments in the dark as compared with the wild type (260).

Triphoric acid:

Mucorales are either homothallic or heterothallic. According to Blakeslee (317) in heterothallic species the zygote results from a fusion of two different but compatible mycelia, whereas in homothallic species zygospores are produced in one thallus. There are two fixed mating types in heterothallic species, designated as plus and minus strain, then the plus and the minus strains of a heterothallic fungus grow near each other, they form specialized hyphal branches—the zygophores, to initiate the sexual process. On the mutual contact of the zygophores, wall between them is lysed and the
resulting heterokaryotic compartment develops into a zygospore (318-320).

Zygophore induction is caused by the production of a series of hormones, the triparic acids (TA) which are characteristically produced only by mixed cultures of plus and minus strains. A combination of column chromatography and thin layer chromatography has separated various forms of TA. About 80% of the total TA from *O. triparis* and *M. mucida* is a C_{13} alcohol which is known as TA C while about 15% is in the form of C_{13} ketone, known as TA B. Minute amount of C_{13} deoxy derivative -TA A are encountered (321-323). A C_{13} deoxy derivative and a C_{10} ketone have also been reported (324-326). The natural product is a mixture of cis trans isomers (322, 327).

Tracer studies by Bu'Lock et al. have shown that 14C labelled \( \beta \)-carotene, retinol and a number of C_{20} and C_{19} derivatives are efficiently incorporated into TA (329, 321, 324, 325, 328), particularly a C_{18} ketone and its 4-hydroxy derivative appeared to serve as good precursors for TA in mated cultures. In TA biosynthesis the hydroxylation at C_{4} might be an early step. The exact nature of these early intermediates is not known. There is no direct evidence of the nature of the enzymes involved in TA biosynthesis, but a
series of mixed function oxygenase seem to be involved.
Barbiturate, an activator of oxygenase system stimulates
TA synthesis in B. trispora (323). Sutter et al. (329, 330)
have reported that both plus and minus mycelia secrete
small amounts of sex specific metabolites which are
converted to TA by the mating partner only (Fig. 9). They
suggested that the complete biosynthesis of TA is only
accomplished by the cooperative action of both mating
types. Later on, the metabolites have been identified.
Plus cultures produce the methyl 4-dihydrotrisporins
and minus cultures produce the trisporins and trisporoles.
These are the major TA precursors. The plus derived
methyl 4-dihydro-trisporates are converted to TA by
minus cultures only (331). The oxidation of methyl 4
dihydrotrisporins to methyl trisporates is mediated
by a NADPH specific dehydrogenase which is present in
the minus strain only. An esterase is involved in the
conversion of methyl trisporates to TA. Both the de-
hydrogenase and esterase enzymes have a Km of 1 mM and
a pH optima of 7.8. The dehydrogenation proceeds the
demethylation because methyl 4 dihydro trisporate
esterase activity could not be detected in vitro.
moreover, 4 dihydrotrisporic acid could not be converted
to TA by minus mycelium. A homogenate of minus mycelium
efficiently oxidized the 4 dihydrotrisporins into
Fig. 9: Proposed pathway for the collaborative synthesis of trisporic acid by (+) and (-) cultures of *R. trispora*. 
trioporins. This reaction was NADP dependent. It has been assumed that 4 dihydrotrisporin is the natural precursor of the trioporins as well as the methyl 4-dihydro triospore. No studies on the enzymatic level have been reported of plus specific reactions (332, 322, 333).

The precursors of TA also known as pheromones, stimulate the development of zygoophores in bioassays with (-) cultures of Rhizopus oryzae and R. mucida (334). Plus cultures normally do not form zygoophores in the absence of a sexual partner because they inactivate sexually self stimulatory triospore precursors by removing a 3-carbon unit from their side chains (335).

Single cultures of R. trisporora produce minute quantities of TA (0.1% or less of the mated culture level) suggesting that they seem to possess all the enzymatic machinery required for TA synthesis (336). The synthesis in mated cultures results from the conversion of specific precursors by each mating type. The newly synthesized TA, then accelerates its own synthesis by increasing the synthesis of its precursors (329, 337-339). The biosynthesis of TA has been reviewed by Bu'Lock (339), Sutter (340), Ende (332, 341) and Gooday (342).

In Mucorales, the mating type character is determined
by a single locus, the mating type (MT) locus, which has two functioning alleles MT\(^+\) and MT\(^-\) (343). As TA is a breakdown product of \(\beta\)-carotene, the factors regulating carotenoid products also control TA synthesis. Two regulating 'blocks' are known to be present in the synthesis of TA from \(\beta\)-carotene (343). The first is a strong repressed step in plus and minus cultures so as to yield TA precursors methyl-4- dihydrotriosporates in plus and trisporol and trisporin synthesis by minus cultures which can be converted by the opposite mating type to TA. These are probably permanent repressions. The second moderate repression at the first step (rate limiting step) of TA biosynthesis, which controls the synthesis of TA by mated cultures and its precursors by single cultures (343). It was shown later that there was an involvement of cyclic AMP in TA synthesis as cyclic AMP levels increased on mating (315). Cyclic AMP and TA could stimulate the synthesis of TA precursors (316). It was concluded that TA acts on its own synthesis by stimulating intracellular cyclic AMP levels and the first step of TA biosynthesis was catabolically repressed and this repression was lifted by cyclic AMP so as to stimulate TA production (316). It has been suggested
that macromolecules (344) or volatile metabolites (345,346), play a role in the induction of TA biosynthetic pathway.

TA has a principal function in the induction of sexual reproduction but its ability to derepress the carotene pathway has aroused considerable interest. In fact TA was first isolated as a stimulator of carotene synthesis instead of as a hormone (37). In mated cultures of A. tricolor and M. mucida an increase in the utilization of acetyl CoA, NADP and ATP has been observed along with an increase in carotene synthesis compared to single cultures (64,347). The utilization of glucose and K+ increase but the uptake of Pi and Mg++ is not affected in mated cultures (348). Increased RIIA kinase activity on mating suggest the overall activation of the carotene biosynthetic pathway. In mated cultures, along with carotenoids, sterol, polypronol and ubiquinone levels were also higher compared to single cultures (219,349).

The action of TA is a part of positive feedback since it greatly stimulates its own synthesis by increased carotenogenesis (350). The outer layer of the zygophore wall of M. mucida and P. lakoslegangae has aperosollenin, an oxygenated derivative of carotenoids.
(351,352). It has also been suggested that the increase in the content of sterols, polypronols and ubiquinones may be necessary for the requirement of the membranes, surface polymers and respiration of the zygophores and later stages in sexual reproduction (349).

**Present investigation:**

There is a 10-15 fold increase in the amount of carotene synthesized by mated cultures compared to single cultures of *G. trispora*. This is attributed to the production of a sex hormone triacetic acid (TA), a breakdown product of β-carotene. TA derepresses the carotene biosynthetic pathway which is known to be repressed in single cultures. Accumulation of β-carotene depends on several environmental factors. A variety of chemicals have been studied for their effect on carotogenesis, some of them being TA, Vitamin A, abscisic acid, ionones, alkaloids, various mutagenic agents and some antibiotics. Thus, until now, much information is available regarding the external factors which affect carotene levels. Objectives of the present investigation are to search for intracellular factors that affected carotene production. The problem was approached in the following manners:

1. Purification and characterization of proteases stimulated by triacetic acid and its structural analogues.
2. Isolation and characterization of penicillin stimulated protease(s).

3. Inhibitor protein of carotene biosynthesis and its interaction with different proteases.

4. Probable site of action of inhibitory proteins and stimulatory factors in the carotene biosynthetic pathway.