Glycerol and beta-Carotene Production by *Dunaliella*

Ami Ben-Amotz

Nature Beta Technologies
Eilat
Israel

Dunaliella

Introduction

&

Background

HISTORY

Year	Species	Author
1823	*Lepraria kermesina*	Wrangel
1836	*Globularia kermesina*	Turpin
1838	*Haematococcus salinus/ Protococcus salinus*	Dunal
1840	*Monas dunalii*	Joly
1841	*Diselmis dunalii*	Dujardin
1865	*Chlamydomonas dunalii*	Cohn
1872	*Protococcus salinus*	Geleznow
1886	*Sphaerella lacustris* var. *Dunalii*	Hansgirg
1891	*Chlamydomonas dunalii*	Blanchard
1905	*Dunaliella salina*	Teodoresco
1906	*Dunaliella viridis*	Teodoresco
1926	*D. kermesina*[1]	Labbé
1935	*D. peircei*	Nicolai&Baas-Becking
1937	1. *D. parva* 2. *D. media* 3. *D. euchlora* 4. *D. minuta*	Lerche
1938	*D. spec.* 1 *D. spec.* 2 *D. spec.* 3 *D. spec.* 4	Ruinen
1956 195	*D. bioculata*	Eddy Butcher
1959	1. *D. tertiolecta*[2] 2. *D. primolecta*[3] 3. *D. polymorpha* 4. *D. quartolecta*	Butcher
1969	*D. turcomanica*	Masjuk
1971	*D. asymetrica*	Masjuk
1973a	1. *D. maritima* 2. *D. granulata*	Masjuk
1973b	1. *D. terricola* 2. *D. gracilis* 3. *D. ruineniana* 4. *D. baas-beckingii* 5. *D. minutissima* 6. *D. carpatica* 7. *D. jacobae*	Masjuk
1973c	*D. pseudosalina*	Masjuk and Radcnenko
1978	*D. bardawil*[4]	Avron and Ben-Amotz
1980	*D. marina*	Kombrink and Wöber

Dunaliella

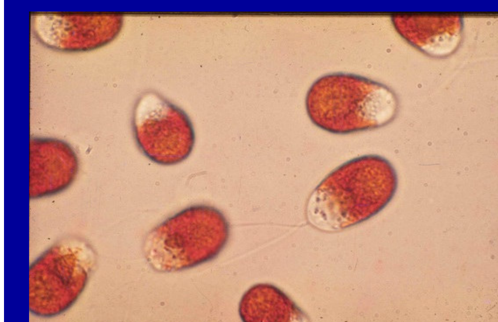
1823-2006

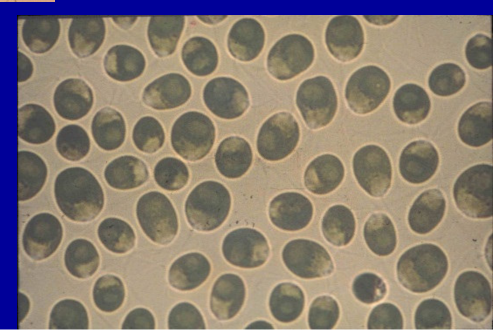
The History Chapter

(Polle, Ben-Amotz, 2006)

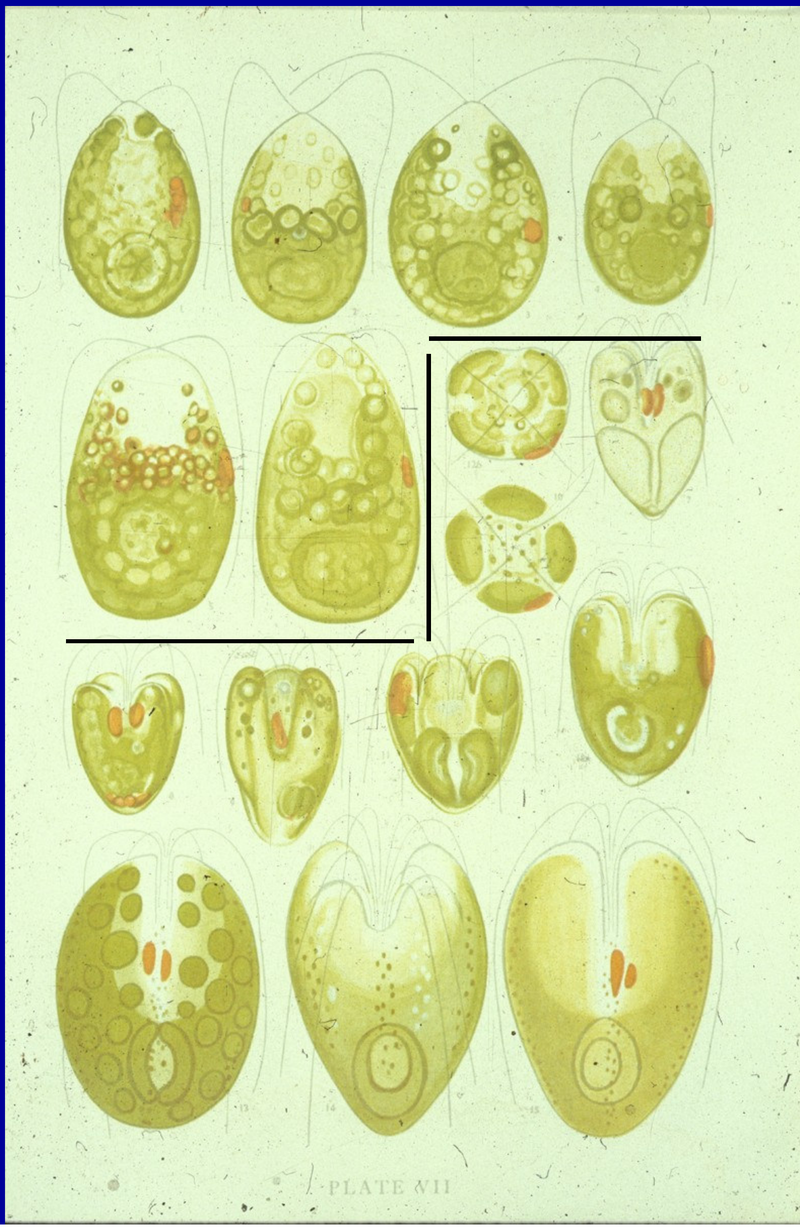
Dunaliella Web Base

http://www.*dunaliella*.org/dunabase/





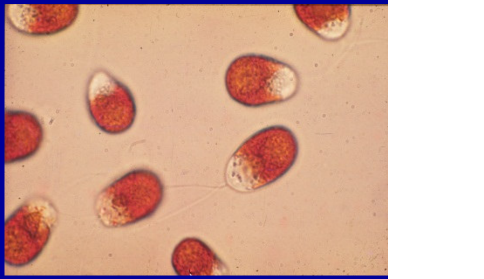
Dunaliella, Chlorophyta, Volvocales



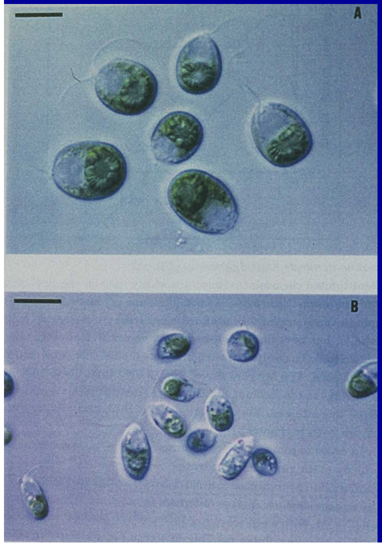
Marine unicellular alga
Butcher, 1959

Natural Bloom of *Dunaliella*

Halotolerant Eukaryotic Alga



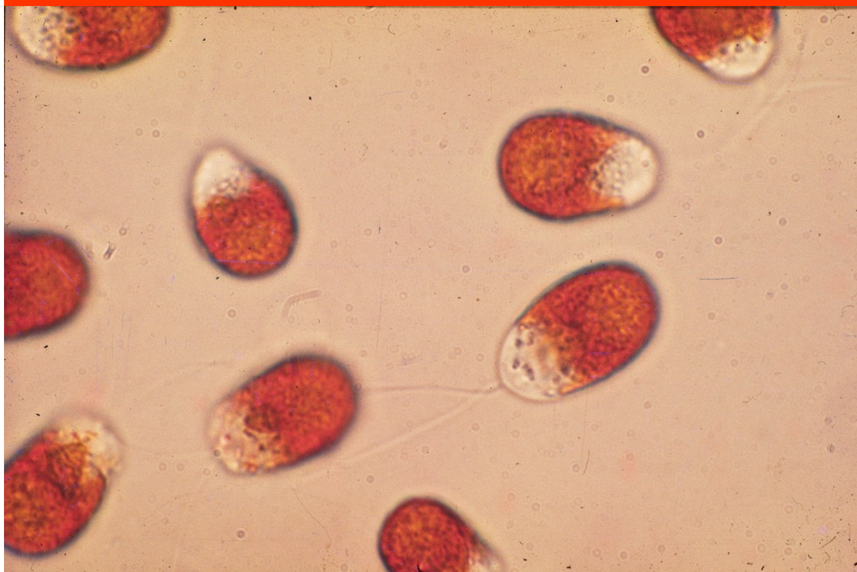
Surviving in saturated salt (>32%)

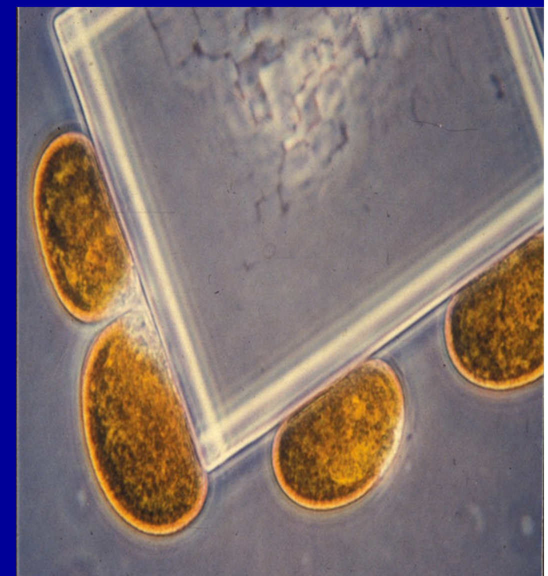


to the Dead Sea

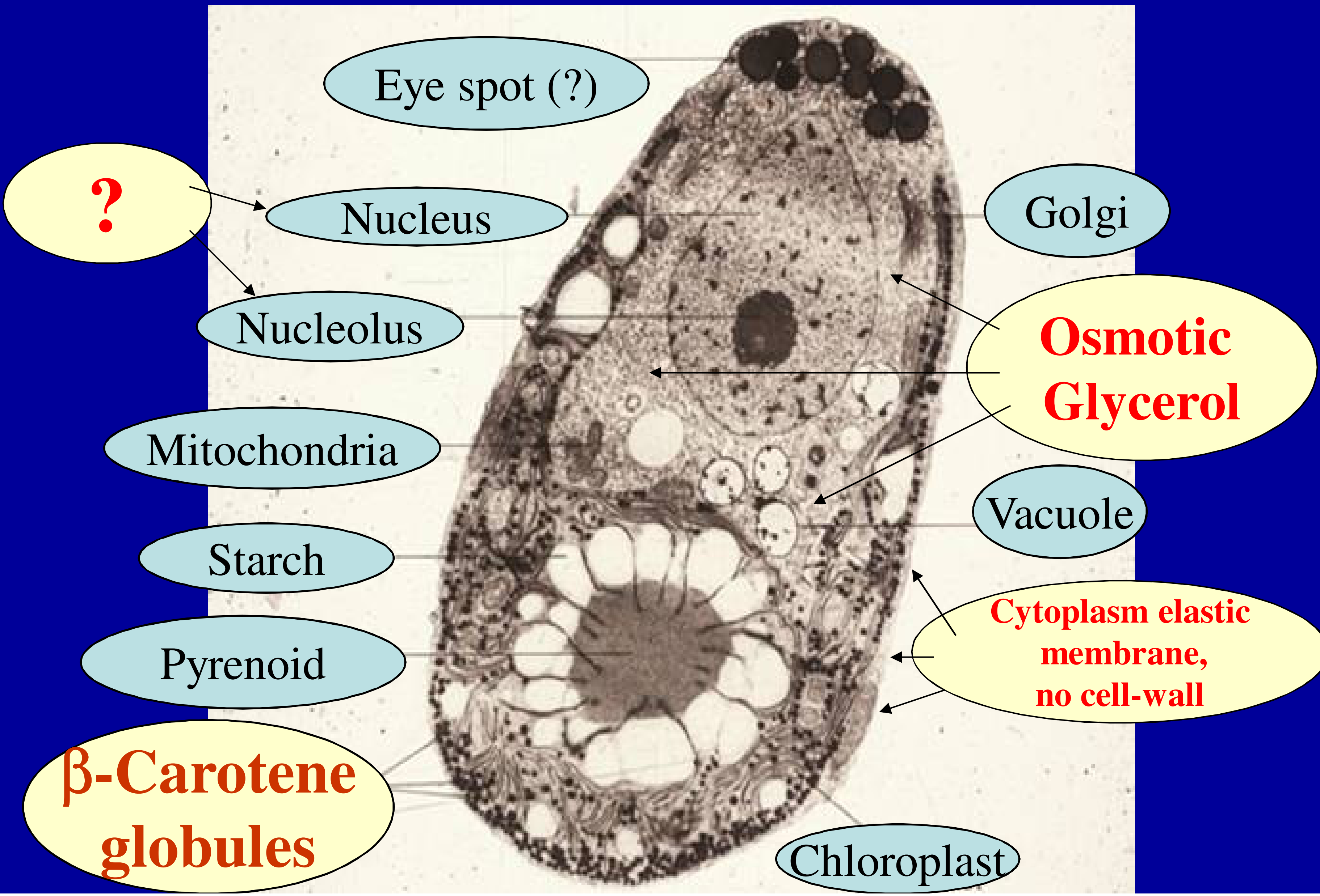
From Sea Water

Dunaliella, "Salt Loving Alga"

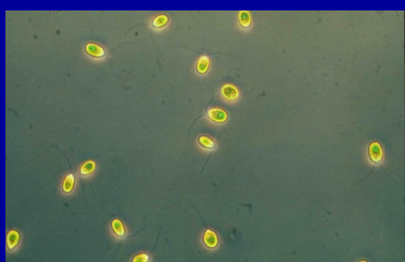


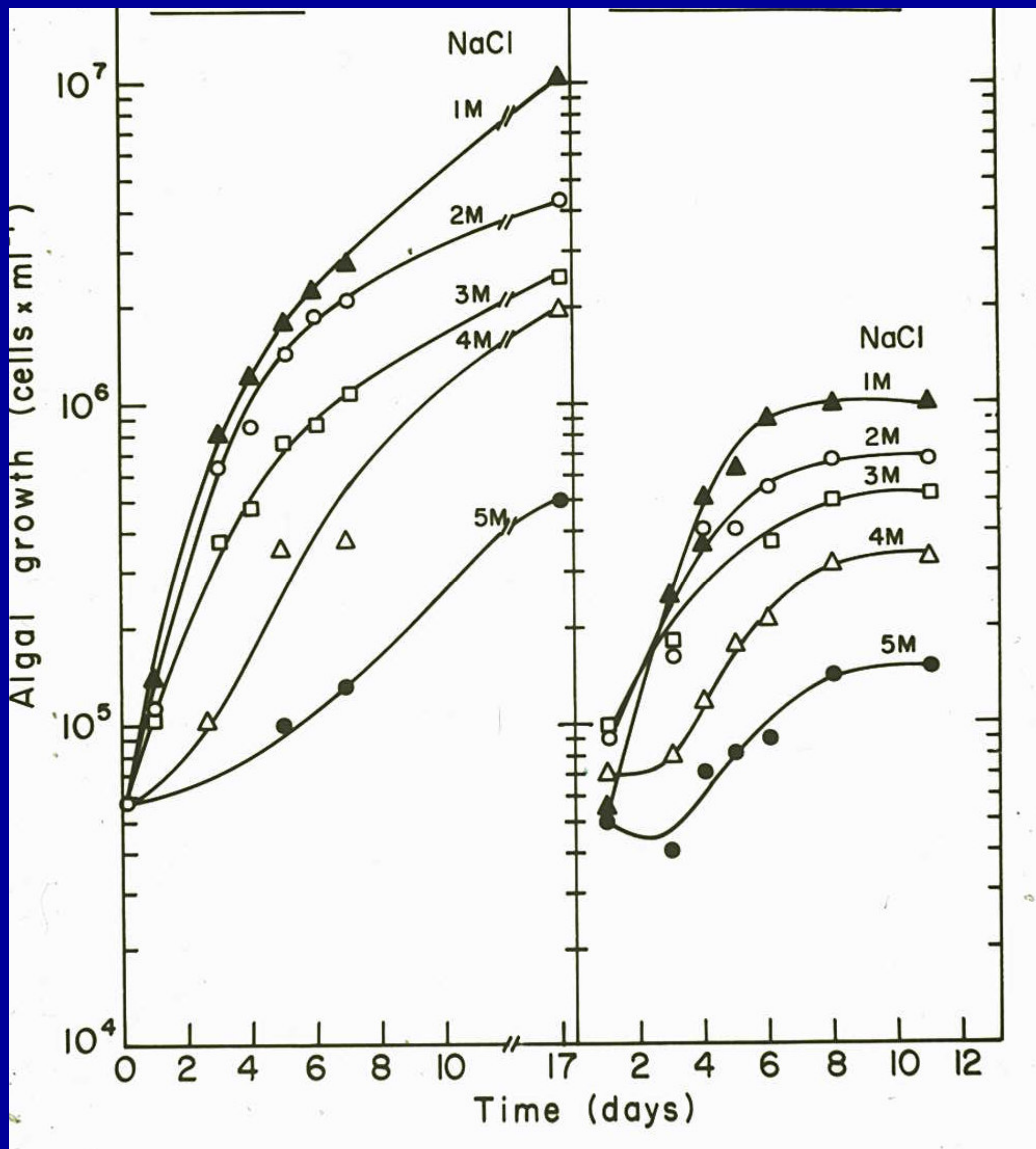


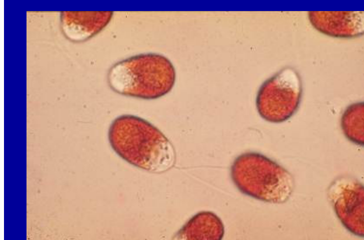
Dunaliella


Eye spot (?)
?
Nucleus
Nucleolus
Golgi
Osmotic Glycerol
Mitochondria
Vacuole
Starch
Cytoplasm elastic membrane, no cell-wall
Pyrenoid
β-Carotene globules
Chloroplast

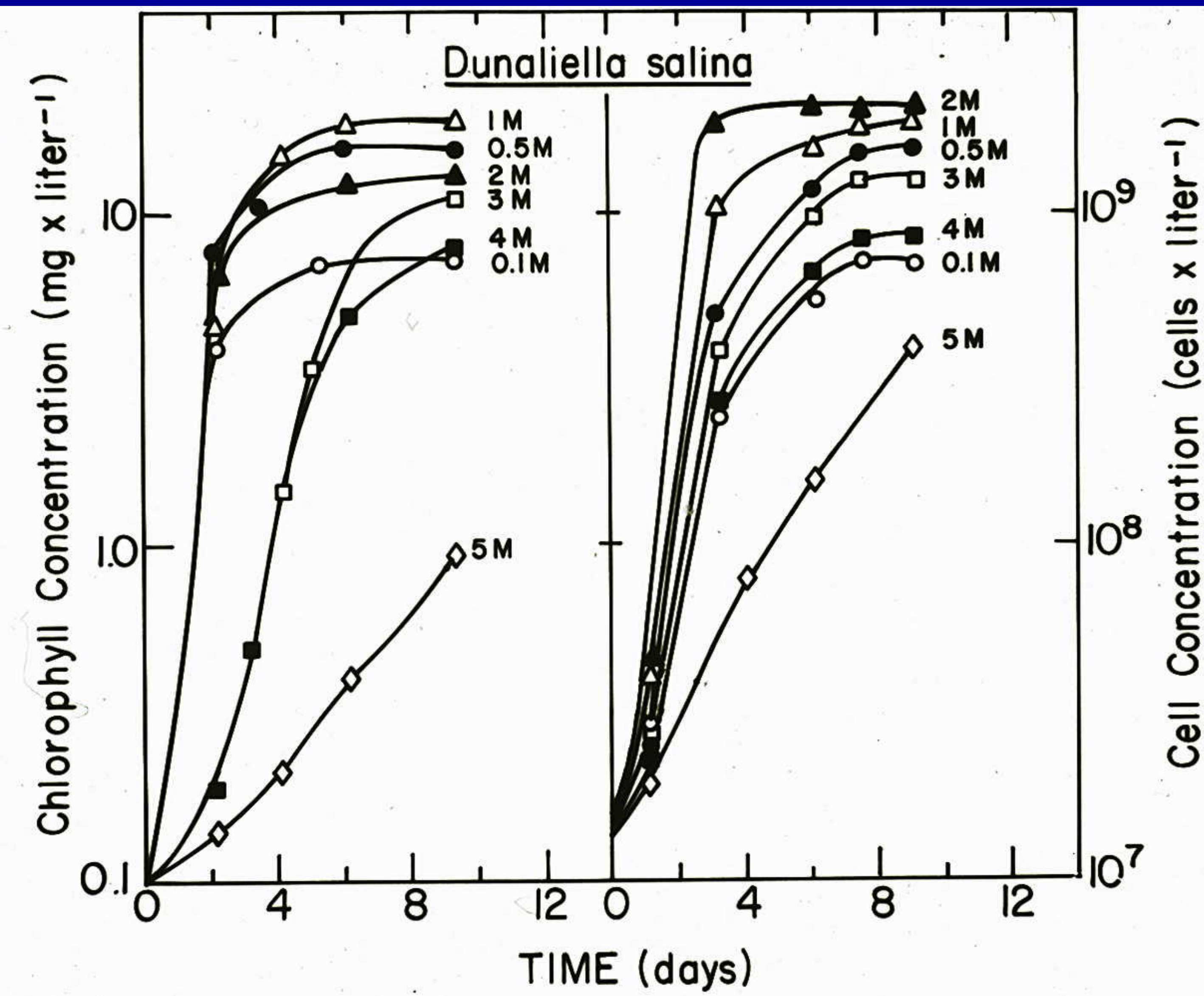
Dunaliella parva *Dunaliella salina*







Effect of Salt on Growth of *D. salina* Halotolerant Alga



Glycerol Content

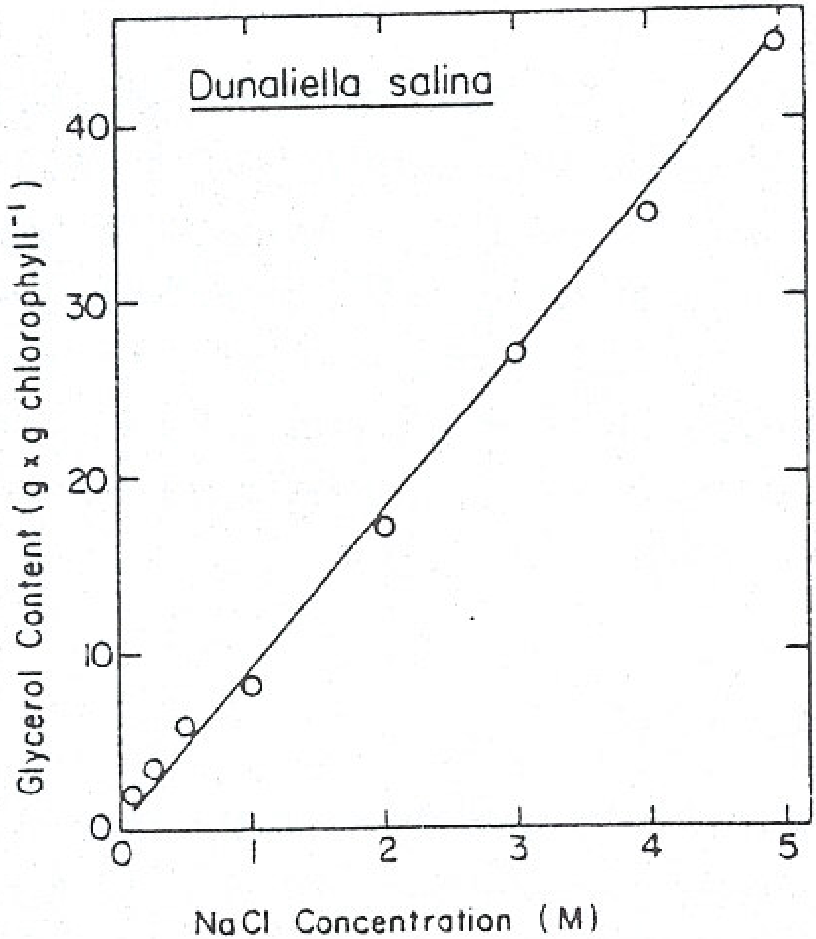
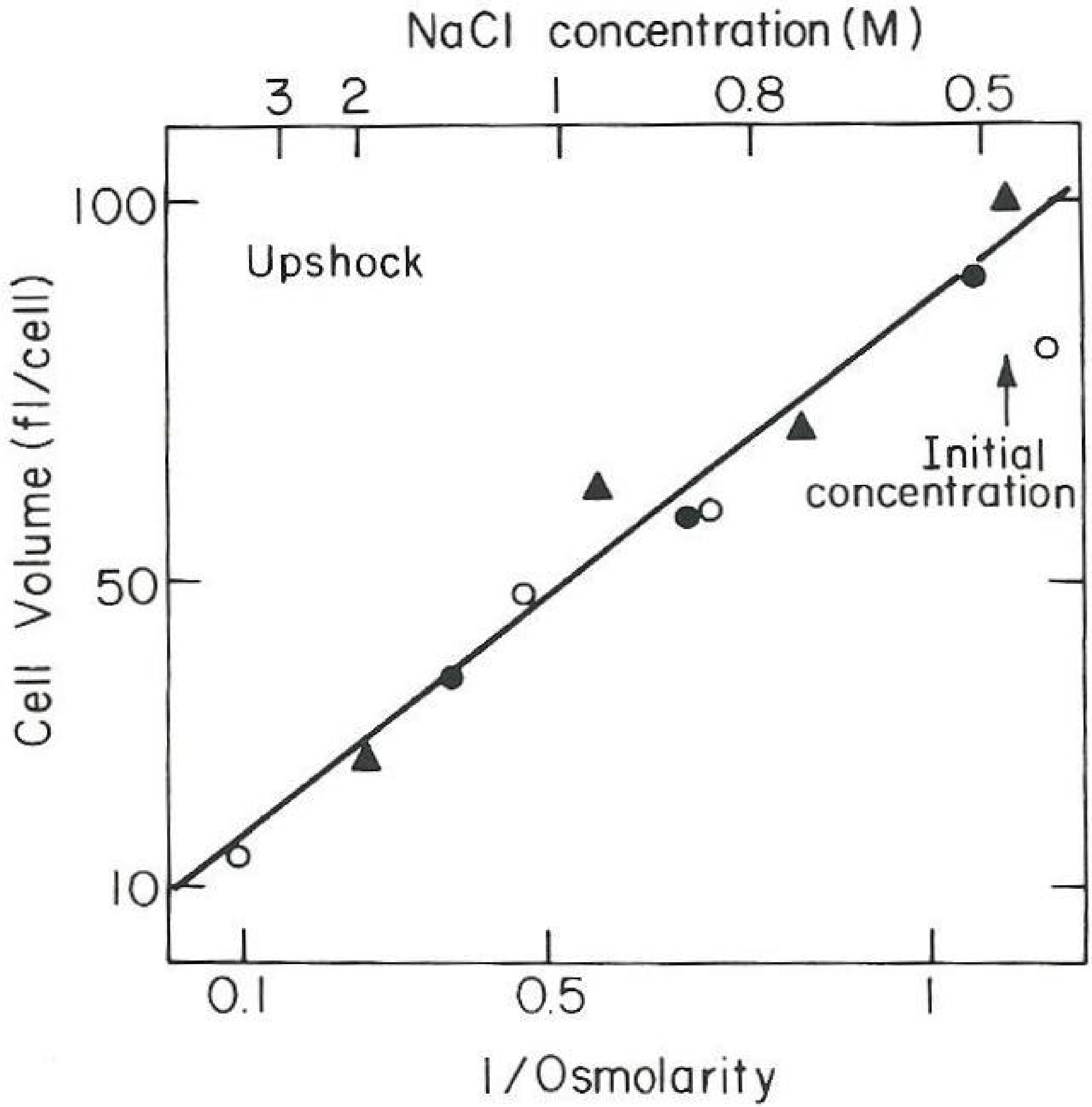
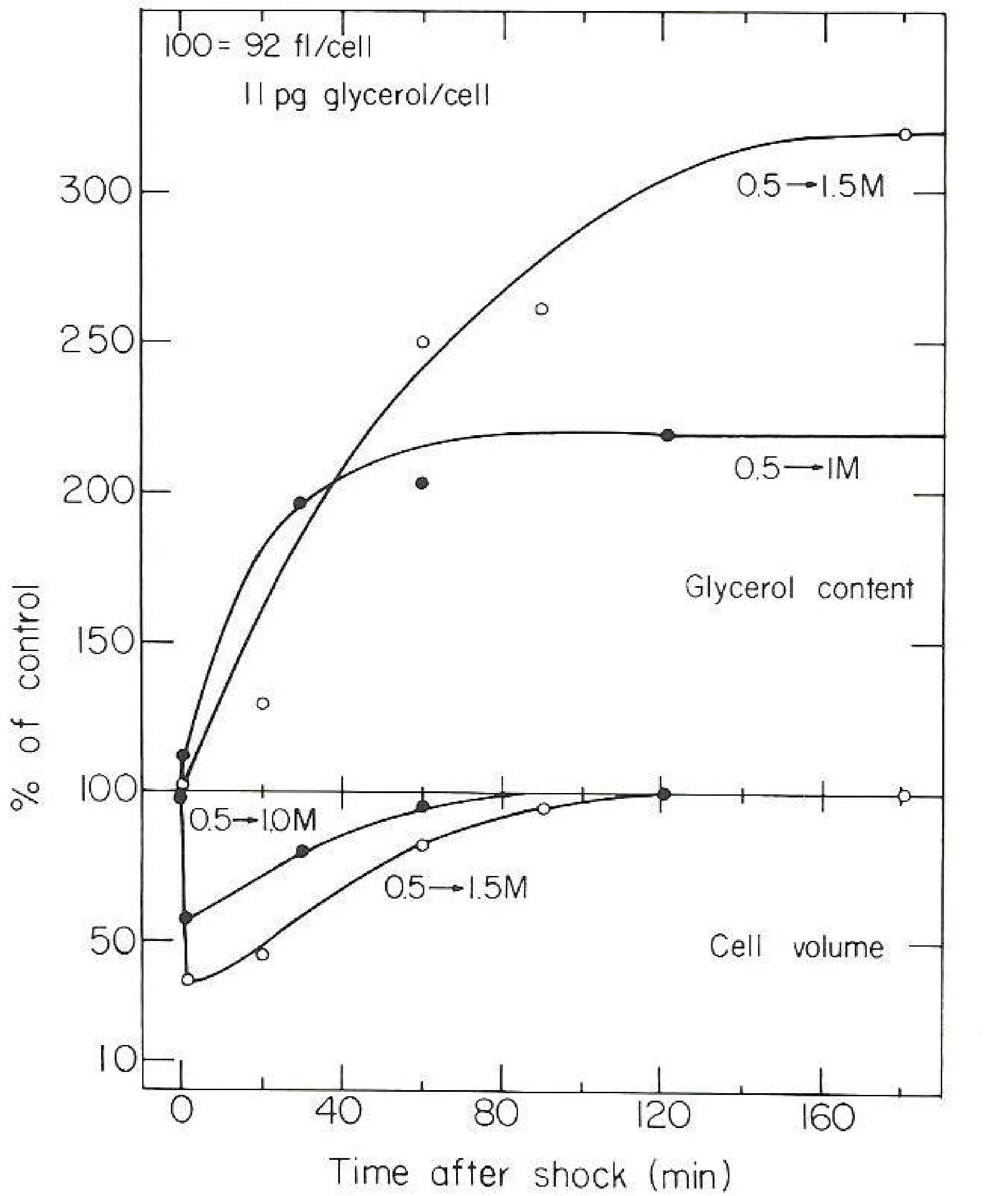


Fig. 4.3. Intracellular glycerol concentration in *Dunaliella* as a function of the medium salt concentration in which the algae were cultivated.

Upshock


NaCl concentration (M)
3
2
1
0.8
0.5
100
50
10
Upshock
Initial concentration
Cell Volume (fl/cell)
0.1
0.5
1
1/Osmolarity

Glycerol and Volume Kinetic Response to Salt



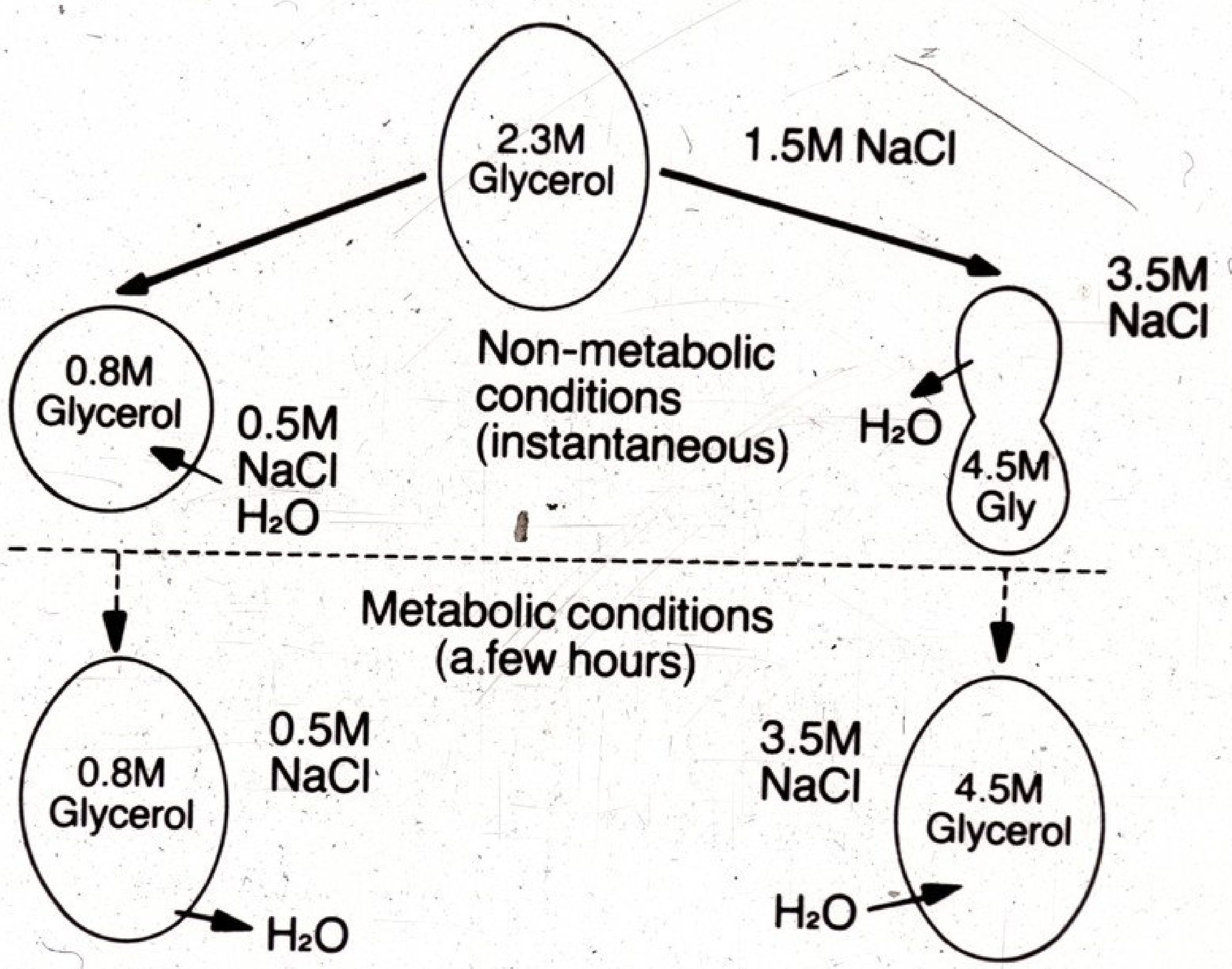
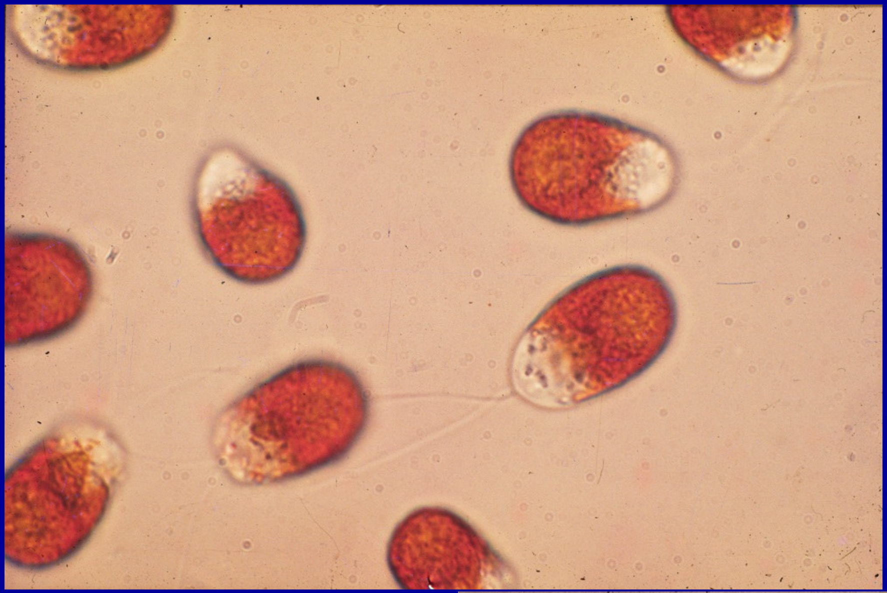
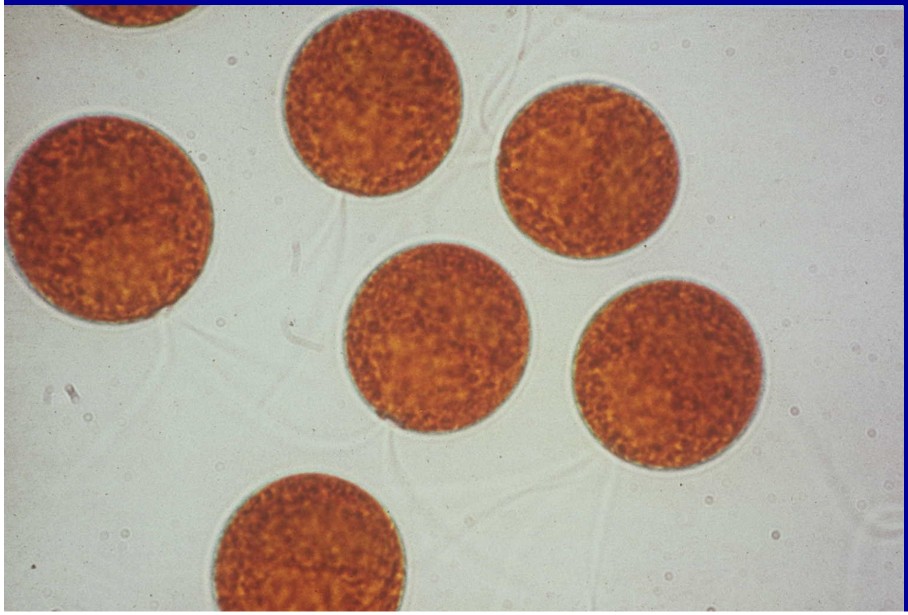


Fig. 4. Schematic representation of the volume changes of Dunaliella *in response to hypertonic or hypotonic conditions.*

Dunaliella

Osmotic Down Shock





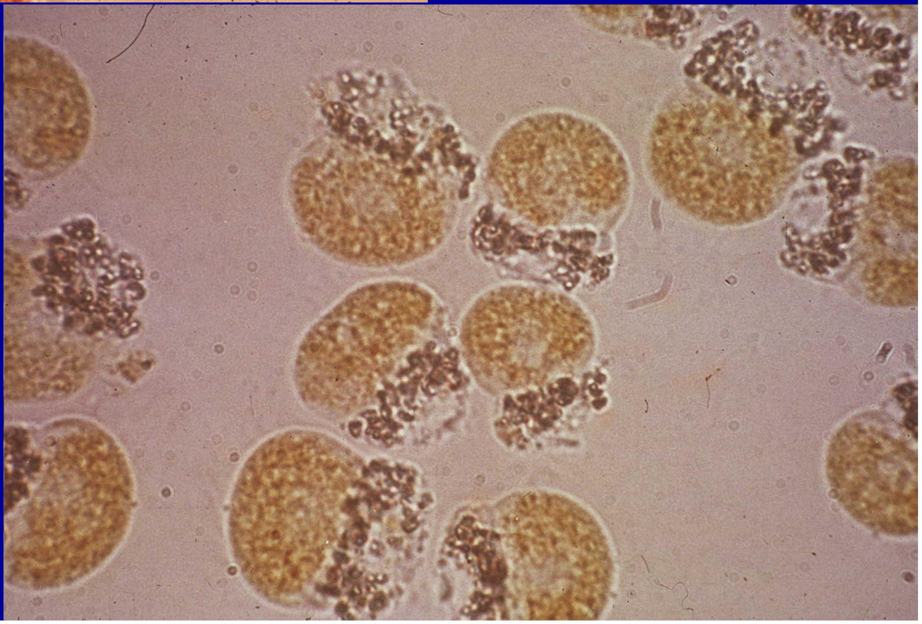
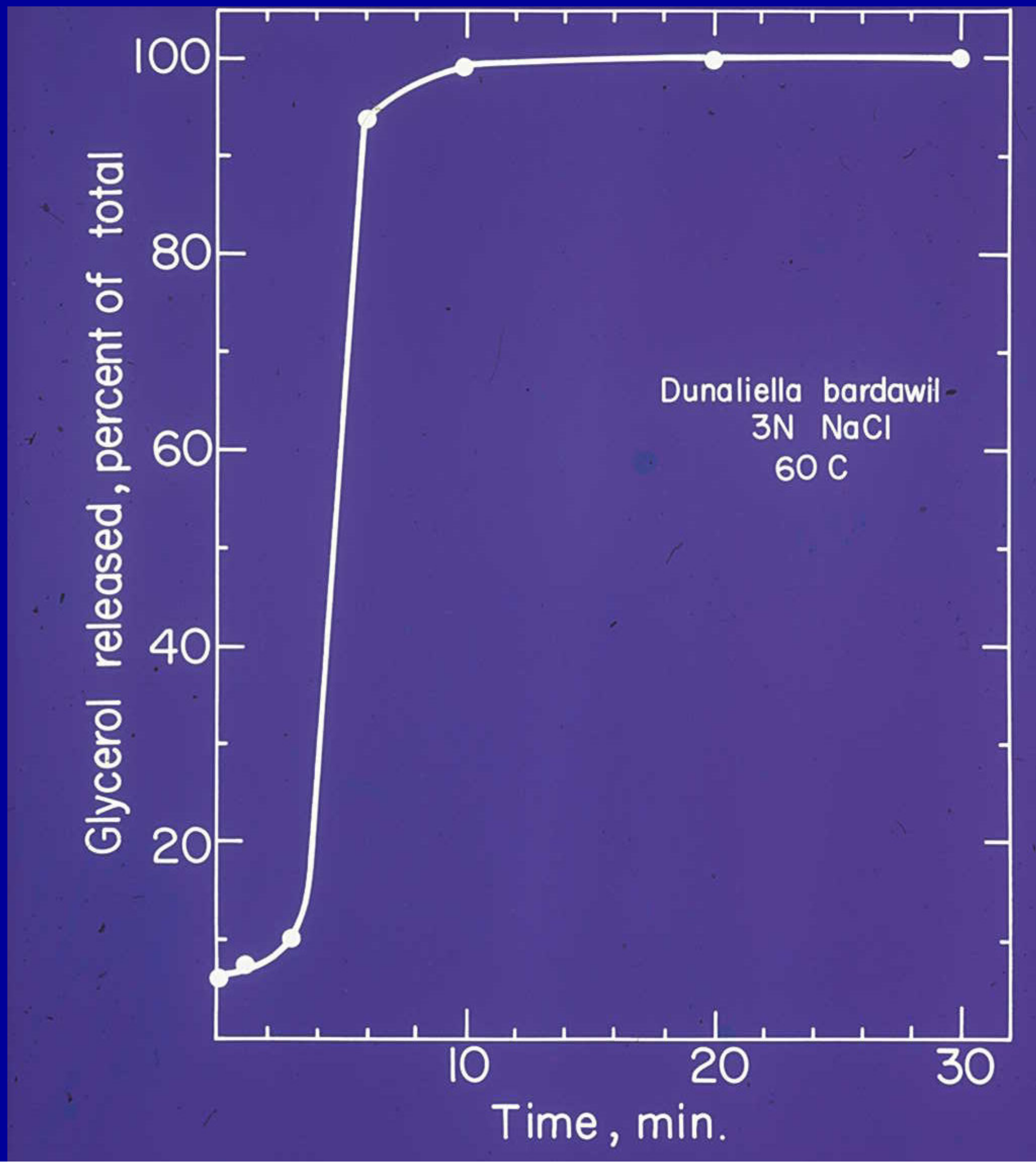


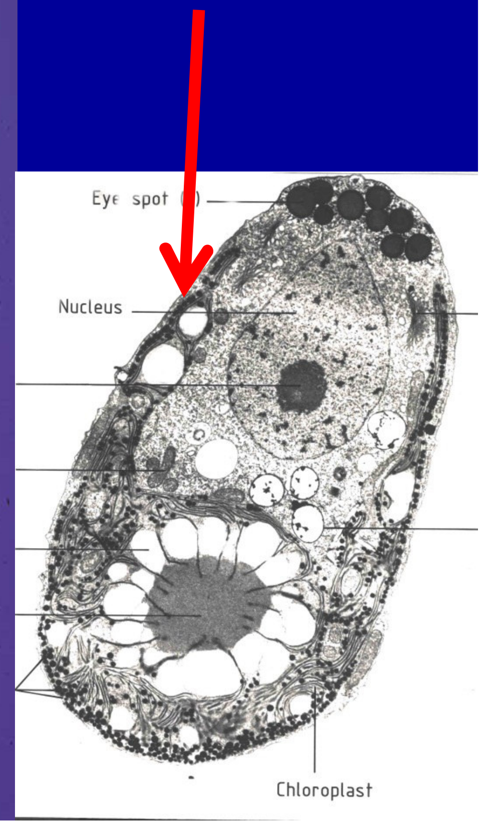
TABLE 2
Salt Responses in *Dunaliella*

Response	Remarks	Ref.
Plasma membrane phenomena		
Fusion with cytoplasmic vesicles	do, f, t	112
Infoldings	up, f, t	113
Lipid ordering	up, f, t	115, 116
Permeability changes	up and do, f, t	114
Na^+ influx	up, f, t	107, 108, 116
Hyperpolarization	up, f, t	76
Inositol phospholipid turnover	do, f, t	79, 93, 112
Choline phospholipid turnover	up, f, t	93, 112
Phosphorylation, 45-kDa polypeptide	up	—
Synthesis of 150-kDa polypeptide	high salt, slow adaptation	132
Enhanced carbonic anhydrase	high salt, slow adaptation	87, 131
Internal factors		
pH drop	up, f, t	108
pH rise	up, s	105, 127, 128
Pi rise	up	41, 28
Polyphosphate metabolism	up and do	109, 114, 129
ATP, NAD(P) changes	up and do, secondary	74, 91, 102, 107, 109

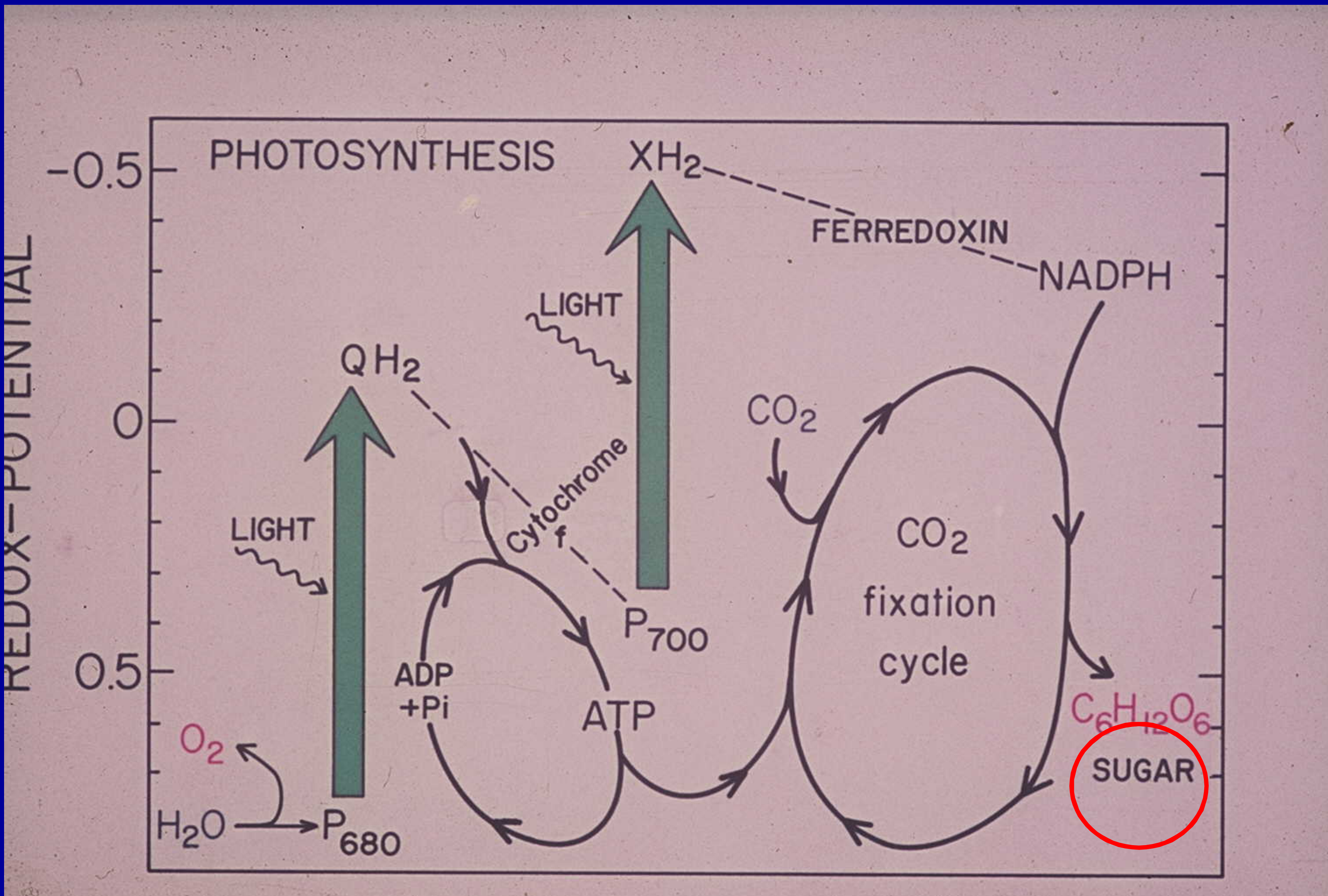
Note: do, downshock (hypoosmotic); up, upshock (hyperosmotic); f, fast; t, transient; s, slow.

Dunaliella Plasmalemma Biphasic Membrane Temperature Effect

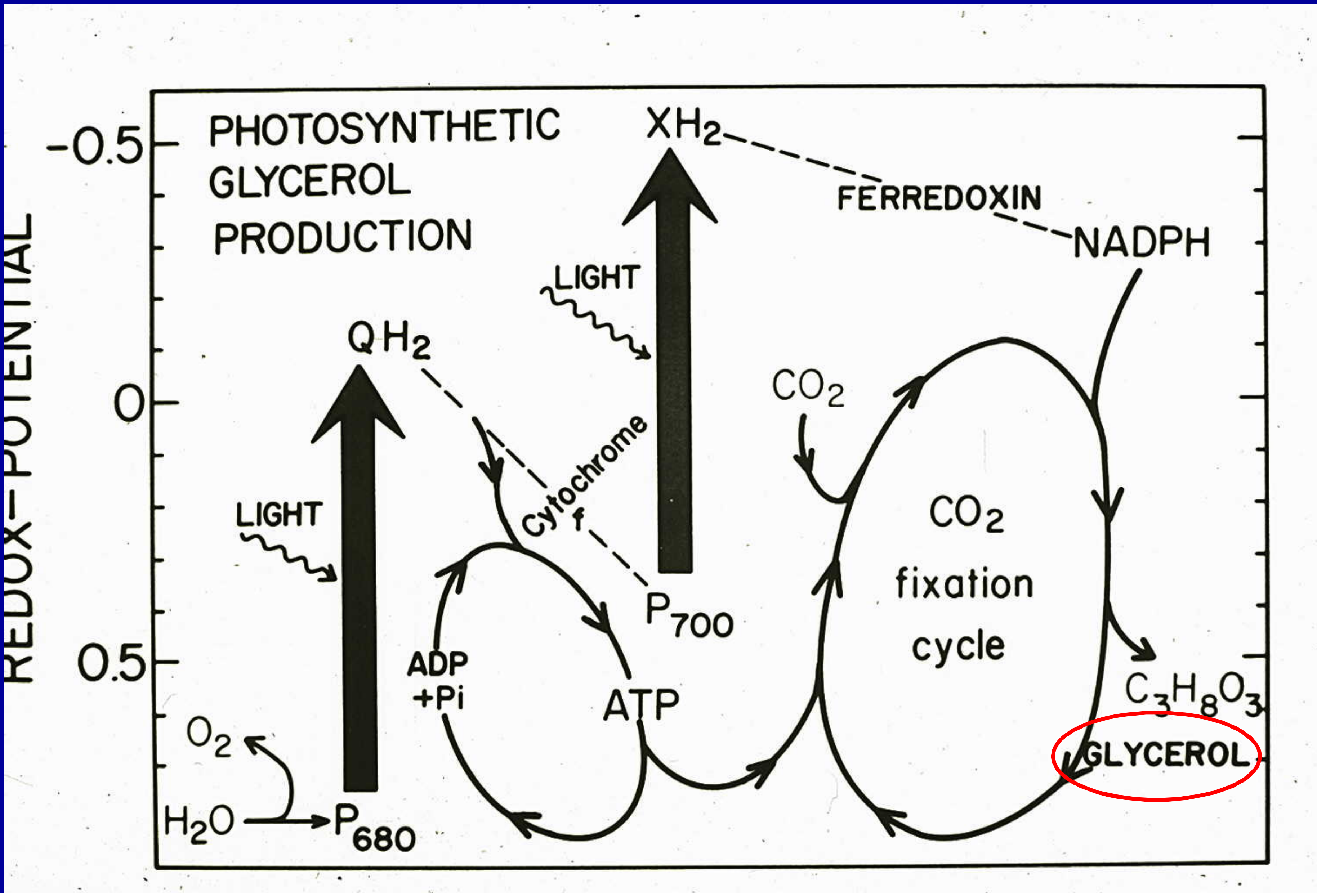




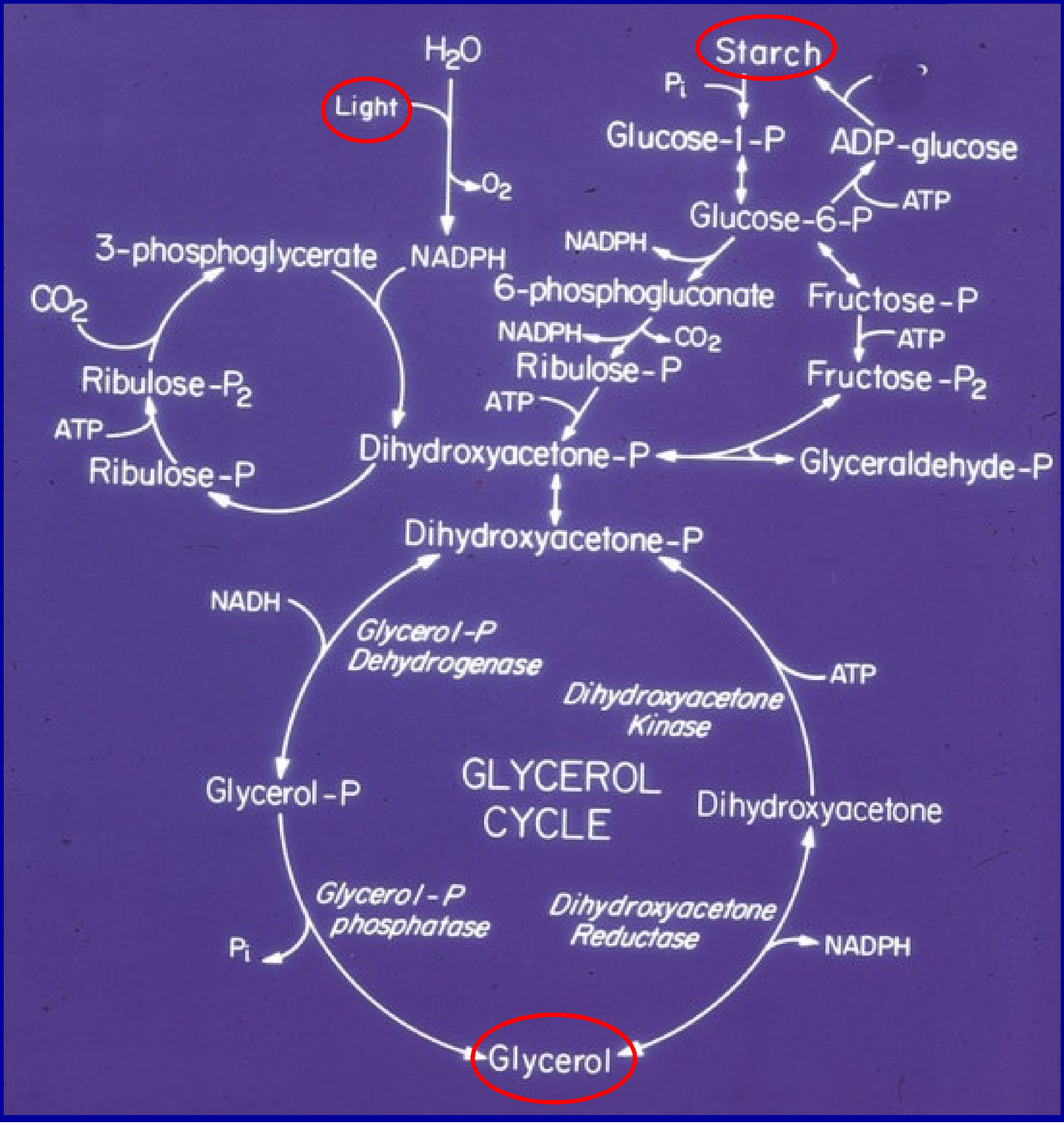
Classical Photosynthesis and Sugar Biosynthesis



Glycerol Biosynthesis



Biosynthesis of Glycerol in *Dunaliella*



Glycerol Biosynthesis and Degradation Under Light and Dark

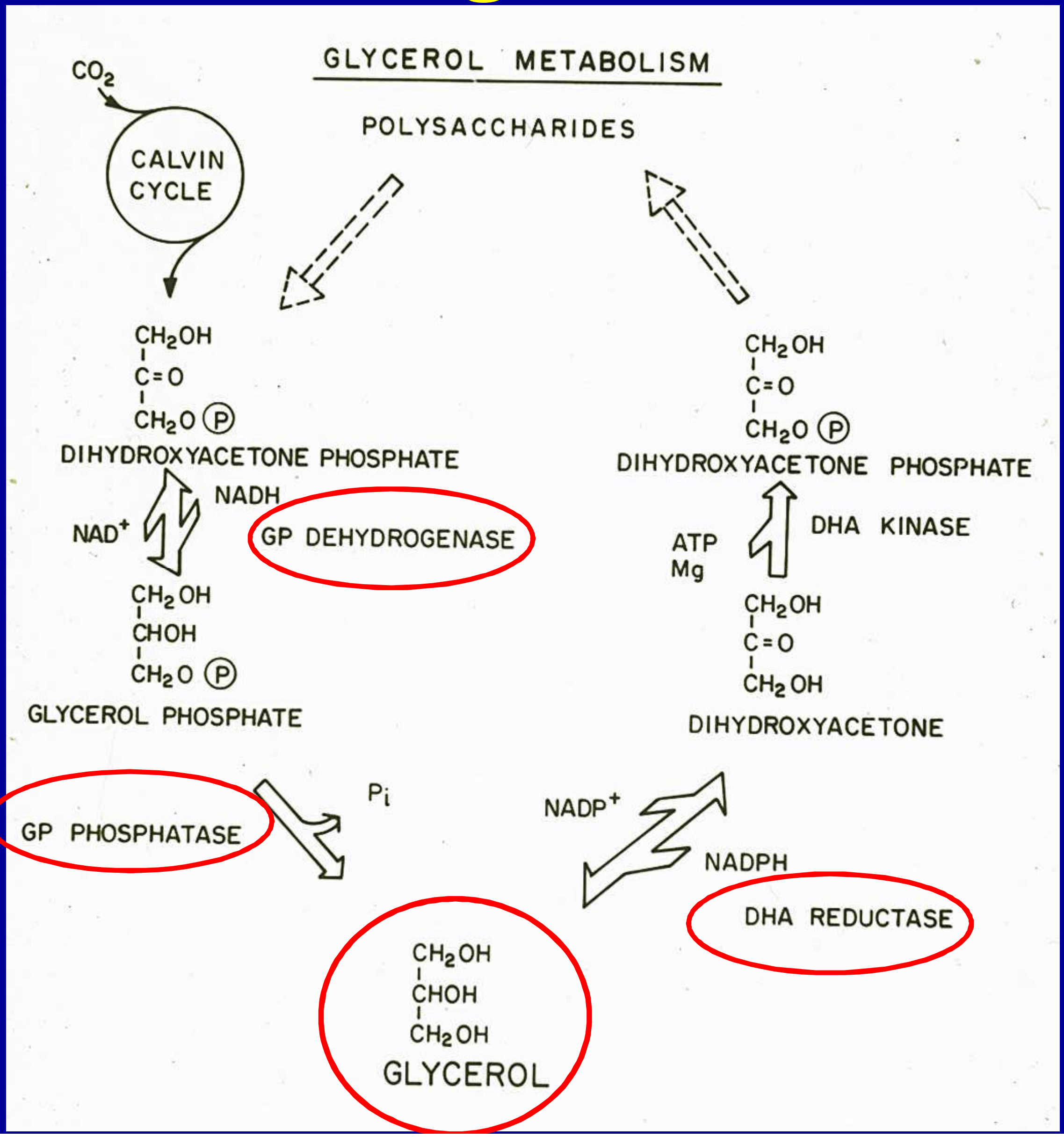
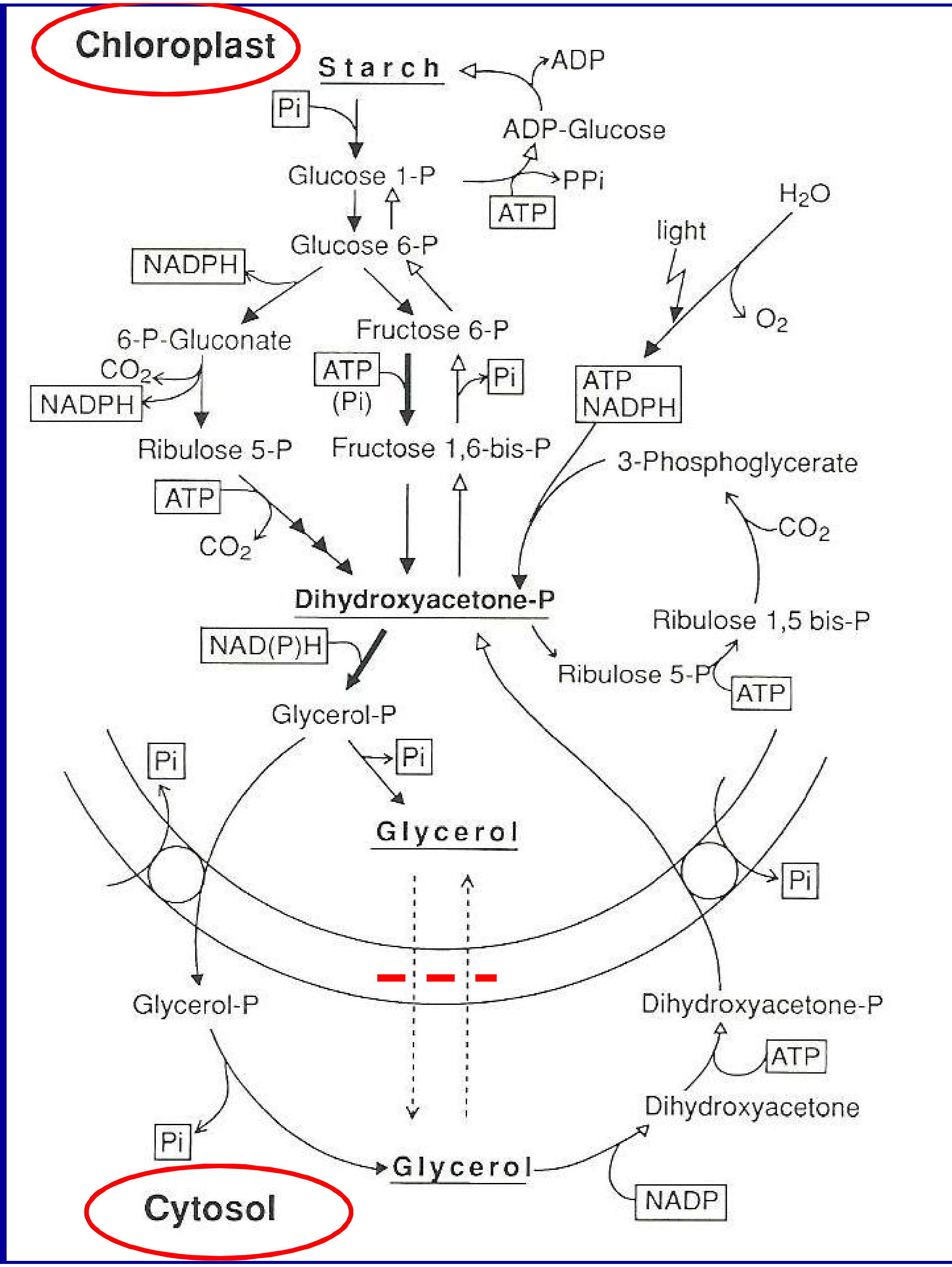


TABLE 1
Enzymes Involved in Glycerol Metabolism in *Dunaliella*

Enzyme	Special characteristics	Ref.
Starch mobilization		
Starch phosphorylase	Pi ↑ , pHac ↑ , NaCl ↑	105
Amylase	DTT ↑ , pHac ↓	105
Maltase	pHac ↑	105
Starch synthases(3)	—	104
Glycolysis		
Phosphofructokinase[a]	Pi ↑ ↓ , PGA ↓ , PEP ↓ , Gly3P ↓ , ATP ↑ ↓ , NaCl ↓ , pHac ↓	105, 106
Pentose phosphate pathway		
Glucose 6-P dehydrogenase	DTT ↓	105
P-Gluconate dehydrogenase	—	105
Glycerol/DHAP		
Glycerol-3-phosphate dehydrogenase[a]	Pi ↓ , pH ↑ ↓	100—103
Glycerol-3 phosphatase	Pi ↓ , Ca ↓ , NaCl ↓	98, 99
Dihydroxyacetone reductase	NADP sp., high Km (glycerol), pH ↑ ↓	4
Dihydroxyacetone kinase	high subst. sp.	96, 97

Note: ↑ , activator; ↓ , inhibitor; ↑ ↓ , complex regulation; pHac, acidic; sp., specificity.


Chloroplast
Starch
ADP
Pi
ADP-Glucose
Glucose 1-P
PPi
ATP
H_2O
light
Glucose 6-P
NADPH
O_2
Fructose 6-P
6-P-Gluconate
CO_2
NADPH
ATP
(Pi)
Pi
ATP
NADPH
Ribulose 5-P
Fructose 1,6-bis-P
3-Phosphoglycerate
ATP
CO_2
CO_2
Dihydroxyacetone-P
Ribulose 1,5 bis-P
NAD(P)H
Ribulose 5-P
ATP
Glycerol-P
Pi
Pi
Glycerol
Pi
Glycerol-P
Dihydroxyacetone-P
ATP
Dihydroxyacetone
Pi
Glycerol
NADP
Cytosol

VI. SUMMARY

The following observations reflect the work done to date on the genus *Dunaliella:*

1. *Dunaliella* adapts to high extracellular osmotic stress by synthesis of intracellular glycerol.
2. Glycerol is produced either photosynthetically or by degradation of starch reserves.
3. The induction of glycerol synthesis or reassimilation is triggered by volume changes.
4. Activation of glycerol synthesis does not involve *de novo* protein synthesis. Glycerol phosphate dehydrogenase and phosphofructokinase are probably the checkpoint enzymes which control glycerol synthesis.
5. The molecular mechanism which trigger glycerol production in *Dunaliella* is not known. Changes in the plasma membrane, inorganic phosphate, and pH following osmotic shocks suggest that a plasma membrane sensor as well as soluble metabolites are involved in the activation of glycerol synthesis.

Glycerol Productivity

PHOTOSYNTHETIC SOLAR ENERGY CONSERVING SYSTEMS

		Maximal yield
1. Photosynthesis	$H_2O + CO_2 \xrightarrow{h\nu} [CH_2O] + O_2$	10%
2. H_2 production	$H_2O \xrightarrow{h\nu} H_2 + \frac{1}{2}O_2$	12%
3. Glycerol pro-duction	$4H_2O + 3CO_2 \xrightarrow{h\nu} C_3H_8O_2 + 3\ \frac{1}{2}O_2$	10%
4. Methane "	$2[CH_2O] \longrightarrow CO_2 + CH_4$	6%
5. Electricity produced from methane		2%

Photosynthetic Limitation of Long Term Algal Productivity
Theoretical Algal Productivity in Open Ponds

Environment Factor	Reduction	(%)
Solar light	-------	100
Scattering and reflecting properties of surface	10%	90
Absorption spectrum (depth of culture)	50%	45
Photosynthetic efficiency (25%)	75%	11.3
Light saturation (7-95%)	60%	4.5
Respiration, photo-respiration, excretion	5%	4.3
Photo-inhibition	10%	3.8
Temperature	20%	3.1
===================================	============================	
	Productivity	
Mean daily solar intensity	4,000 kcal/m^2/day	
Energy productivity at 3% efficiency	120 kcal/m^2/day	
Algal biomass productivity (5 kcal/g)	25 g/m^2/day	
Glycerol 33%	8.25 g/m^2/day	
Higher Plants Max (sugar cane, corn, wheat, etc.)	5 g/m^2/day	

Photosynthesis & Glycerol Productivity

Autotrophy

1% Photosynthetic Efficiency (PE)

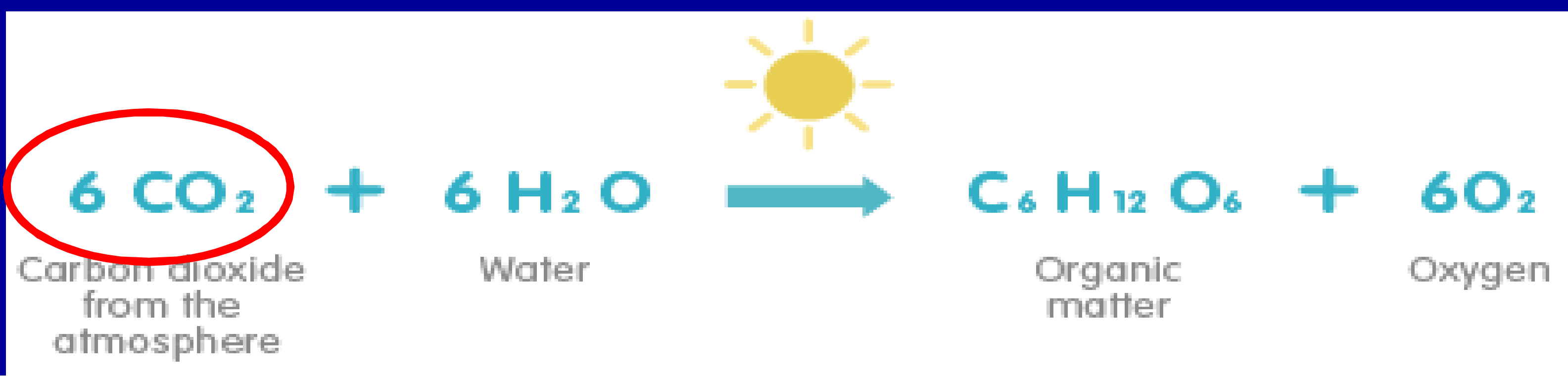
Mean average daily solar intensity: 4,000 kcal/m^2/day

PE 1% = 40 kcal/m^2/day

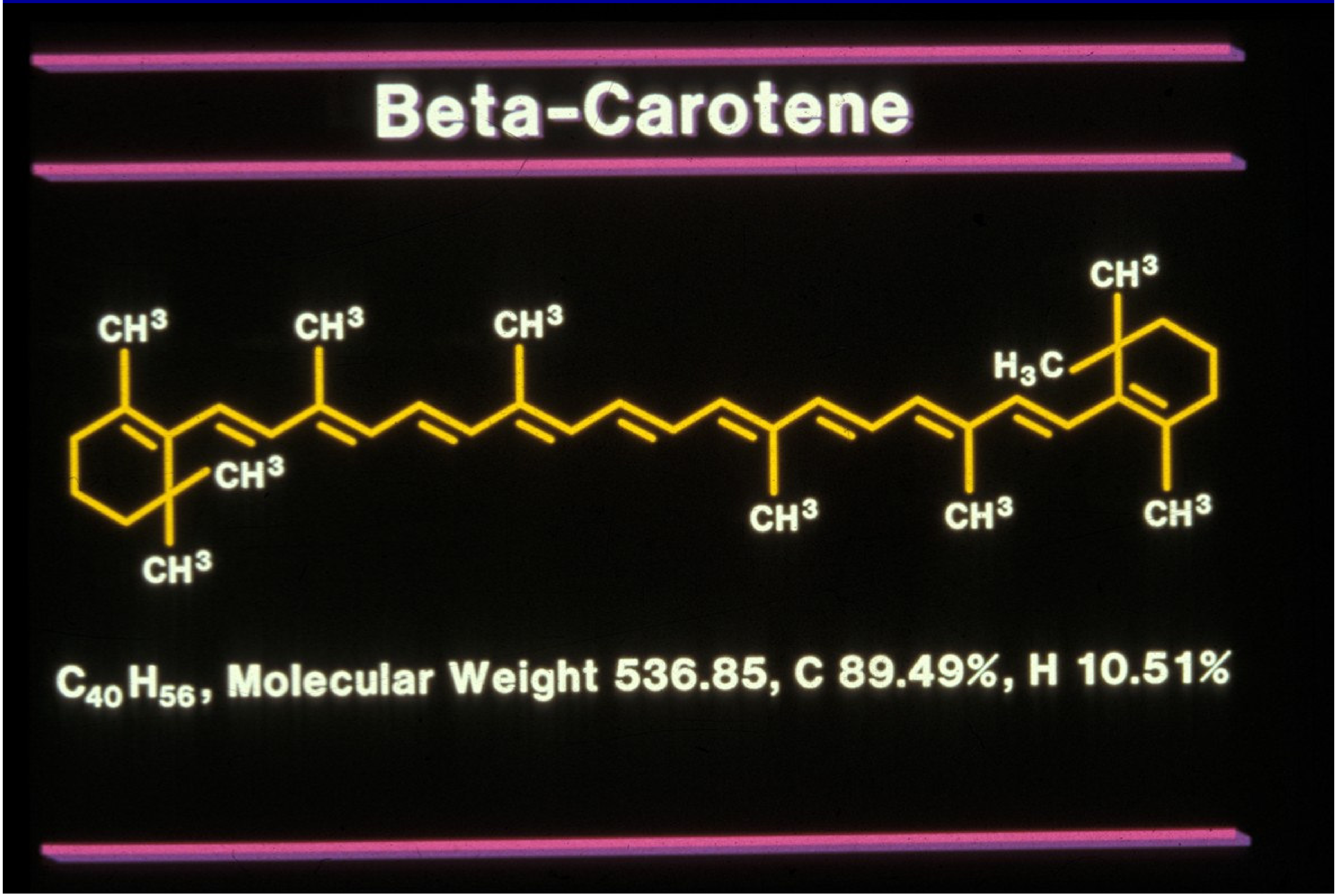
Glycerol caloric value, 4.5 kcal/g

Expected Glycerol Productivity

8.9 g/m^2/day



all-trans β-Carotene



Scheme of Carotenoids Chemical Synthesis
HL Roche 1950: all-trans β-Carotene

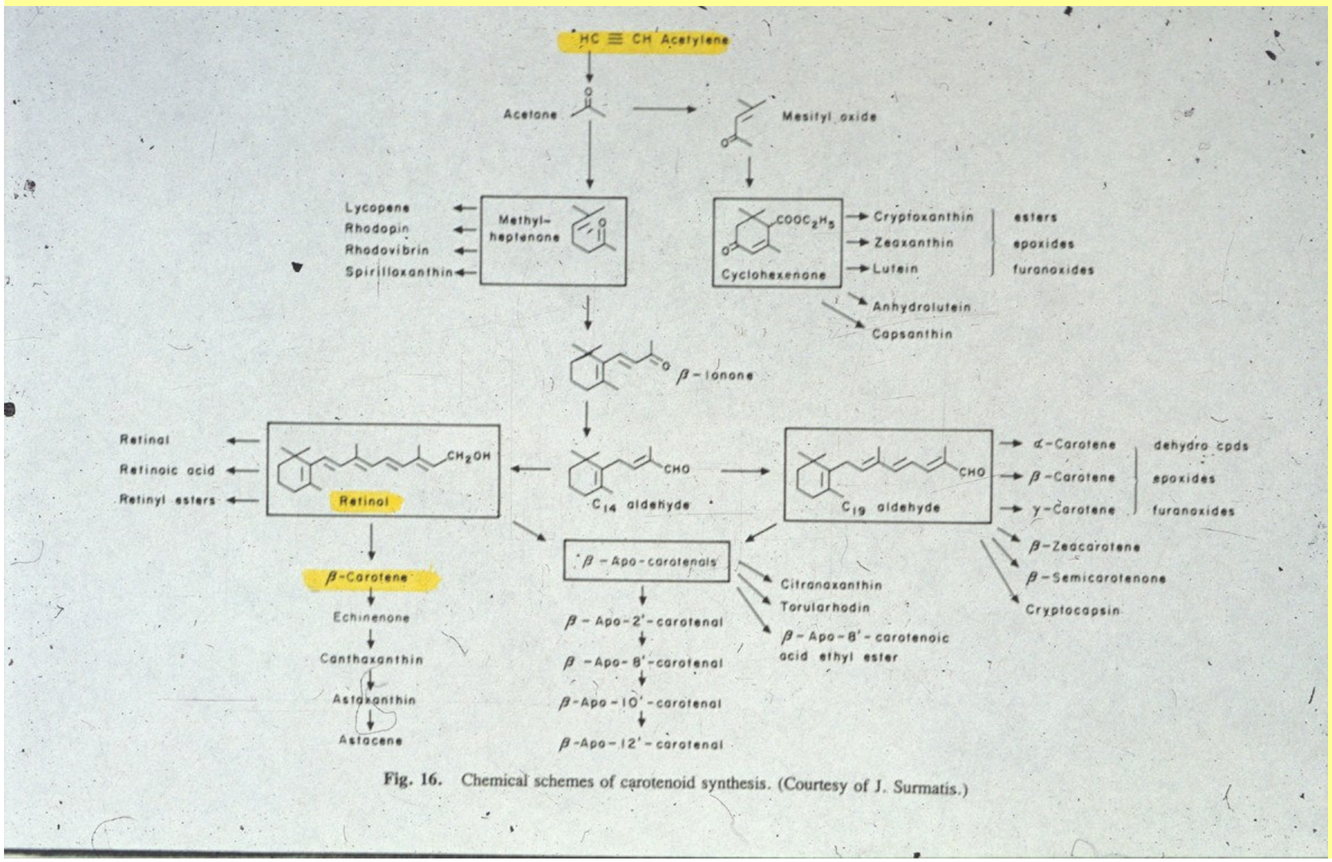
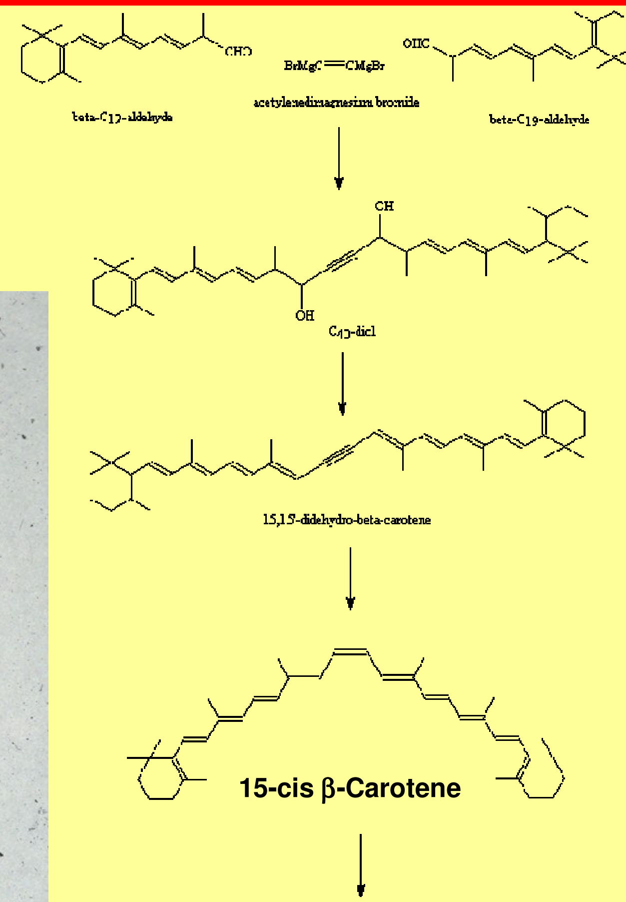


Fig. 16. Chemical schemes of carotenoid synthesis. (Courtesy of J. Surmatis.)

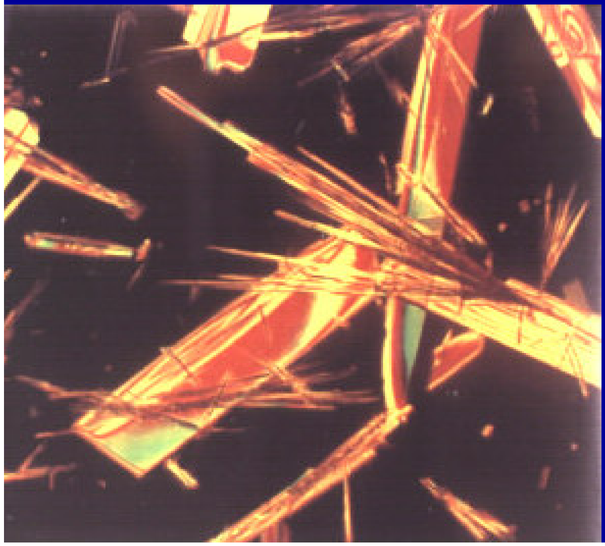


all-trans **β-Carotene**

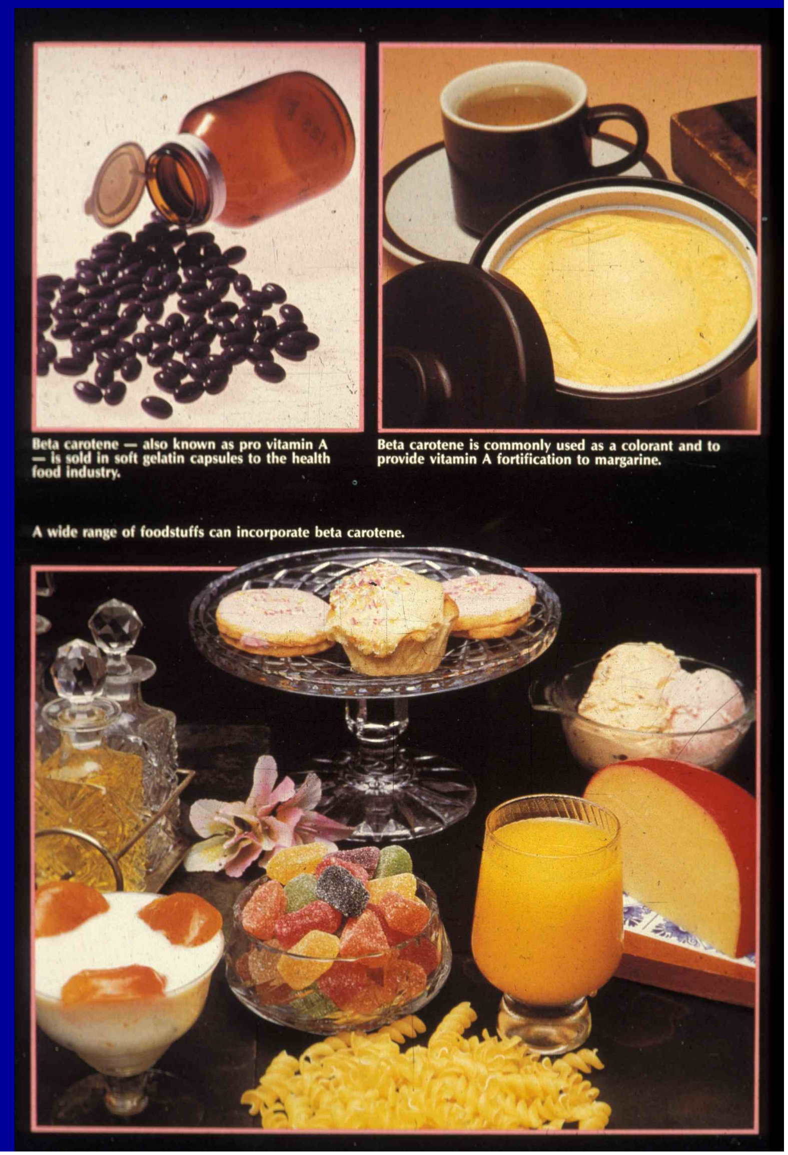
Synthetic all-trans-β-Carotene

Oil emulsion

Low fat solubility
Crystals

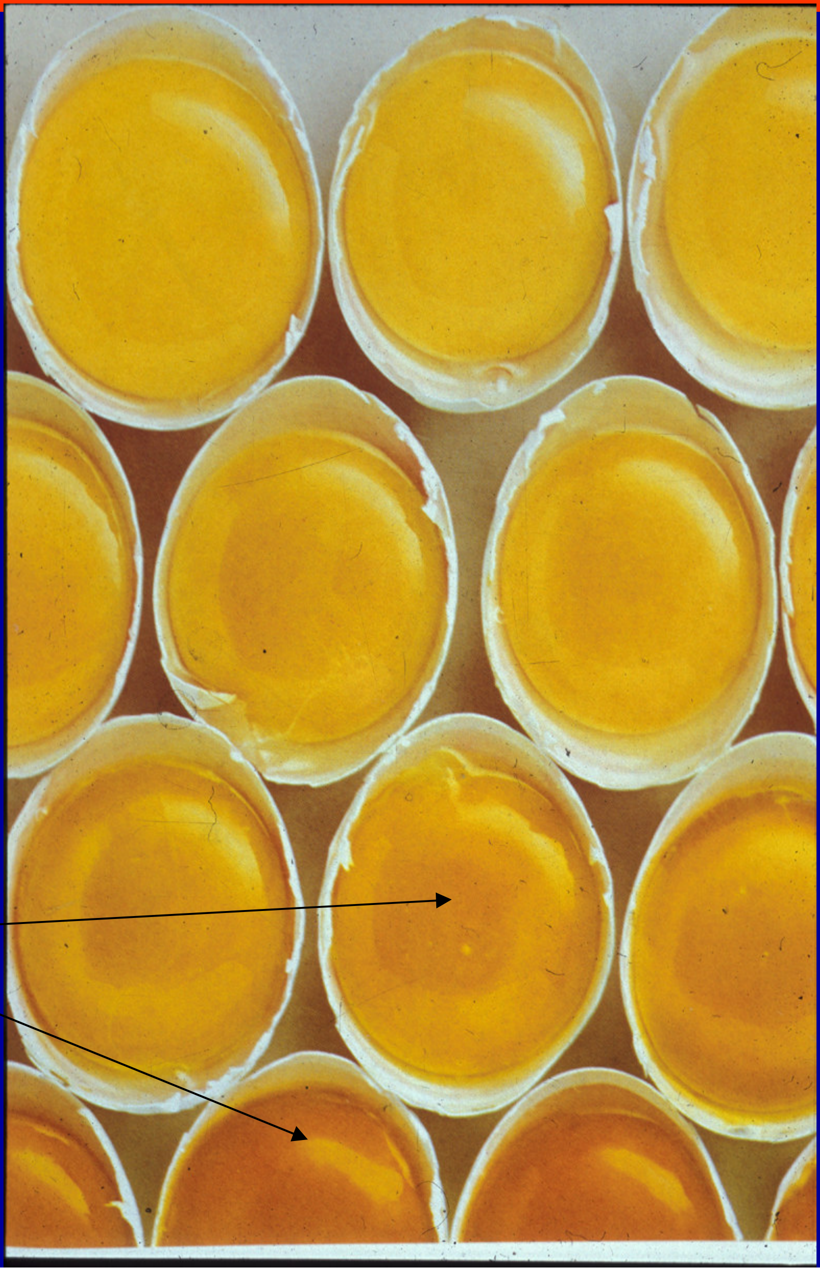


Colored Food
all-trans-β-Carotene



Colored Fish

Colored Eggs



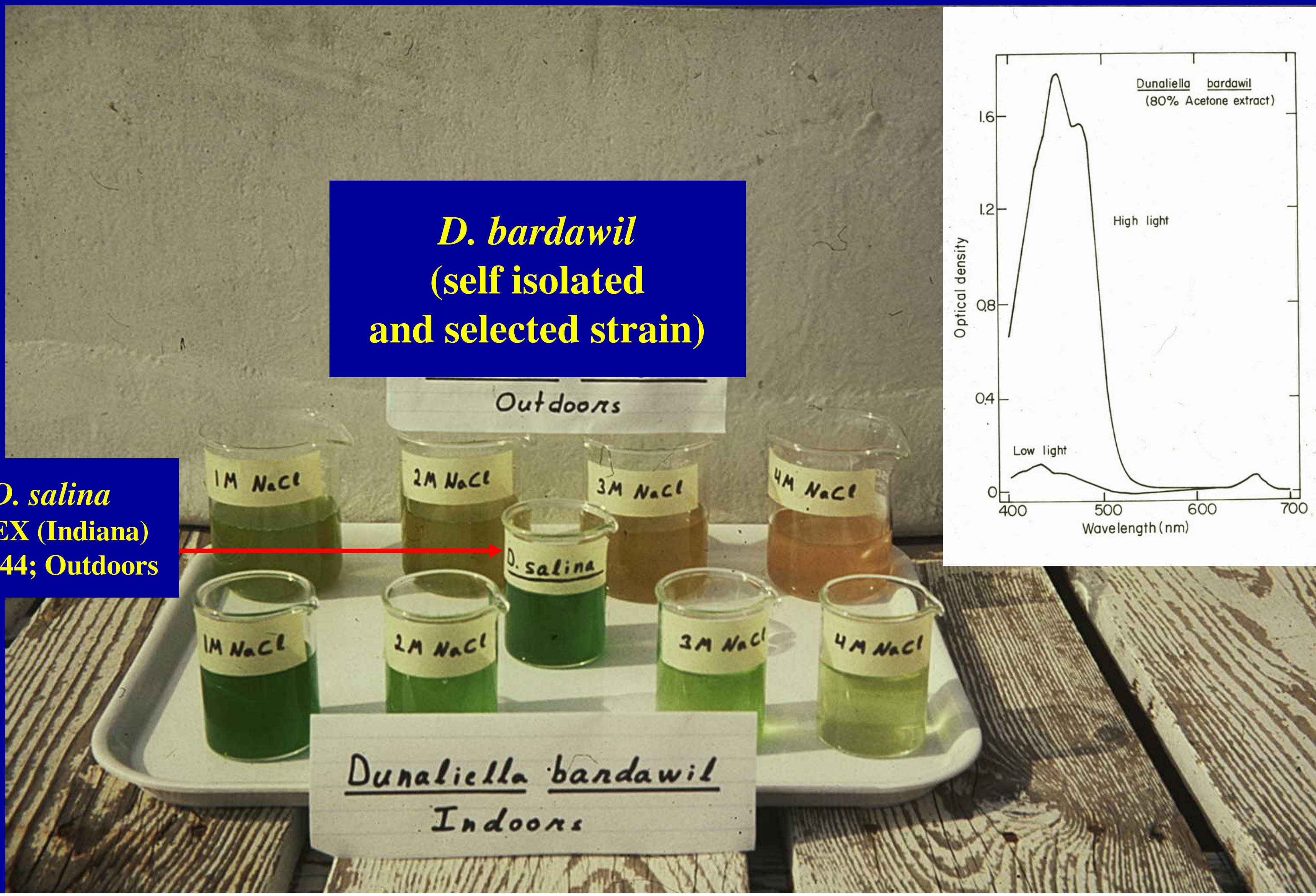
Synthetic
apo-Carotenal

Biosynthesis of β-Carotene in *Dunaliella*

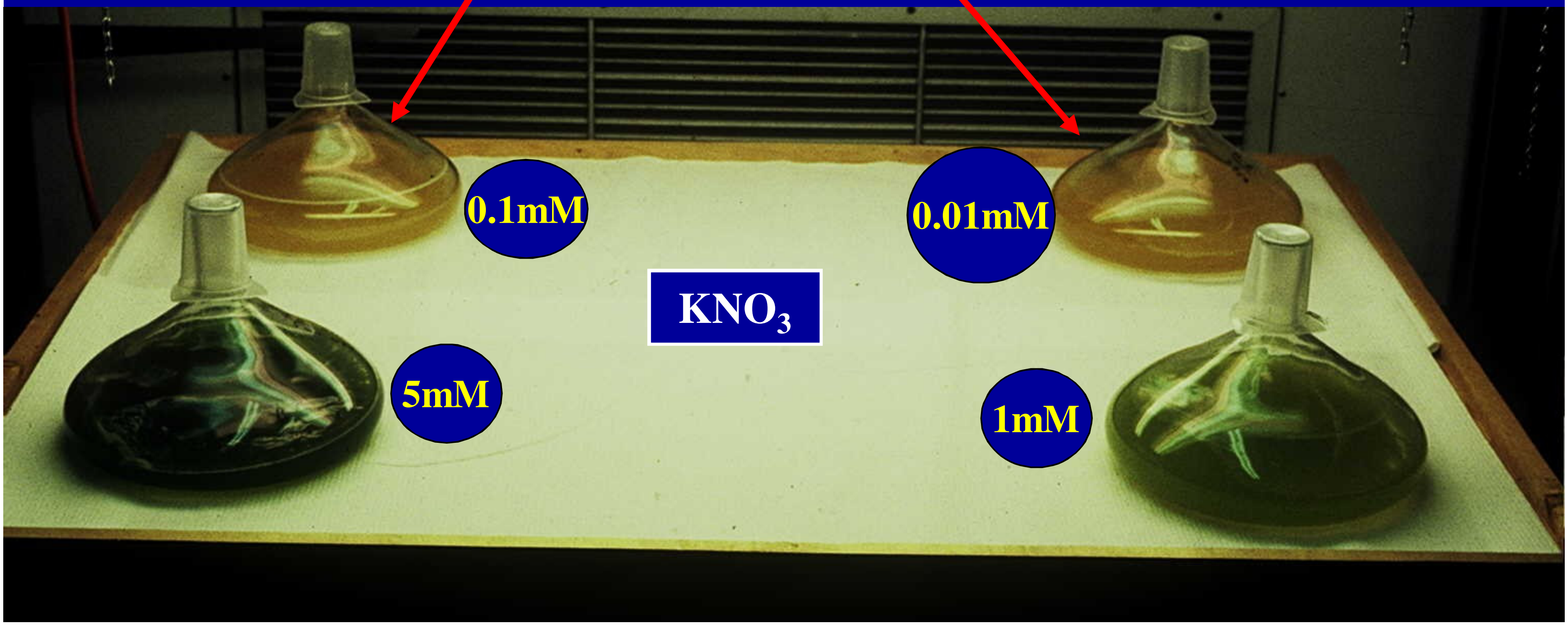
Dunaliella: β-Carotene Biosynthesis
Light & Salt Effect
(5mM KNO_3)

D. bardawil
(self isolated
and selected strain)

D. salina
UTEX (Indiana)
 # 1644; Outdoors



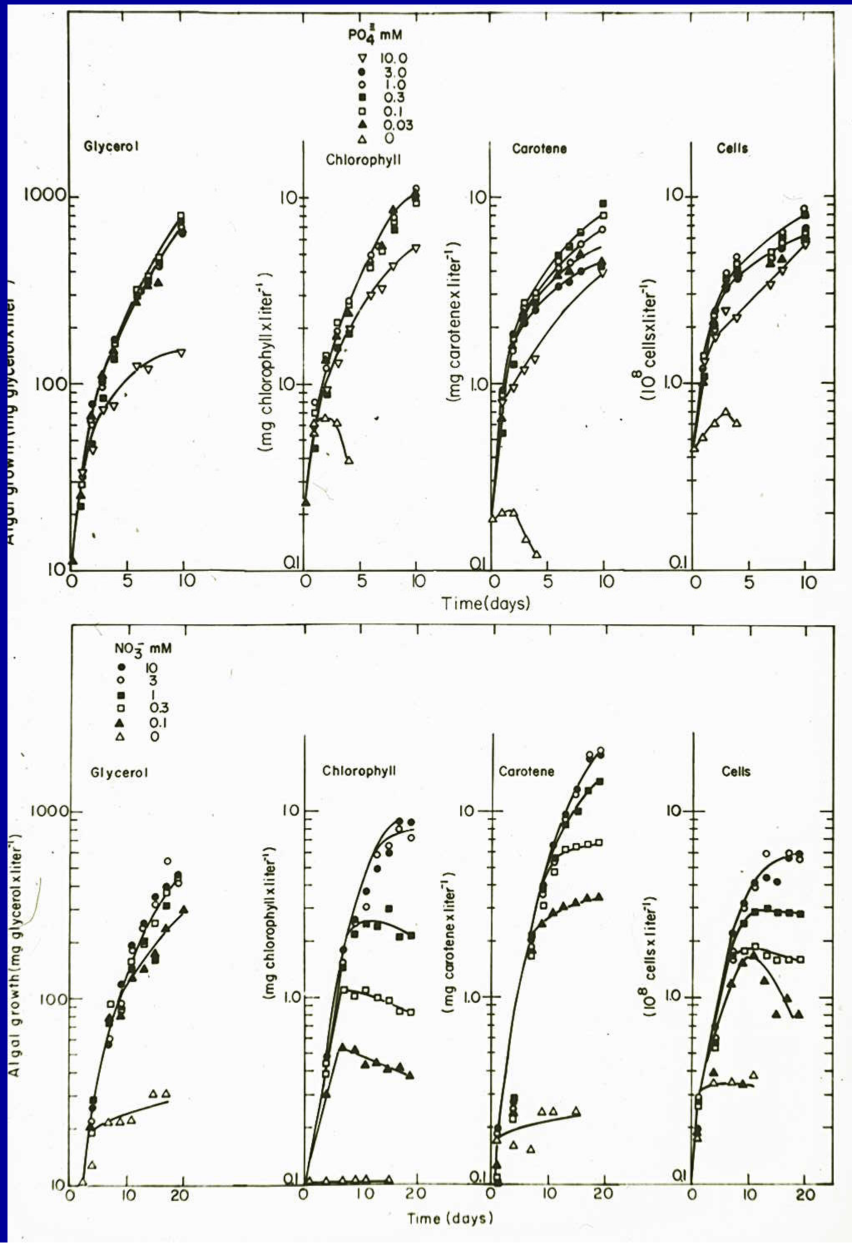
D. bardawil: β-Carotene Biosynthesis
Nitrate deficiency, chlorophyll inhibition



D. bardawil, nutrient protocol: phosphate, nitrate

P

PO_4

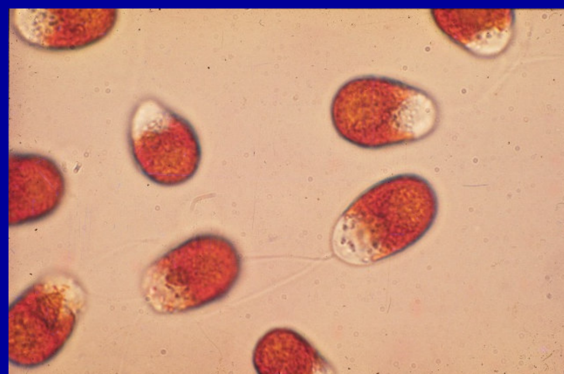


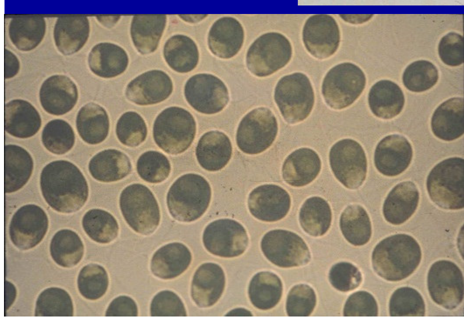
N

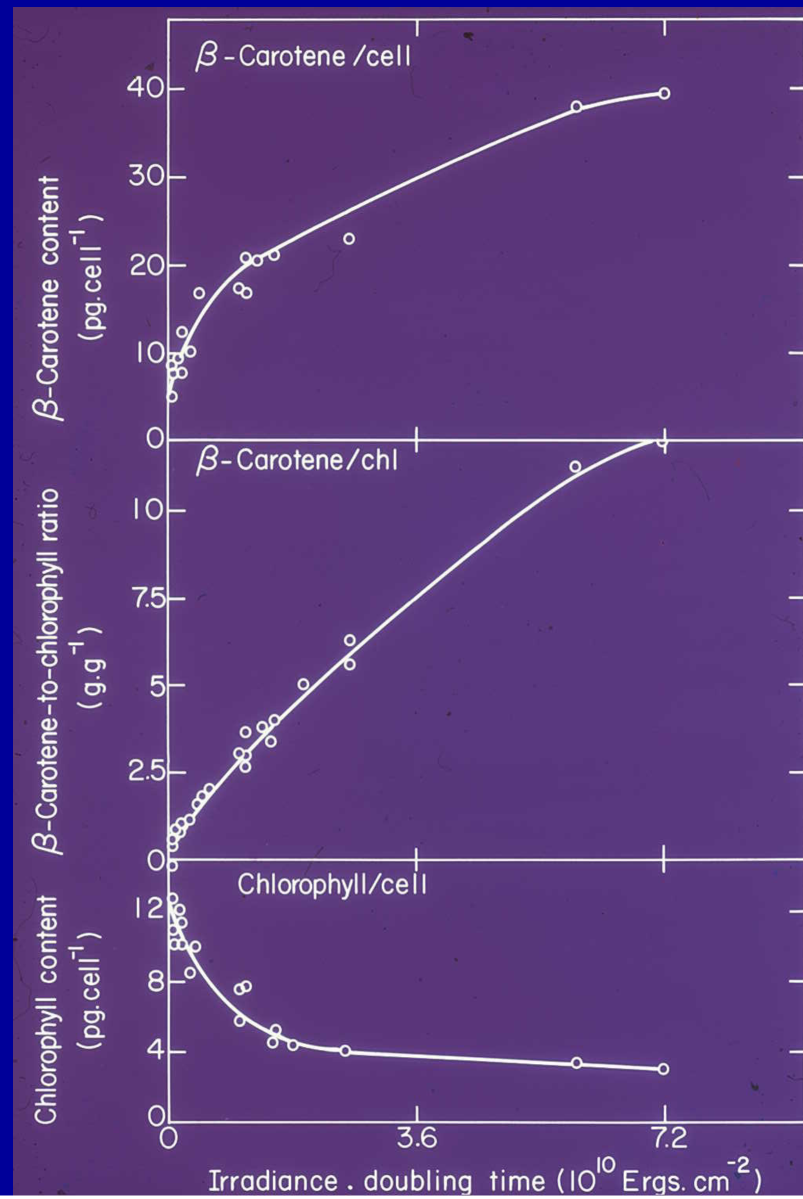
KNO_3

(NH_4, Urea)

Dunaliella bardawil, β-Carotene Biosynthesis
Light & Doubling time; car/chl = $\int h\nu_x\,dt$





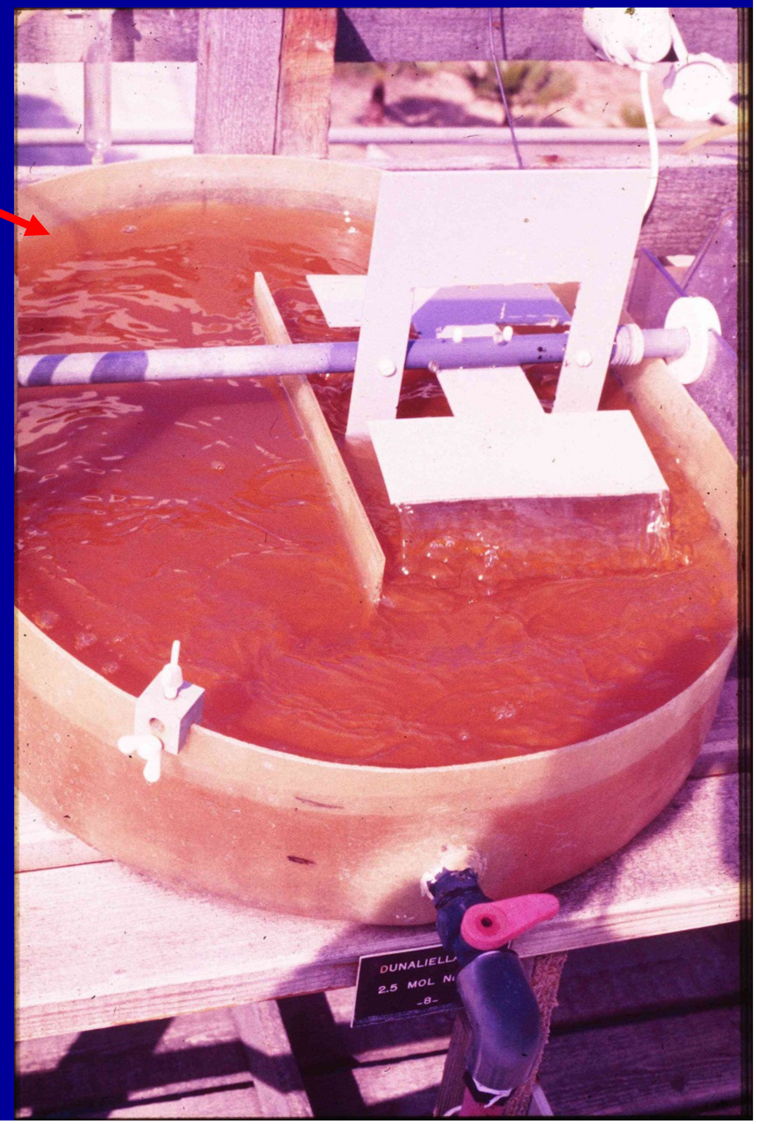


Outdoor Cultivation of *Dunaliella*

> 10% β-Carotene/DW

D. bardawil

low salt, low nitrate, low night temperature, high light
($\int h\nu_x\, dt$) = high lipoid β-carotene

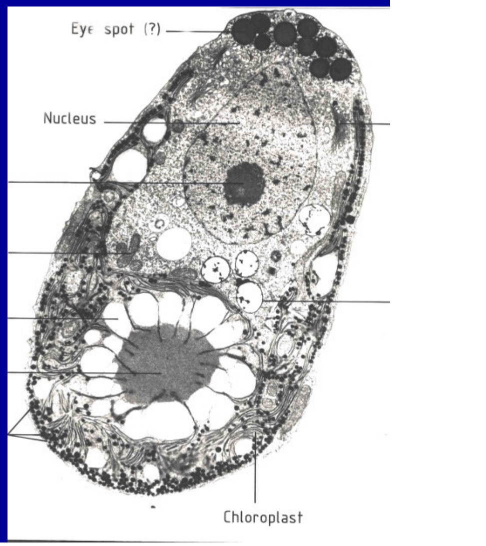


D. salina **UTEX (Indiana) # 1644**

Conclusion:

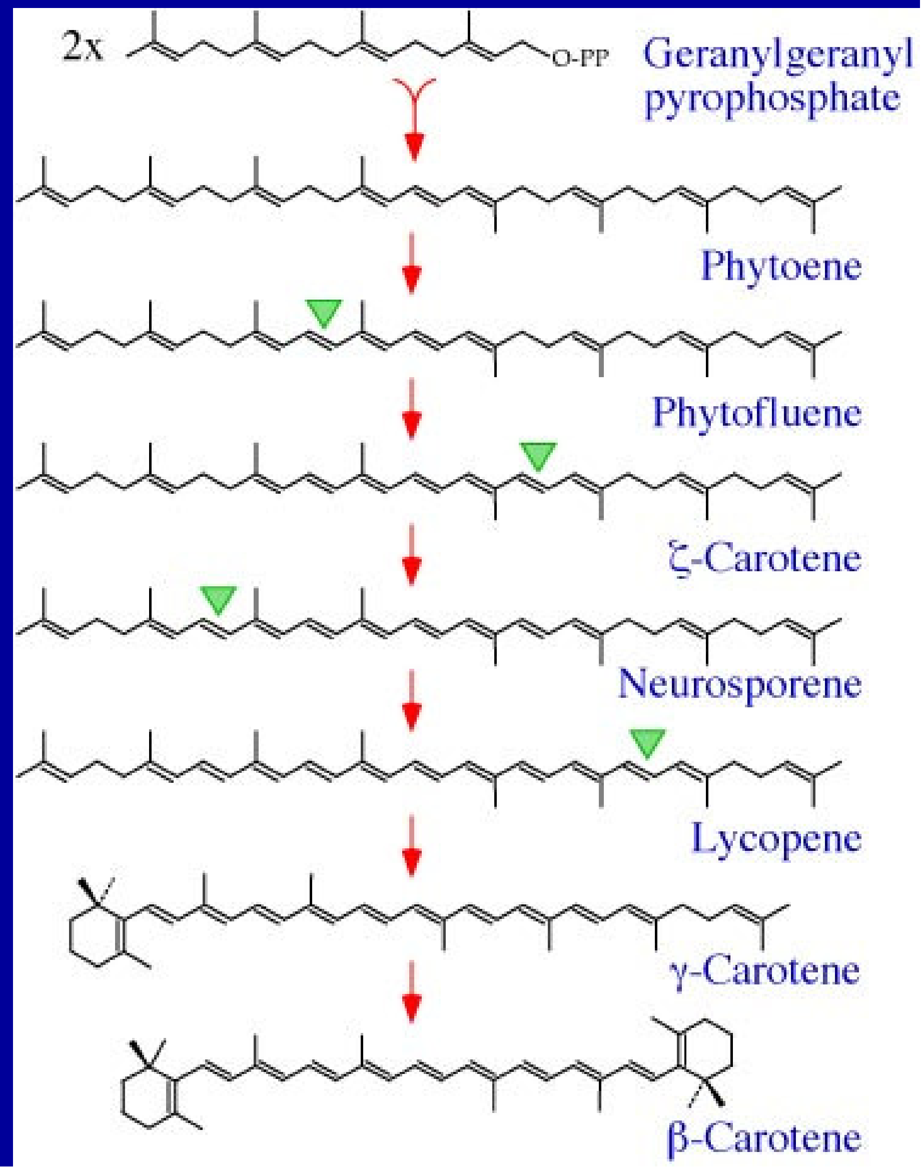
The lipoid β-Carotene in *Dunaliella* protects the alga against damage by high irradiation.

The major function of the β-carotene is light screening

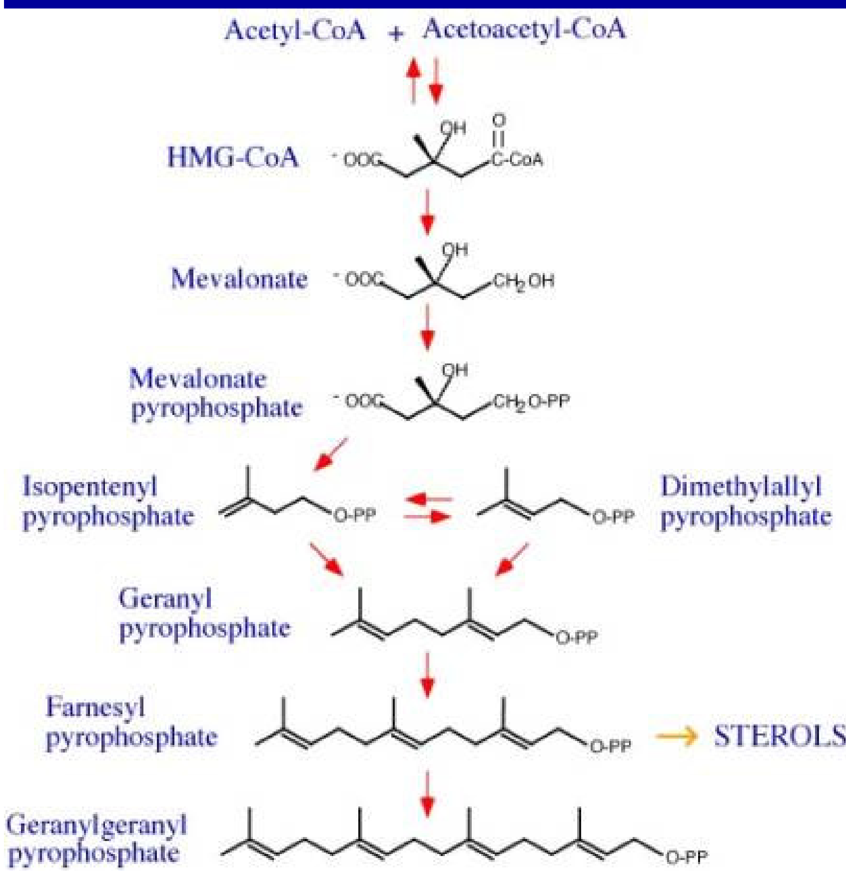


Biosynthesis of Carotenoids

Lipophilic pathway



Polar pathway



Biosynthesis of Carotenoids & Isomers in *Dunaliella*

β-Carotene isomers, isoprene multi structure

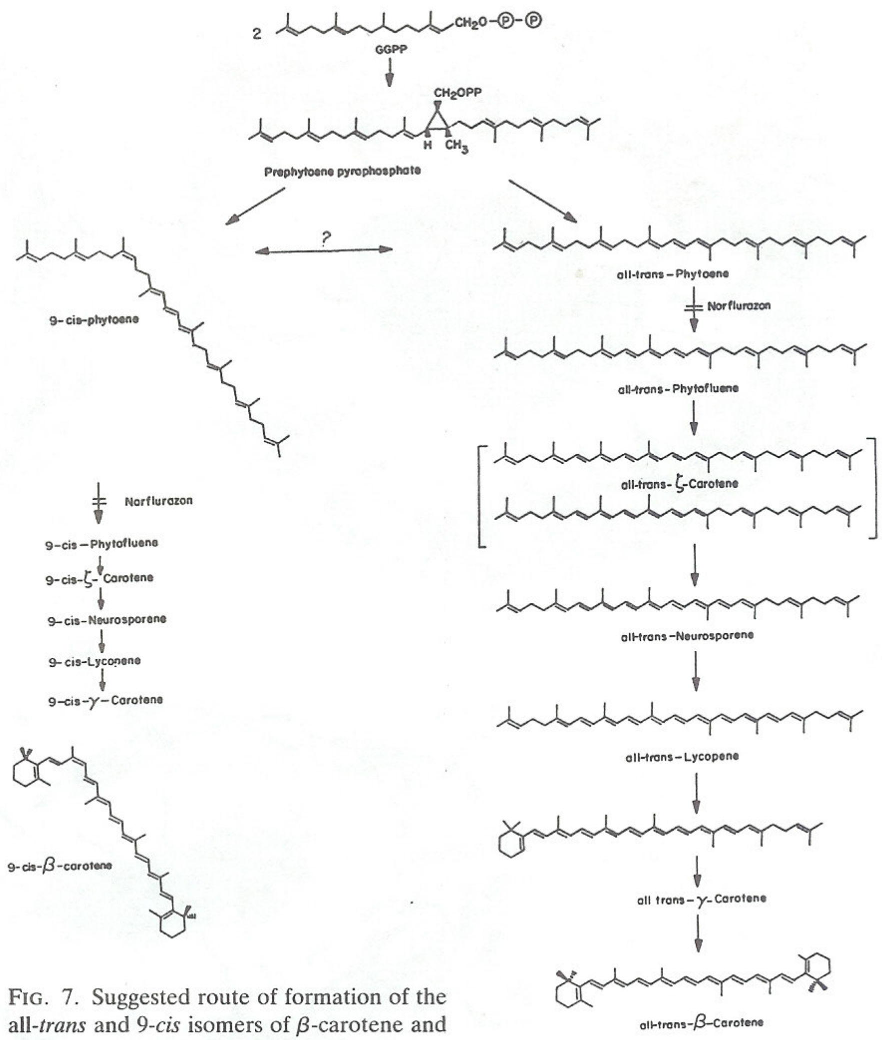
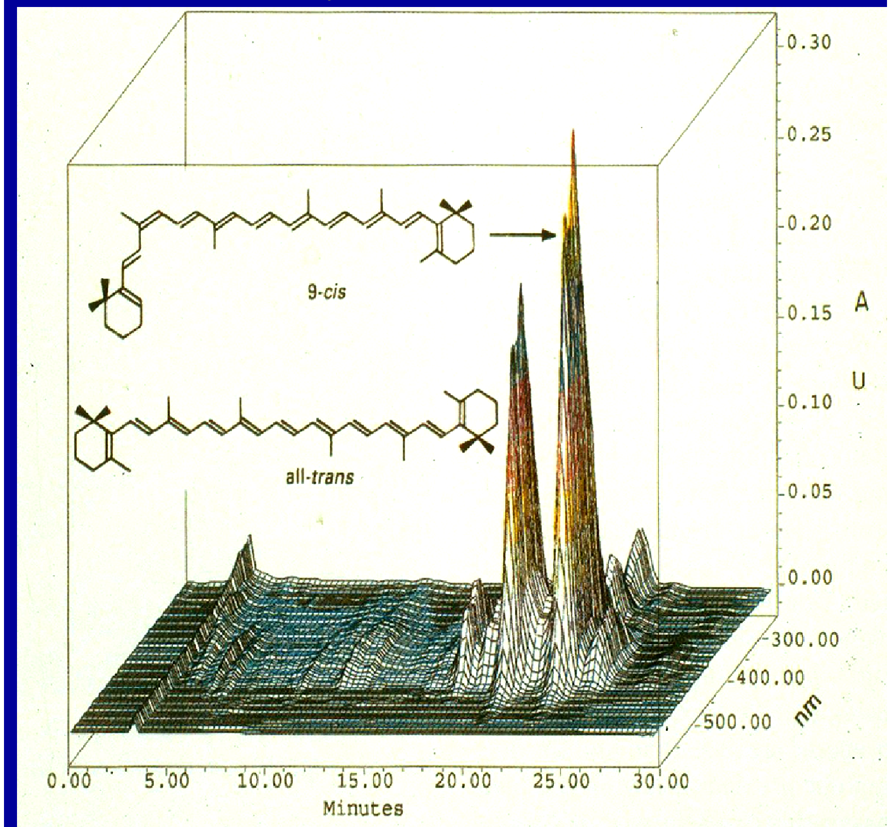


FIG. 7. Suggested route of formation of the all-*trans* and 9-*cis* isomers of β-carotene and phytoene.

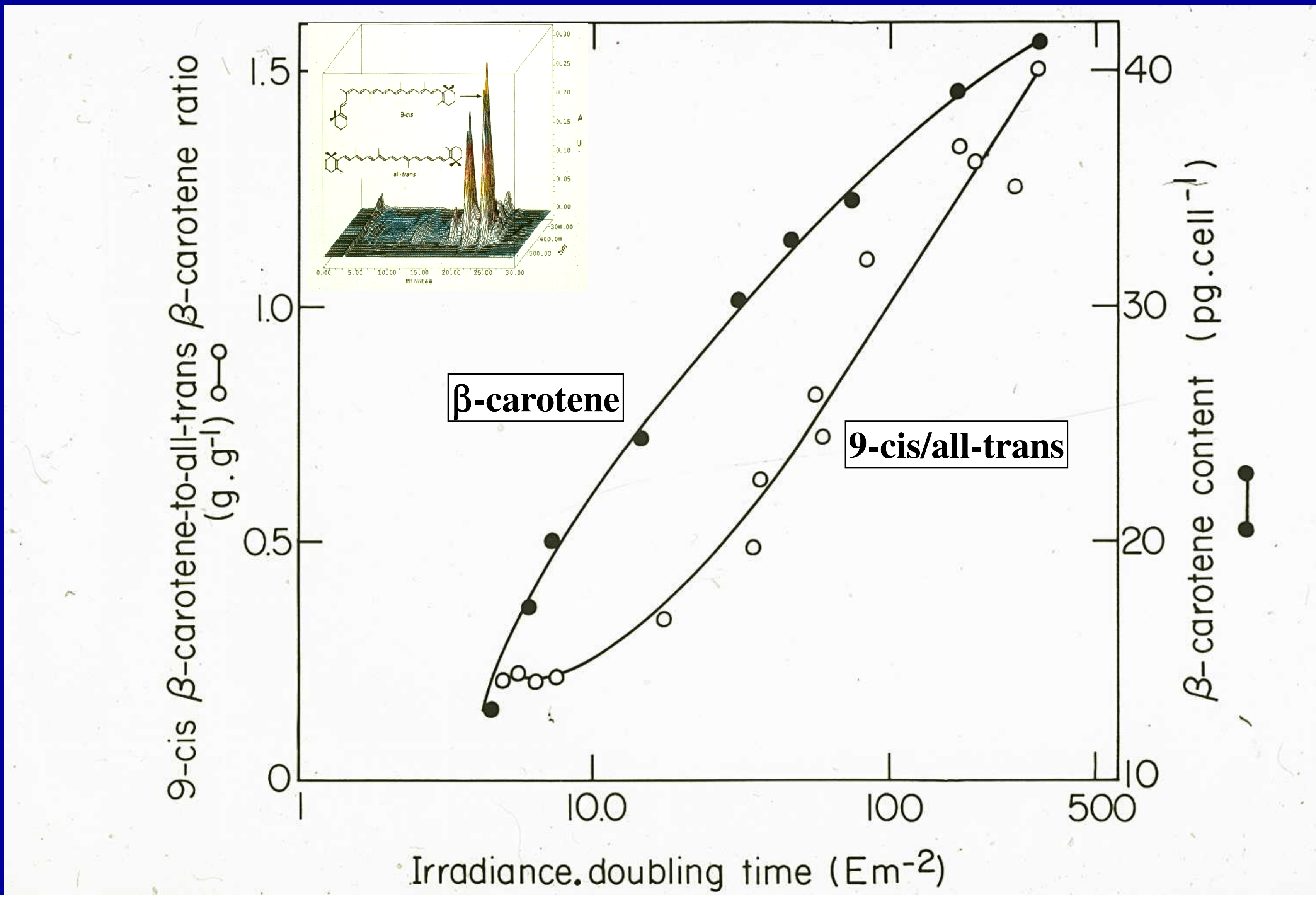
HPLC Analysis

Vydac TP column



Light and β-Carotene Stereoisomeric Ratio

car/chl & 9-cis/all-trans = $\int h\nu_x \, dt$



9-cis β-Carotene
is oily β-carotene
with a major cellular function to
dissolve the crystallized
all-trans β-carotene
in the algal globules

Dunaliella

Biotechnology

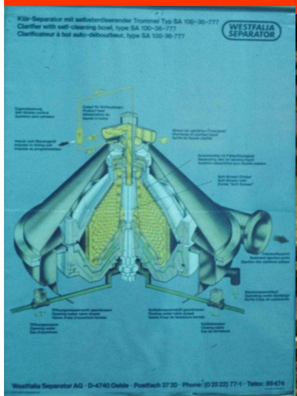
Cultivation & Processing

Dunaliella Biotechnology

Intensive Plant, NBT Ltd., Eilat, Israel, 100,000 m^2

$\int h \nu_x \, dt$

DUNALIELLA Harvesting
Westphalia Ltd., continuous centrifuges



Dunaliella

Spray Dried High β-Carotene Powder

β-Carotene *Dunaliella* Powder Vacuum Packing

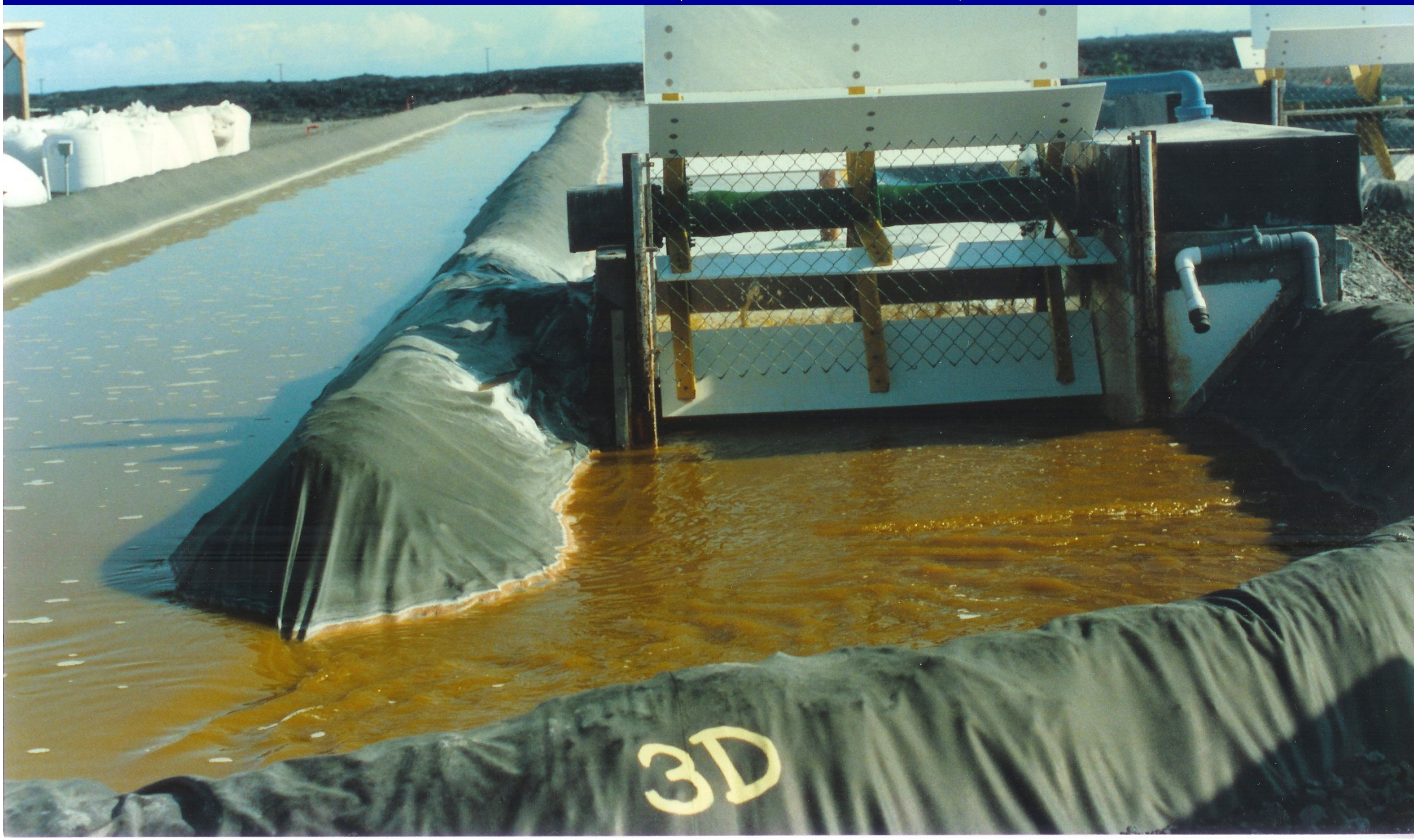
Dunaliella Capsules

300 mg dry powder/cap
20 mg β-carotene
9-cis/all-trans 1:1

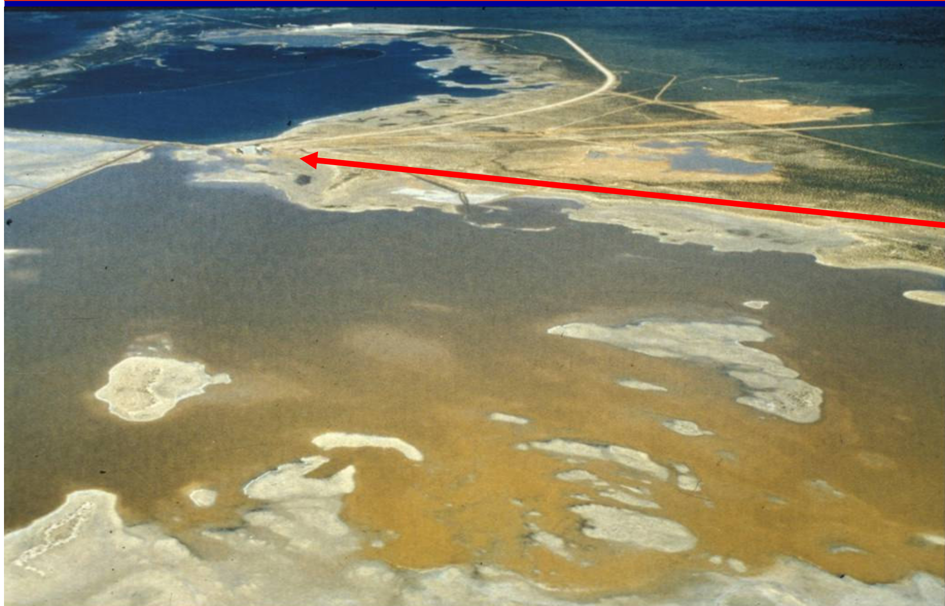
Dunaliella Intensive Plant
Jilantai, China

Dunaliella Plant
Jilantai, Inner Mongolia, China

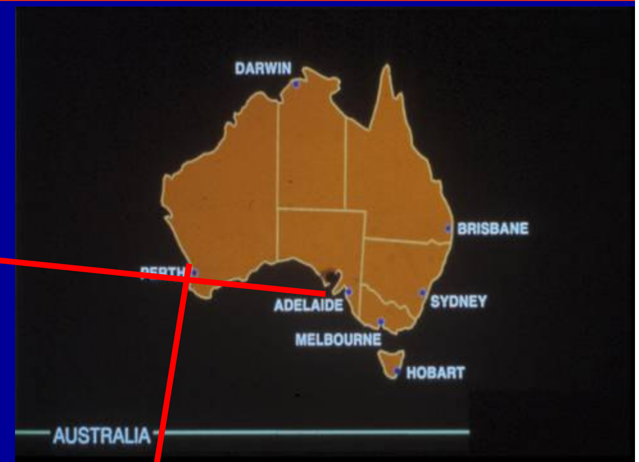
Dunaliella Plant
Kona Island, Hawaii, USA



Extensive Cultivation of *Dunaliella* Australia



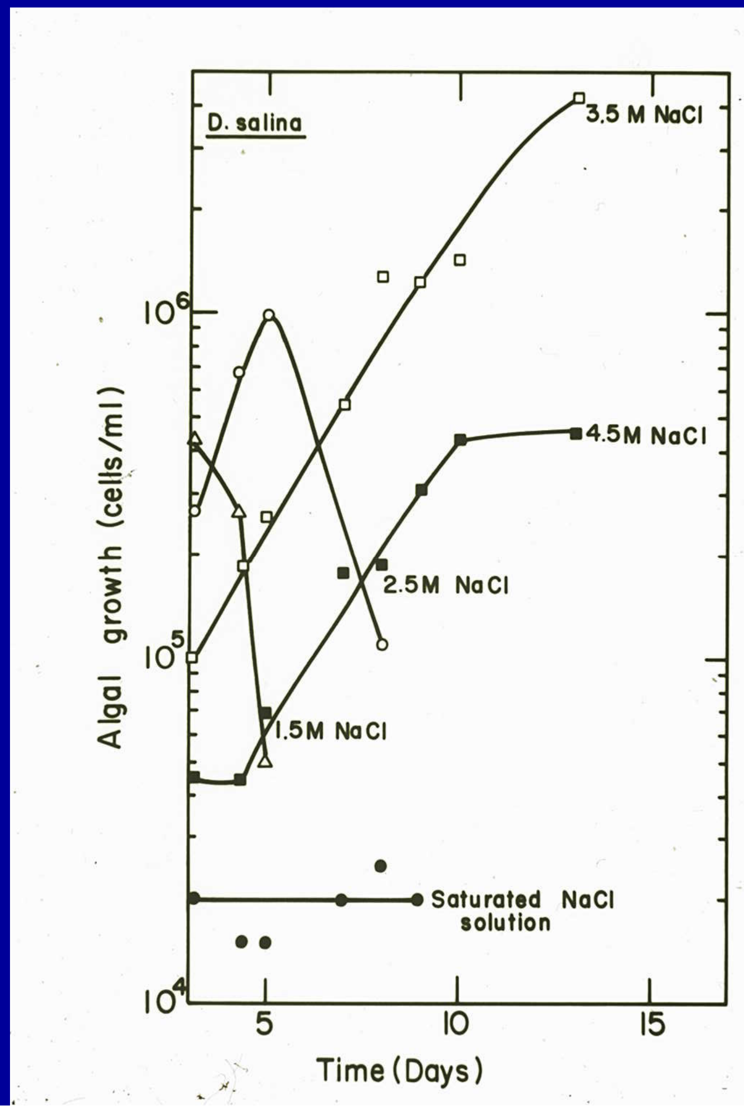
$\int h \nu_x \, dt$



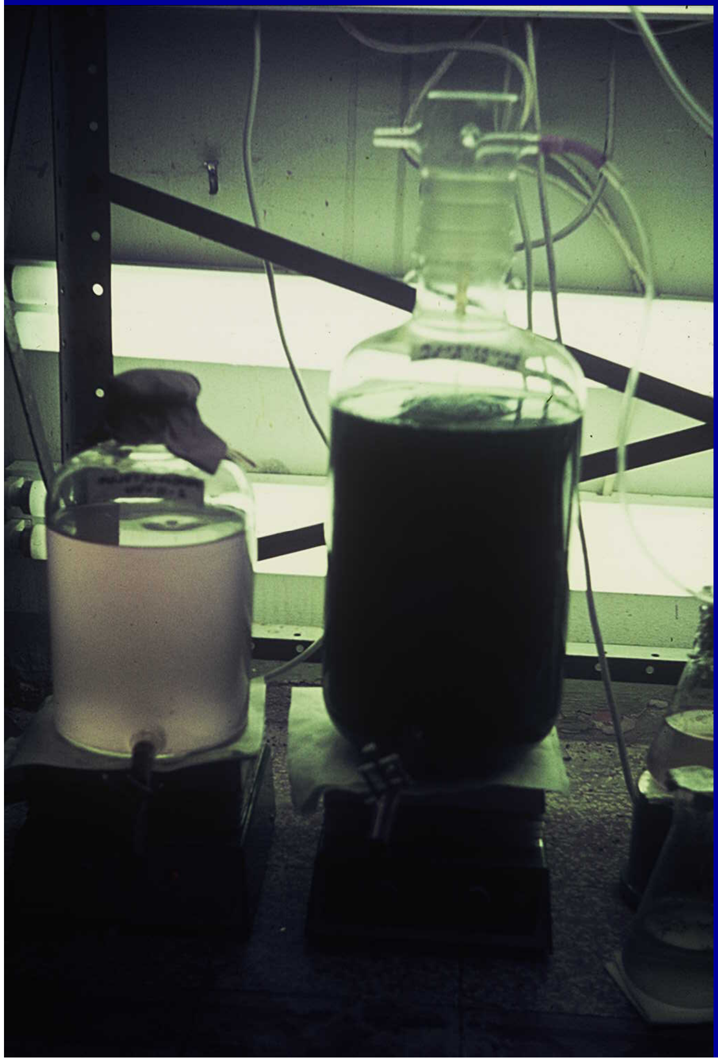
Electro-Magnet Harvesting of non-flagellated hydrophobic *Dunaliella* cysts (spores) by hydrophobic magnetite particles

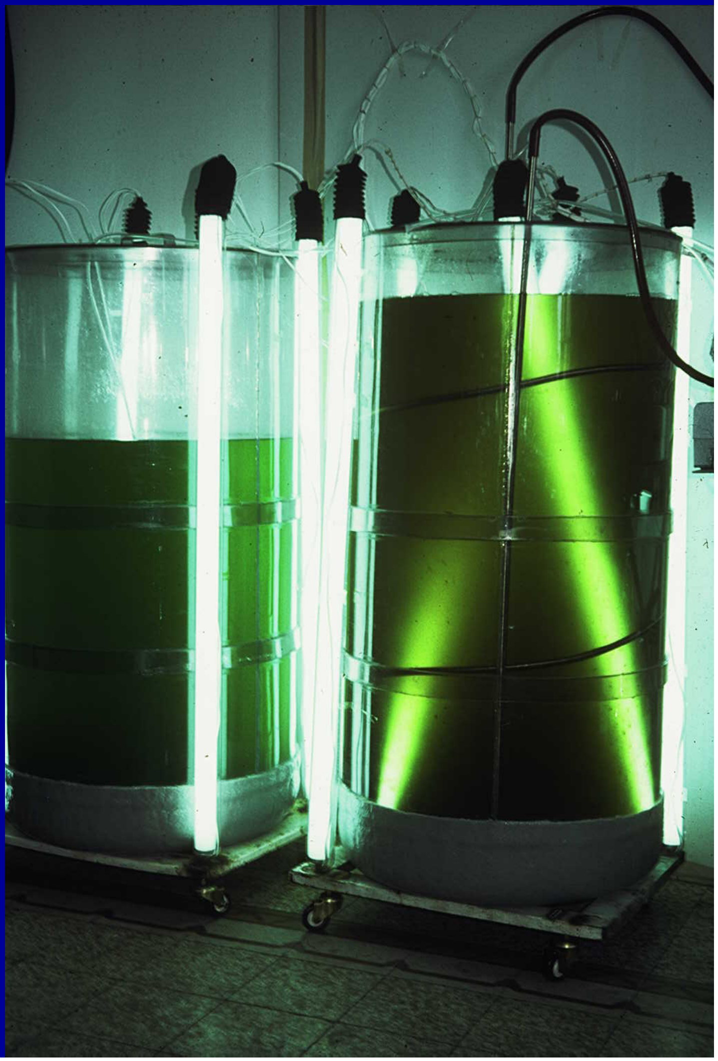
Extensive Cultivation of *Dunaliella*
Australia

Predators (hetero-amoebae) of *Dunaliella*
Salt Elimination Effect



Indoor *Dunaliella* Cultivation
Zooplankton Infestation

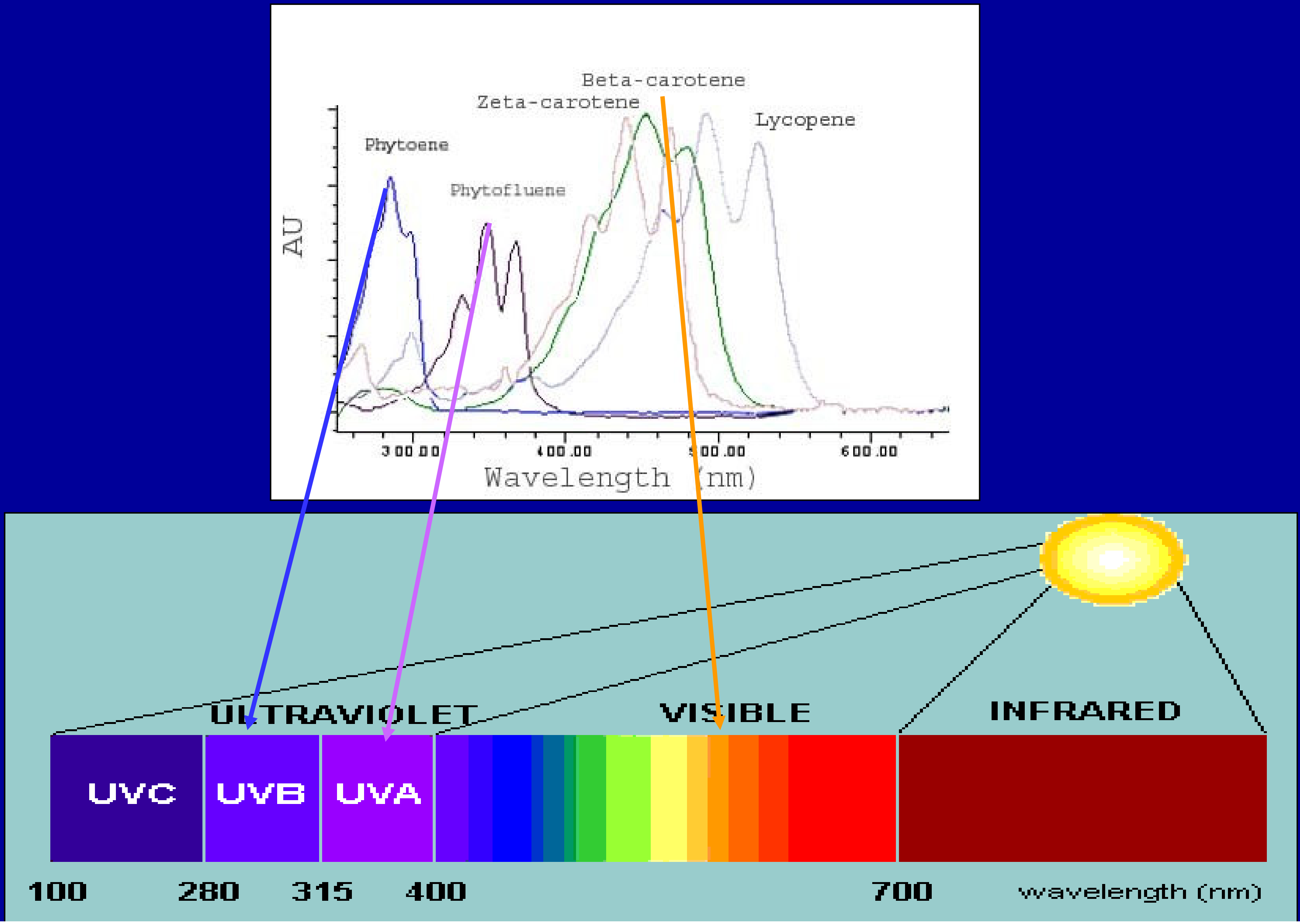




Bacterial Contamination
Halobacterium halobium

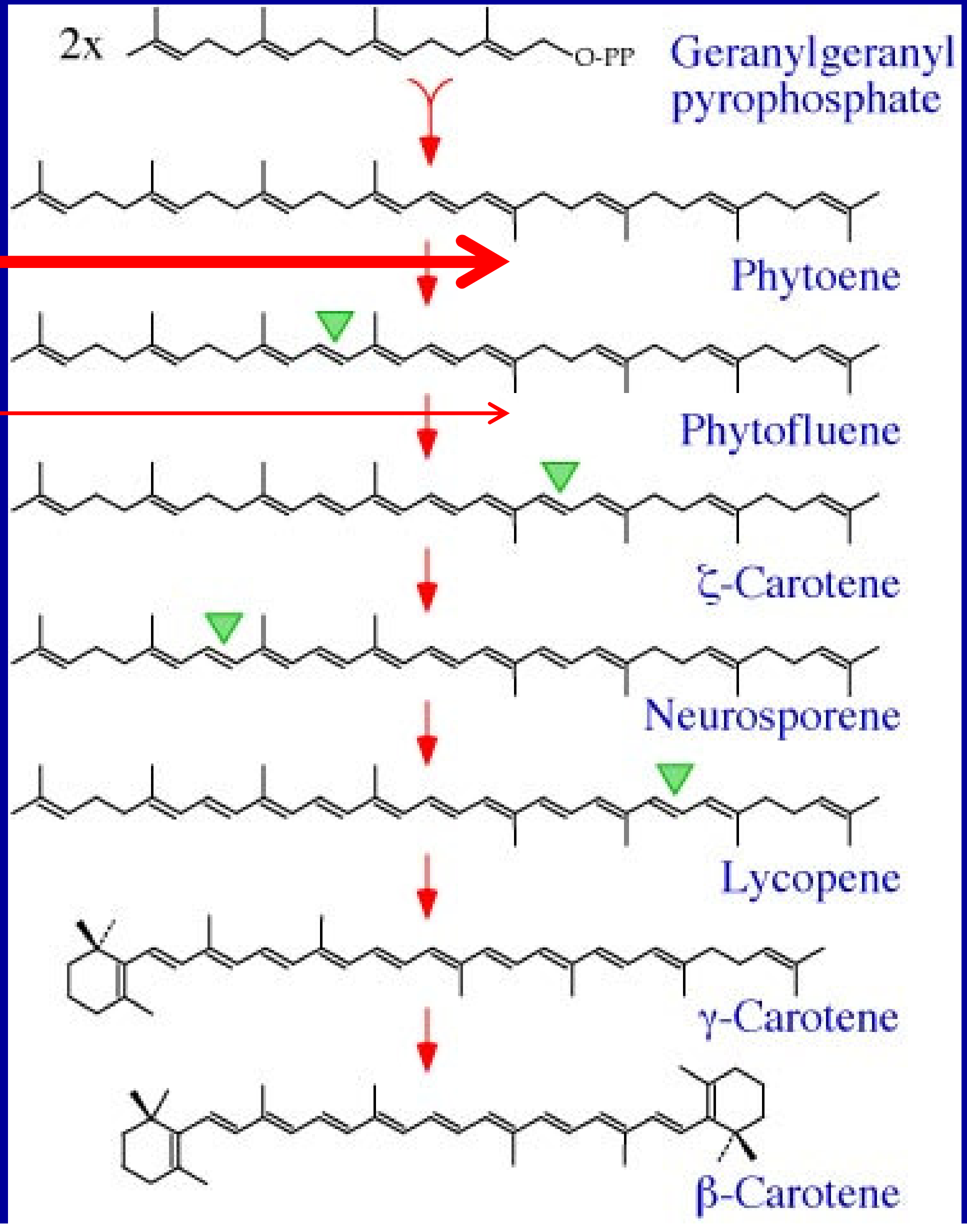
Selection and Production
of
phytoene/phytofluene Colorless Carotenoids
by
Dunaliella

Absorption Spectra of Carotenoids



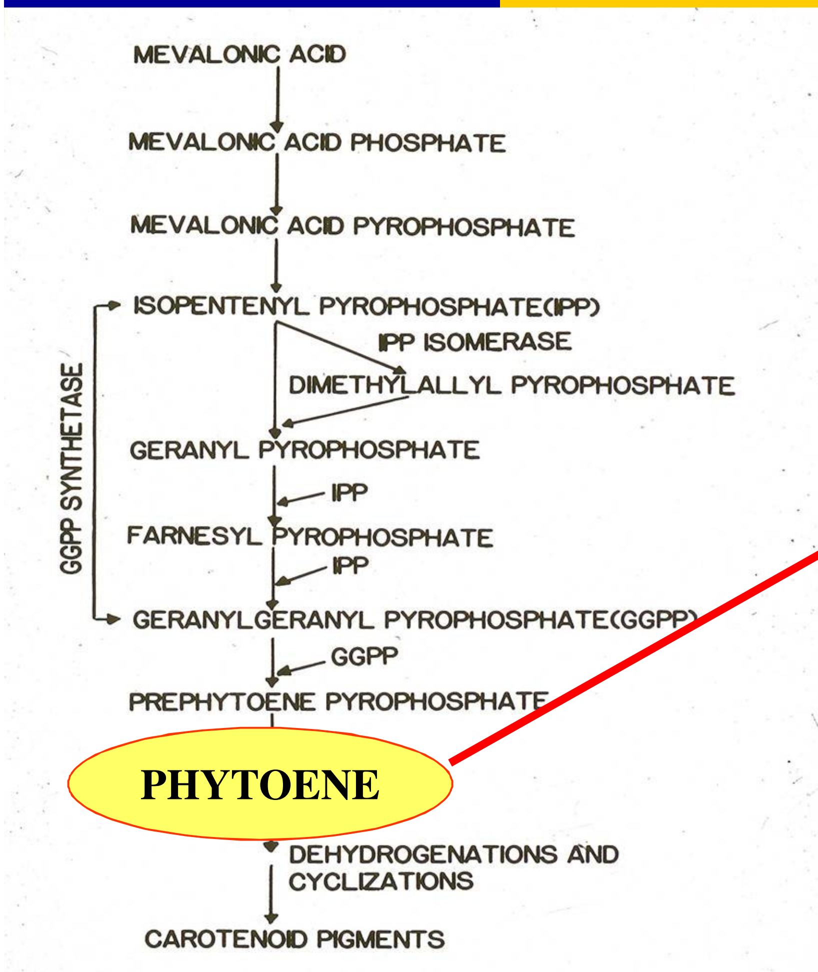
Biosynthesis of Carotenoids

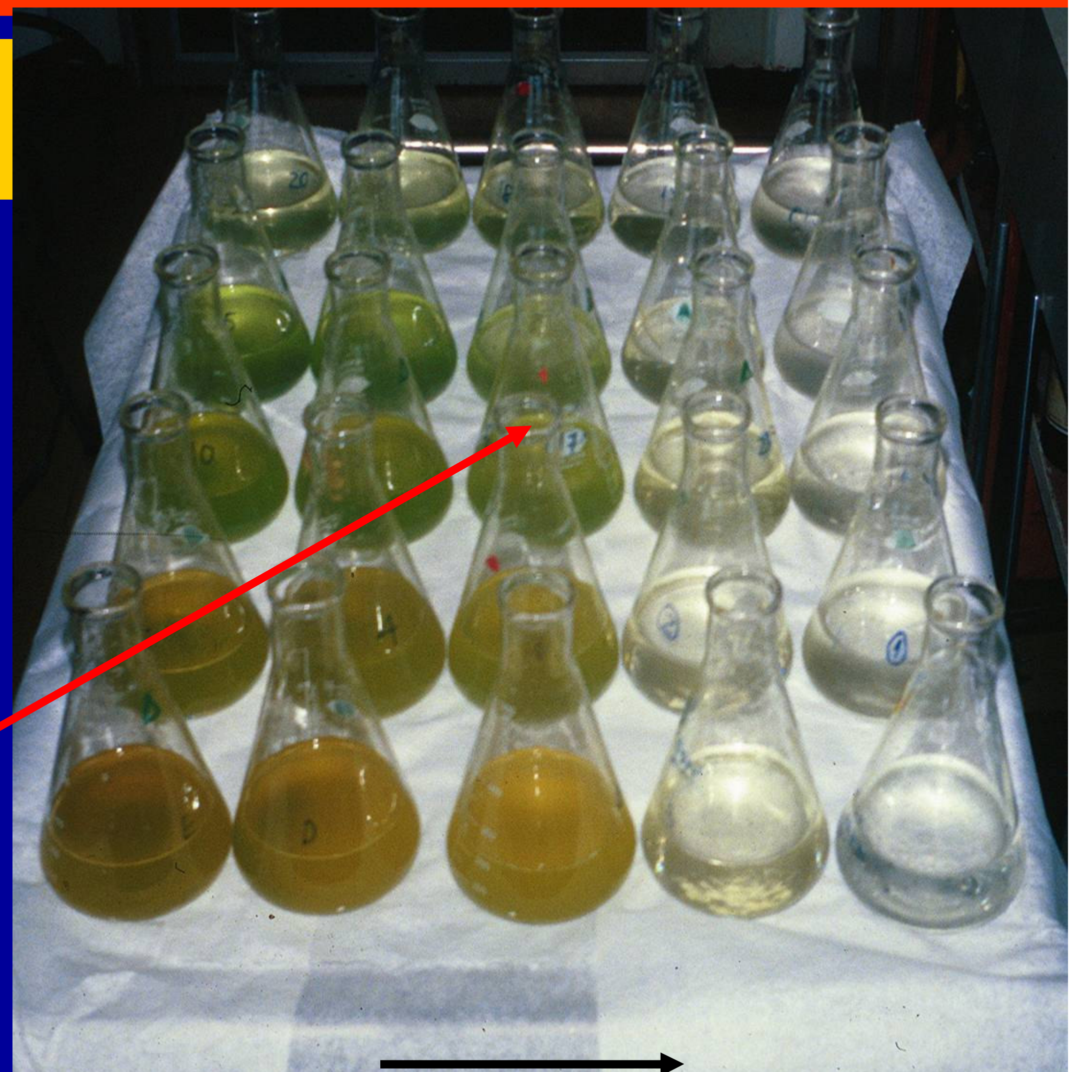
Phytoene desaturase



SELECTION OF PHYTOENE-RICH *DUNALIELLA*

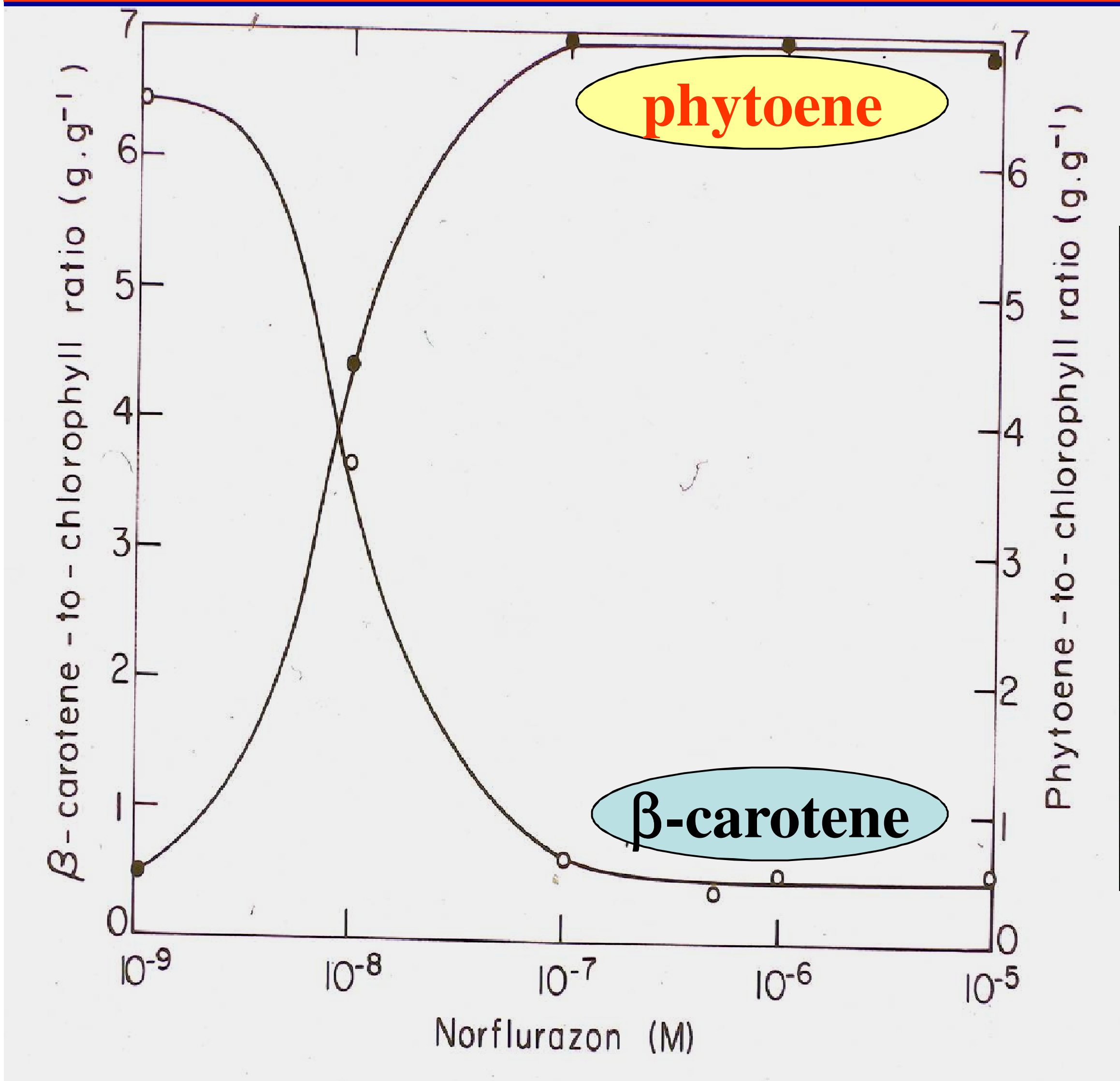
Light intensity

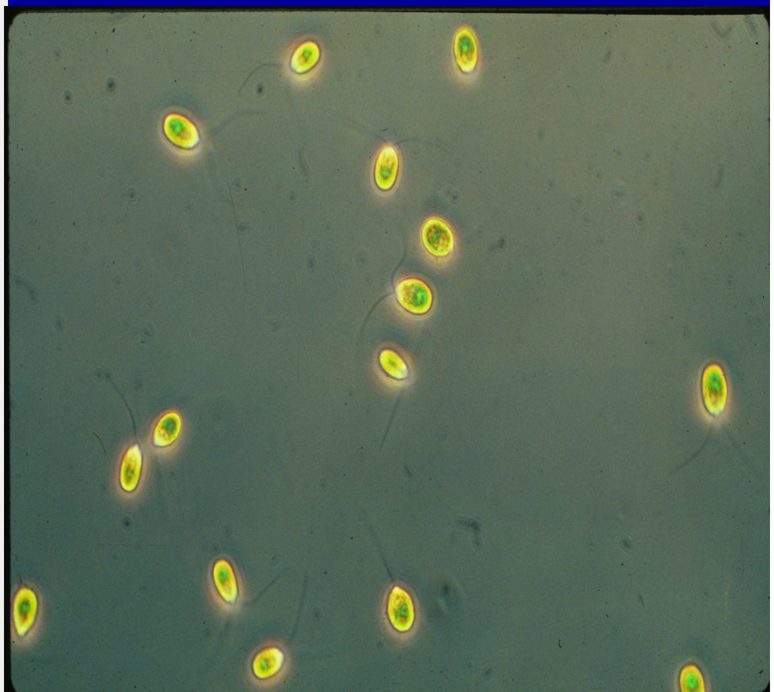




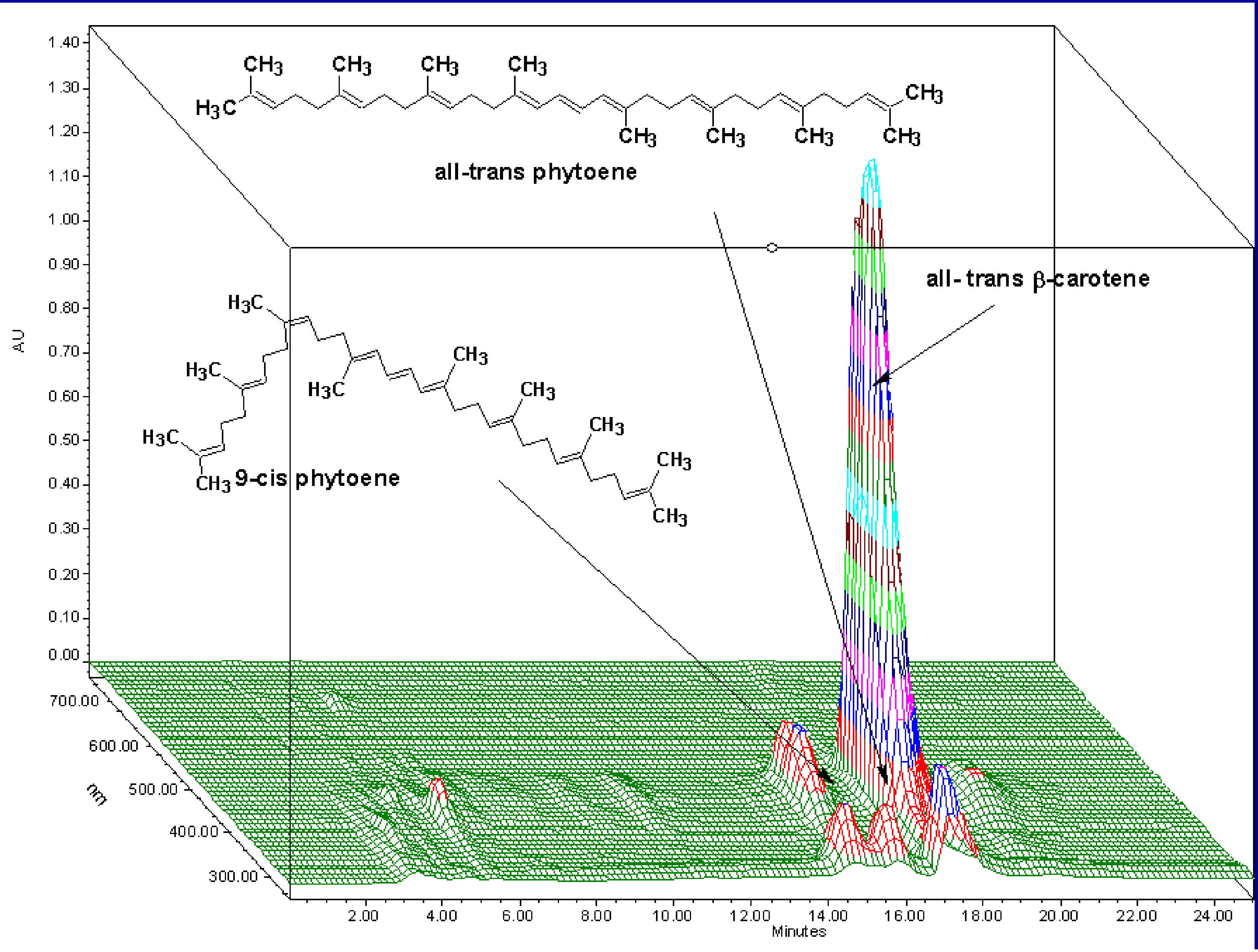
Mutagen & Norflurazon (bleaching herbicide)

Response of β-carotene and Phytoene to Norflurazon

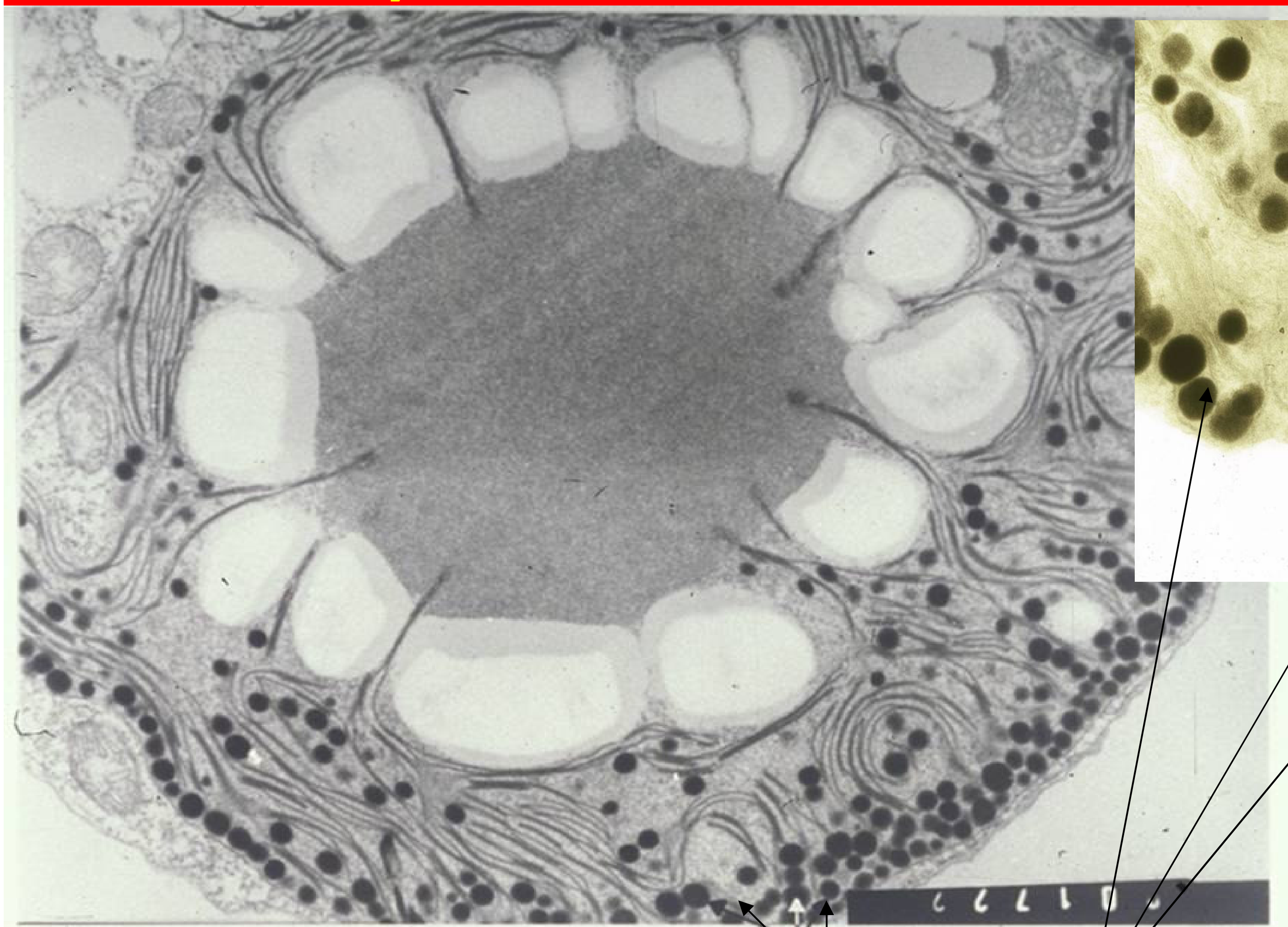


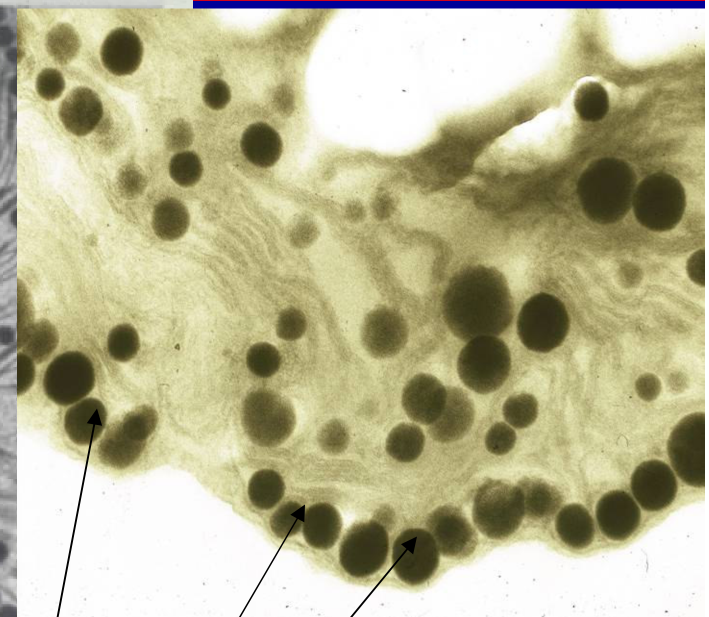


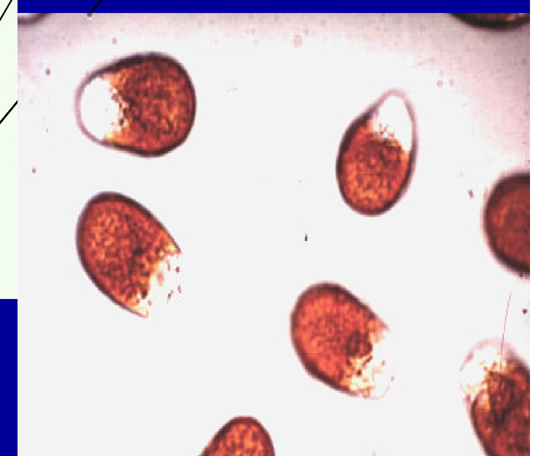
3D HPLC of *Dunaliella* β-Carotene/Phytoene Extract



β-Carotene *Dunaliella*

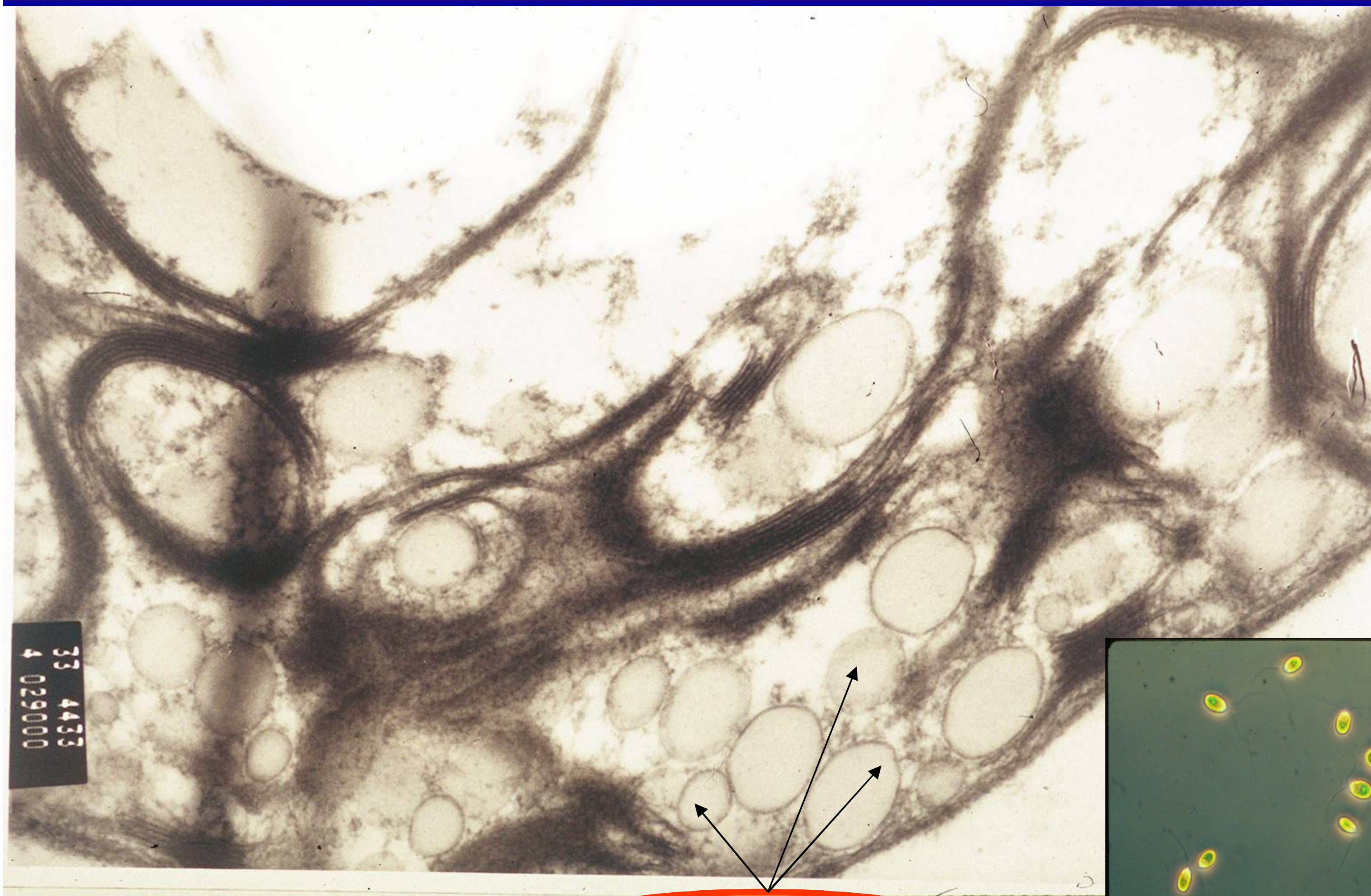


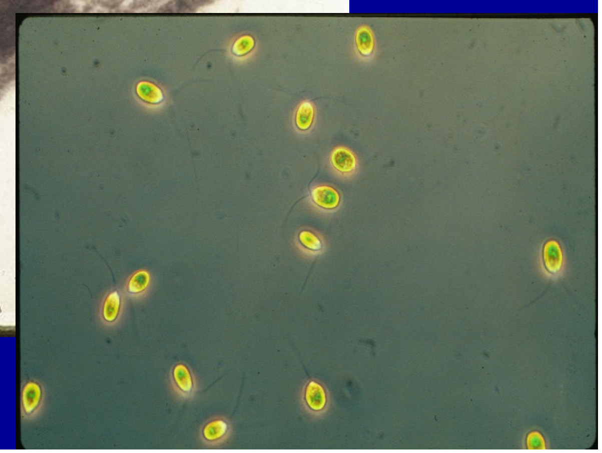




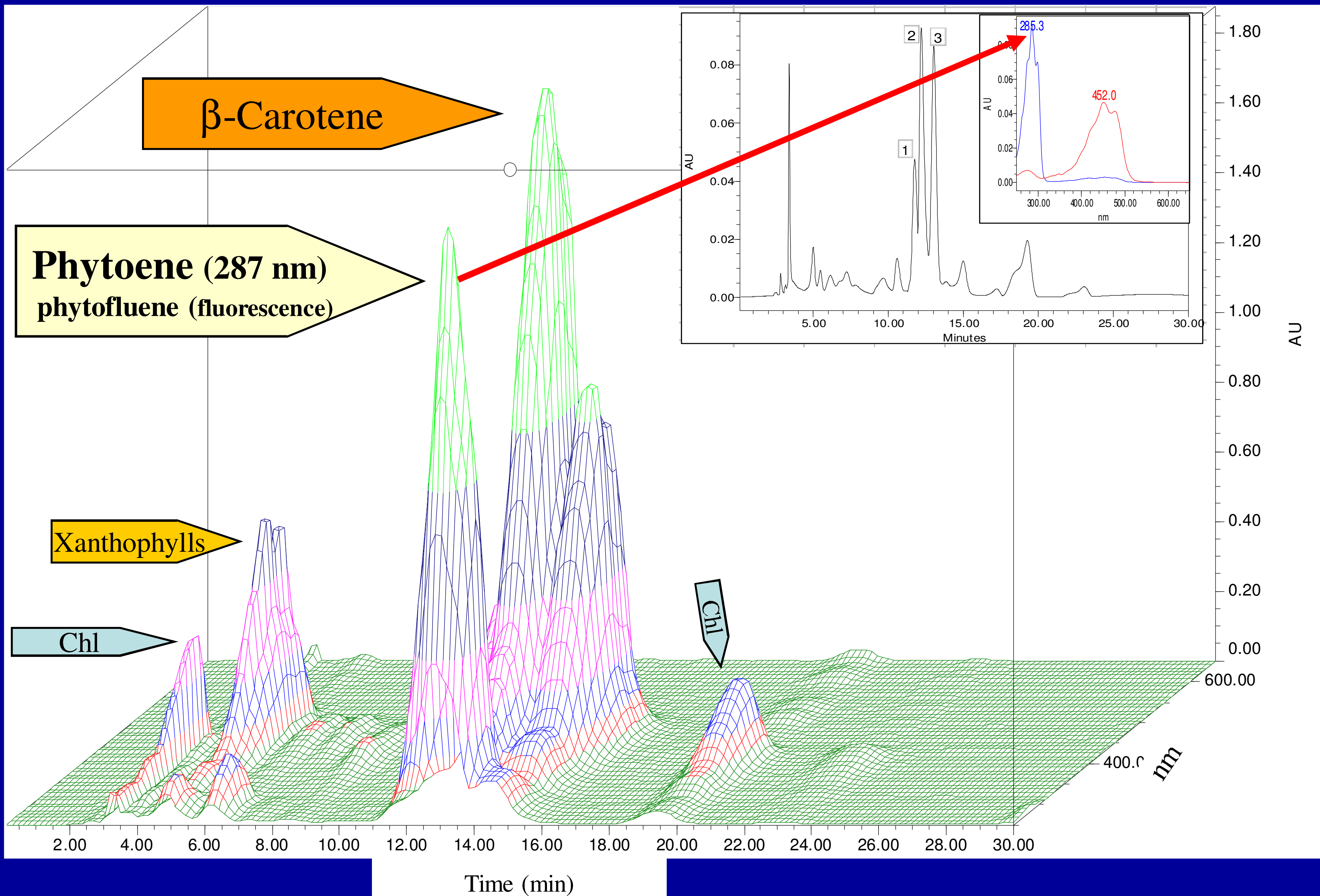
β-Carotene globules

Phytoene *Dunaliella*

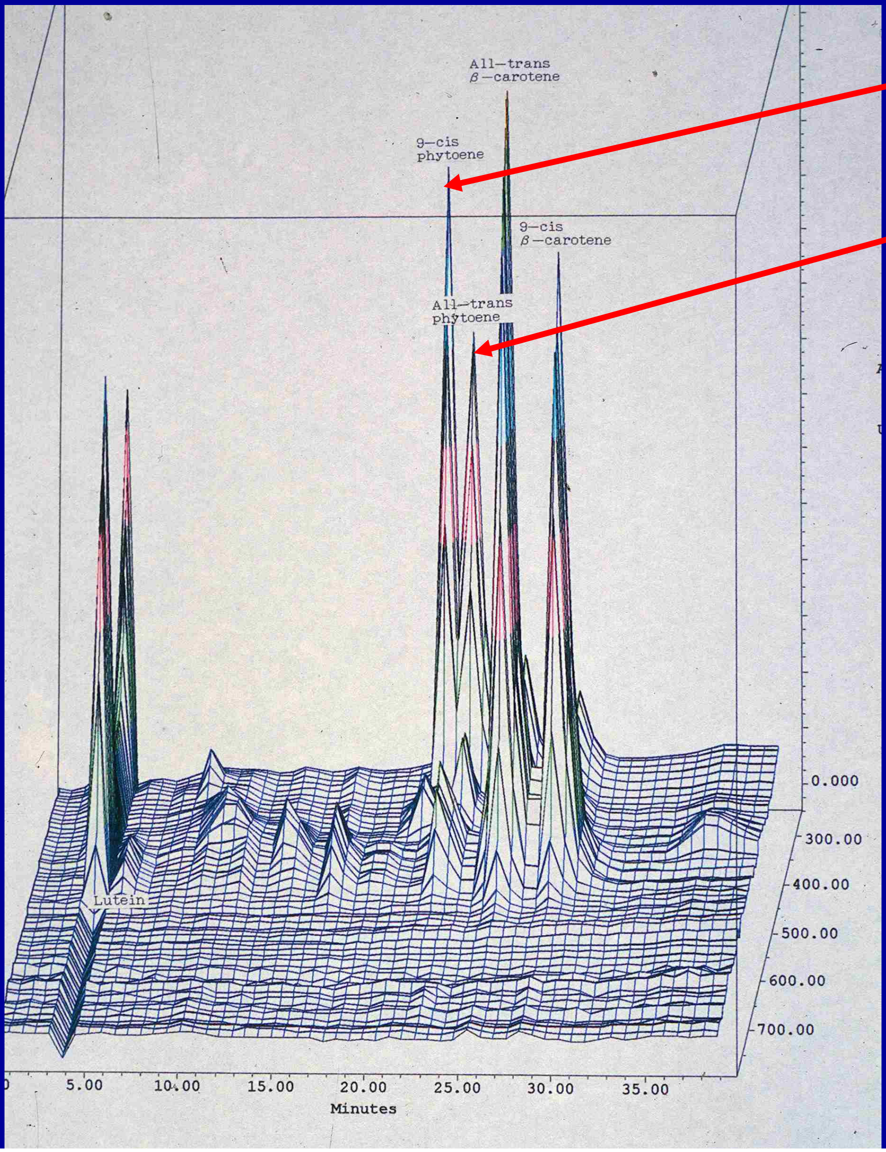




Phytoene, β-carotene-rich *Dunaliella*
HPLC analysis

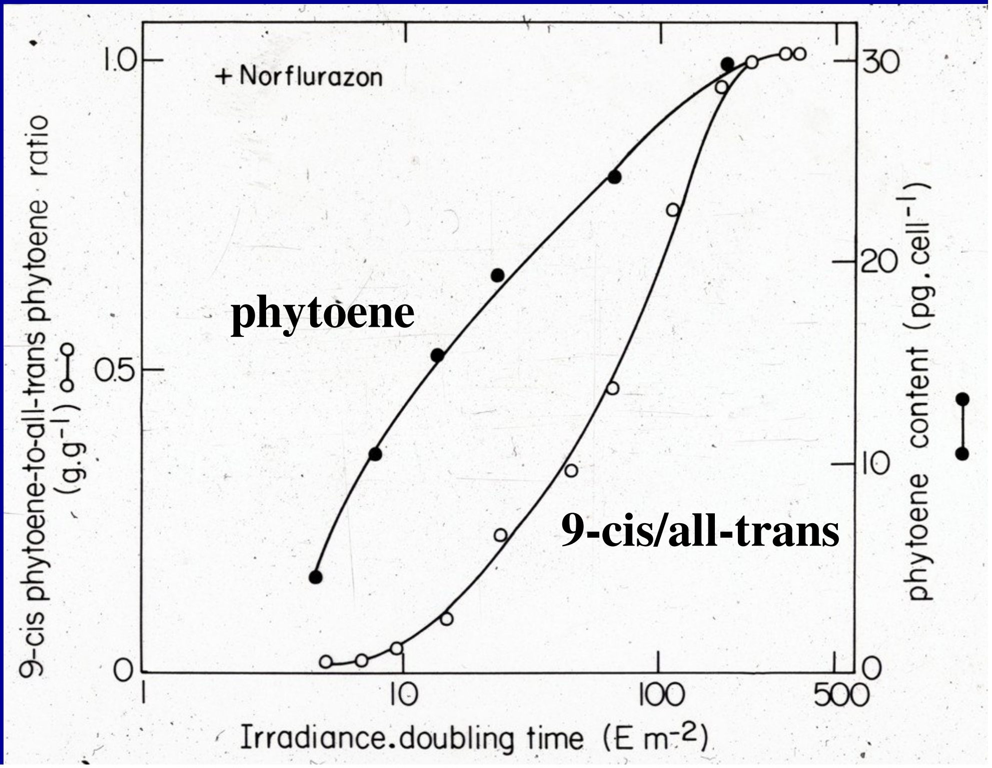


Phytoene, 9-cis/all-trans = ∫hν_x dt



9-cis Phytoene

all-trans phytoene



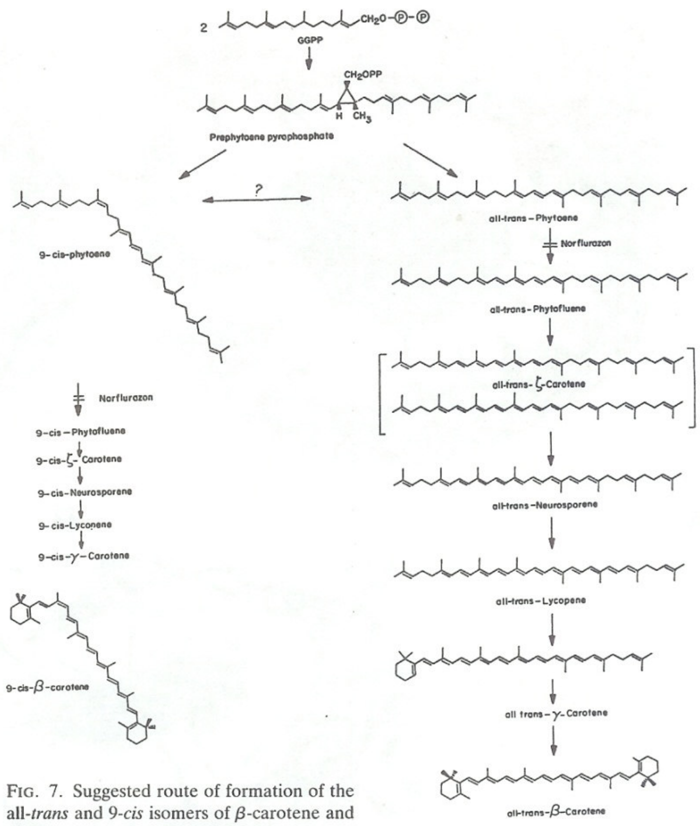
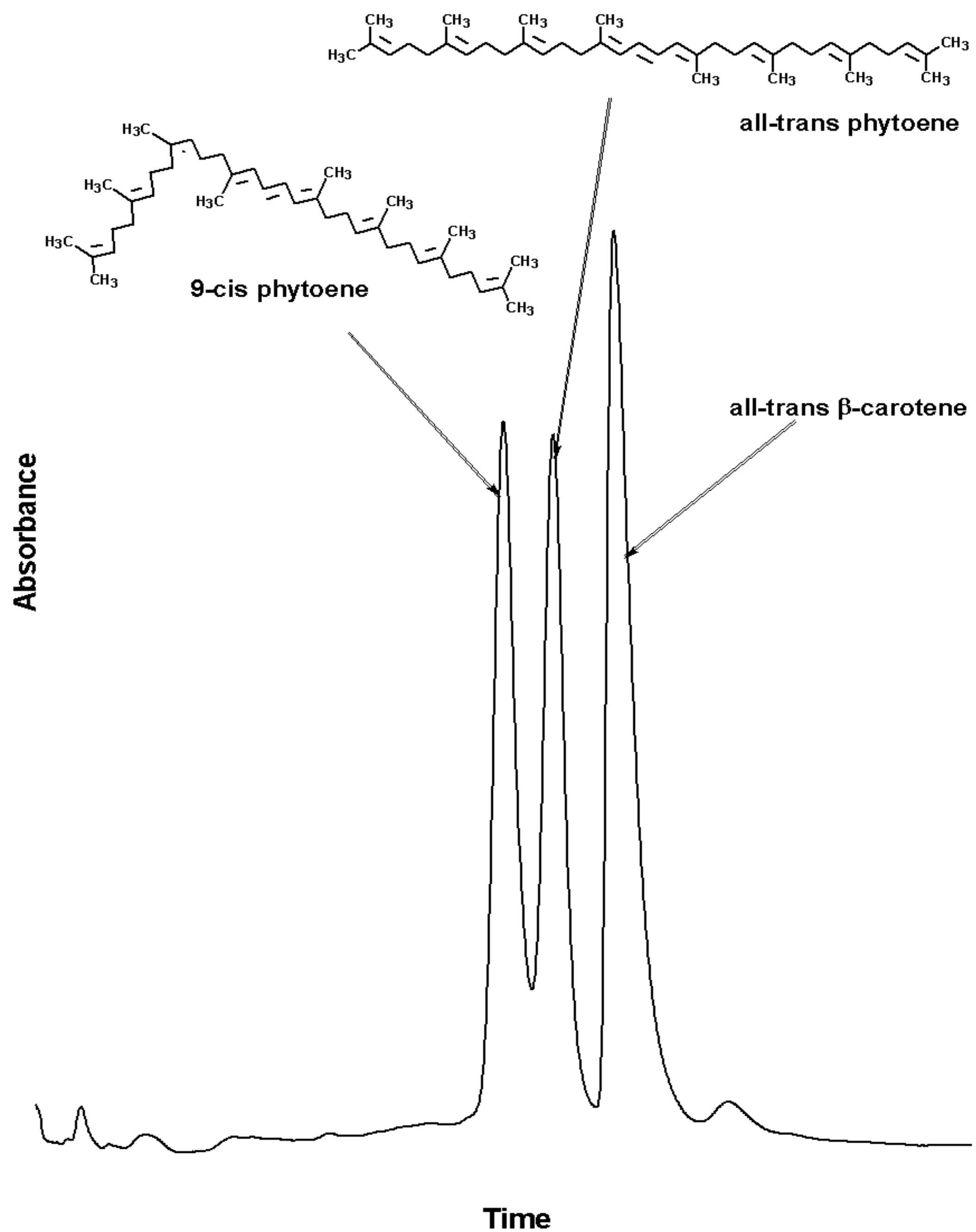


FIG. 7. Suggested route of formation of the all-*trans* and 9-*cis* isomers of β-carotene and phytoene.

2D HPLC profile of *Dunaliella (287 nm)*



Phytoene/Phytofluene
Dunaliella
Outdoors scale-up

Phytoene *Dunaliella* Micro-Ponds (50L)

The Weitzman Institute

Phytoene *Dunaliella*, Light shading

NBT Ltd., Eilat, Israel, Scale-up ponds (60 m^3)

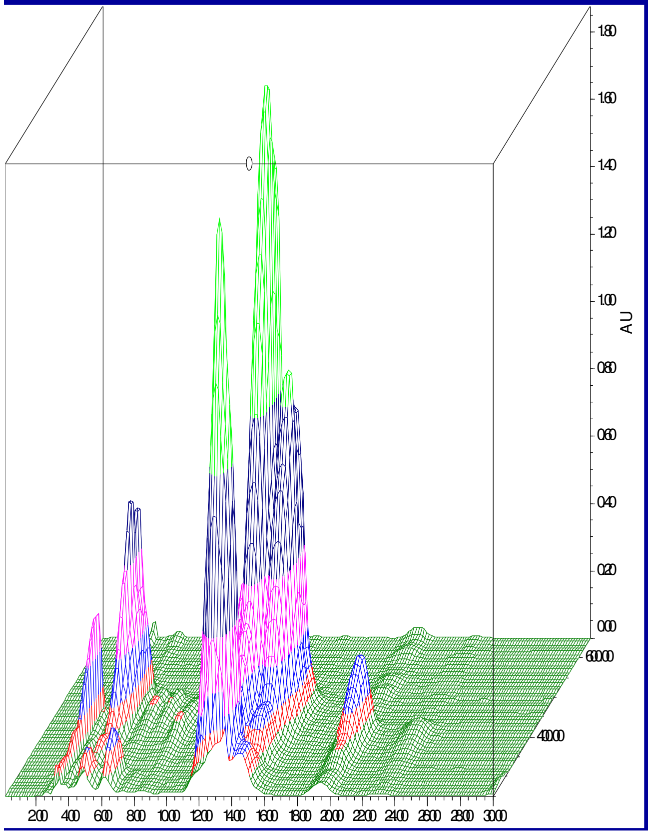
Phytoene
Dunaliella

Phytoene *Dunaliella*,

Production Pond (3,000 m^2, 600 m^3), NBT Ltd., Eilat

Phytoene *Dunaliella*, Spray Dried Powder

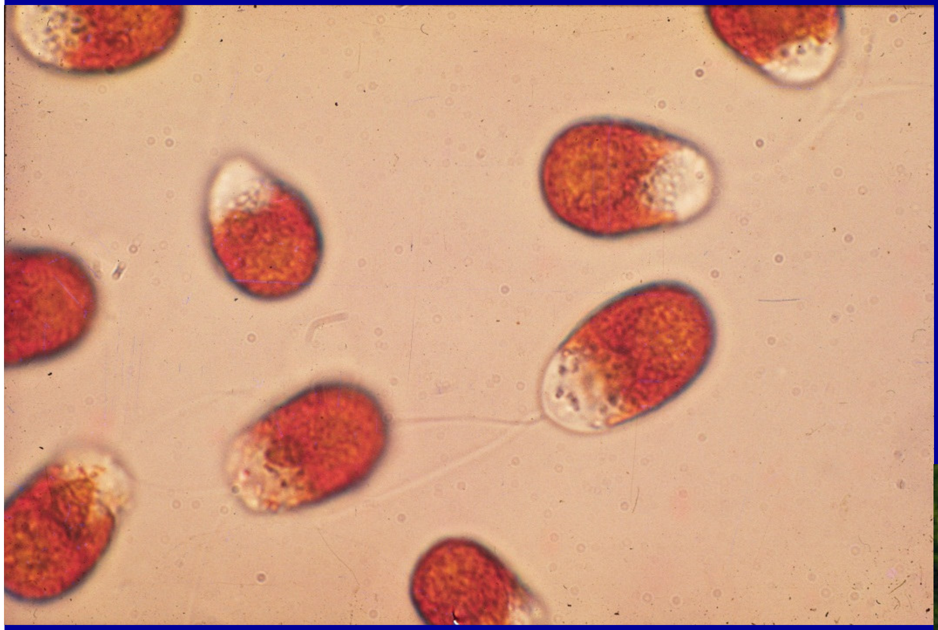
Phytoene isomers



Conclusion

***Dunaliella* can be controlled and manipulated**
to produce large scale of
new carotenoids

The β-carotene in *Dunaliell*a (and in fruits & vegetables) is composed of various stereoisomers of special nutritional, medical and biotechnology interest



β-carotene/phytoene/phytofluene

Dunaliella

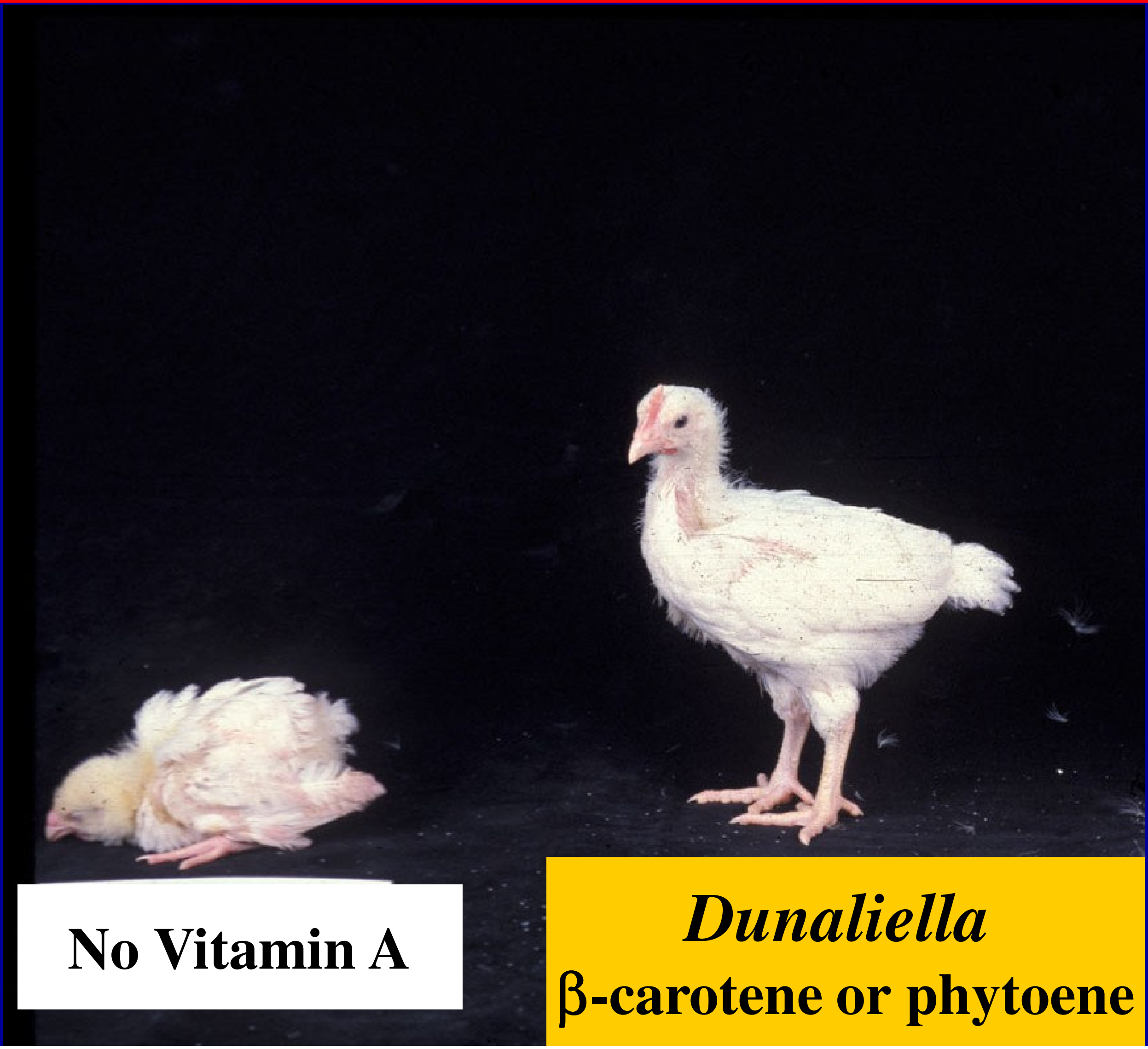
Feeding Studies

Chicks & Chickens

Chicks Study

No Vitamin A | + Vitamin A | + Synthetic β-carotene | + *Dunaliella*

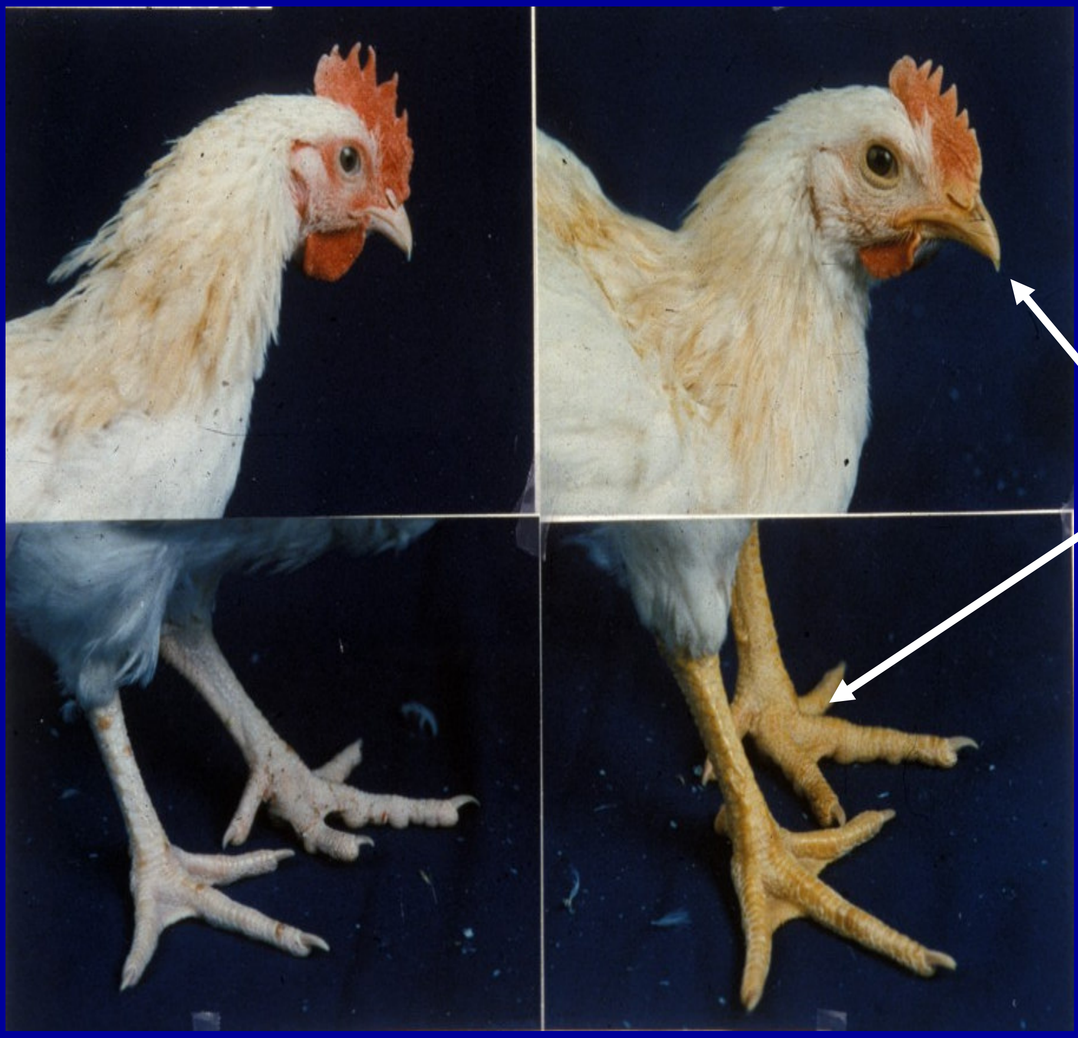
Chicken Study
Vitamin A deficiency & *Dunaliella*



No Vitamin A

Dunaliella
β-carotene or phytoene

Phytoene *Dunaliella*
Chicken Study



β-carotene/phytoene/phytofluene
Dunaliella, Feeding Studies
Mice & Rats

Rats fed phytoene *Dunaliella*

weight gain and hepatic content

Table 1
Weight gain (g) and tissue weight (g/100 g BW) of rats fed on control diet, and on diet supplemented with either phytoene-rich *Dunaliella* or placebo*

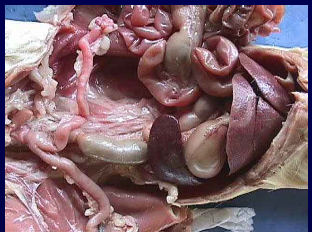
	Control	Phytoene	Placebo
Body weight	71.15 ± 7.34	78.23 ± 4.51	72.33 ± 8.32
Liver	4.22 ± 0.45	4.18 ± 0.53	4.51 ± 0.55
Spleen	0.52 ± 0.23	0.43 ± 0.05	0.43 ± 0.04
Kidney	1.23 ± 0.09	1.31 ± 0.06	1.28 ± 0.11
Heart	0.48 ± 0.04	0.47 ± 0.04	0.48 ± 0.04
Lung	0.78 ± 0.09	0.73 ± 0.08	0.80 ± 0.10
Brain	1.48 ± 0.19	1.41 ± 0.09	1.46 ± 0.07

* Value are means ± SD.

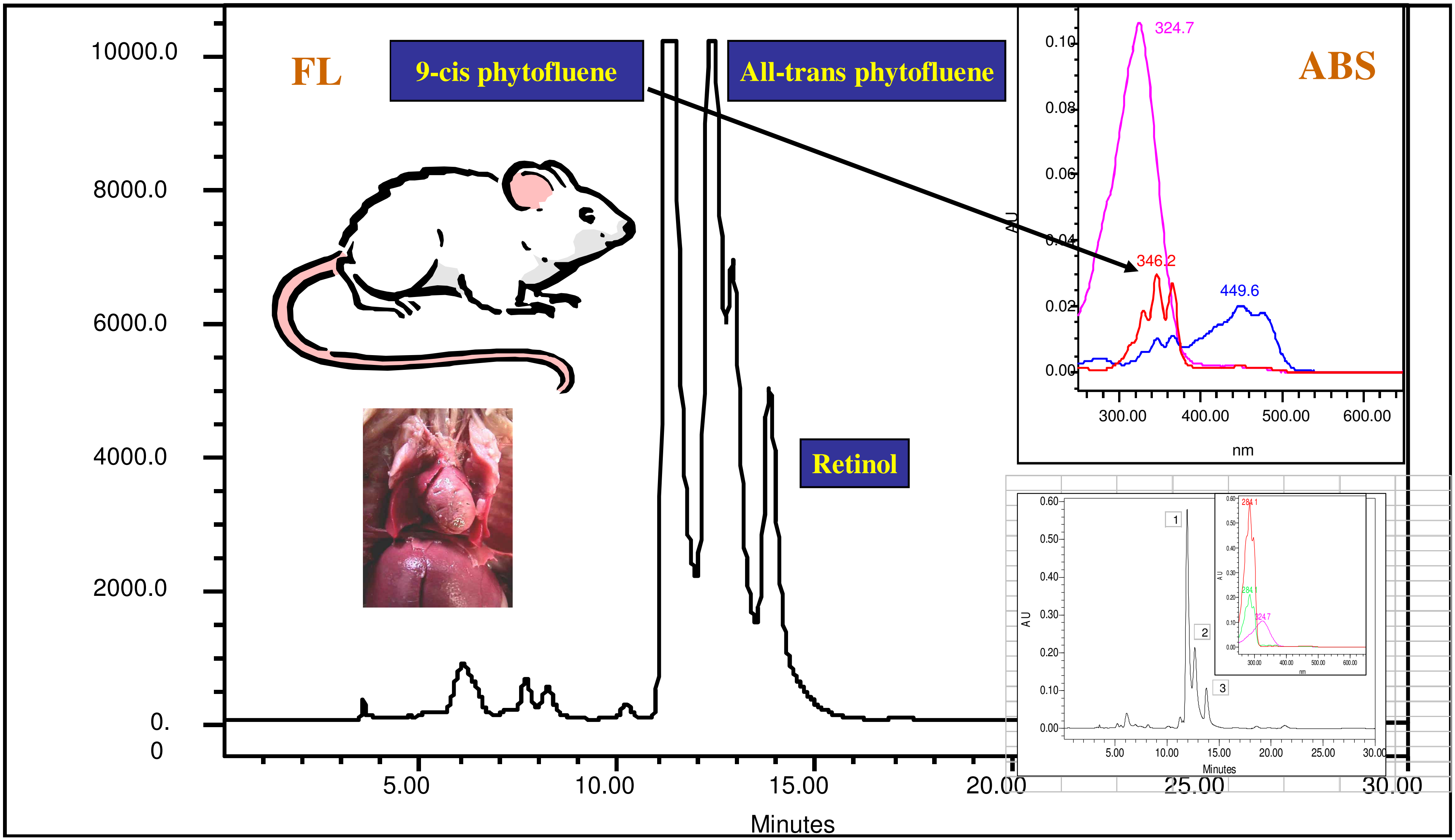
Table 2
Hepatic concentration (μmol/100 mg wt) of phytoene, phytofluene, vitamin A and total carotenoids in rats fed on diet supplemented either with phytoene-rich *Dunaliella* or placebo (ND, not detected)*

	Phytoene	Placebo
Phytoene →	0.440 ± 0.023	ND
Phytofluene	0.022 ± 0.002	ND
Vitamin A ≠→	0.053 ± 0.003	0.011 ± 0.002[a]
Total carotenoids	0.010 ± 0.001	Traces

* Values are means ± SD. Values in a row with[(a)] differ significantly ($P < 0.05$).



HPLC fluorescence (and absorption) analysis of liver extracts of tats fed phytoene *Dunaliella* diet



Phytoene in rat tissues
Total (mg/100 mg wt) and plasma (mg/l)
9-cis & all-trans

	Phytoene	Phytoene Stereoisomers (9-cis-to-all-trans)	Placebo
Liver	242.25 ± 12.75	1/3	ND
Adrenal	9.20 ± 1.21	1/1	ND
Spleen	3.74 ± 0.77	1/3	ND
Kidney	2.68 ± 0.38	1/3	ND
Brain	Traces		ND
Heart	ND		ND
Lung	ND		ND
Plasma	6.50 ± 1.21	1/1	ND
RBC	Traces		ND

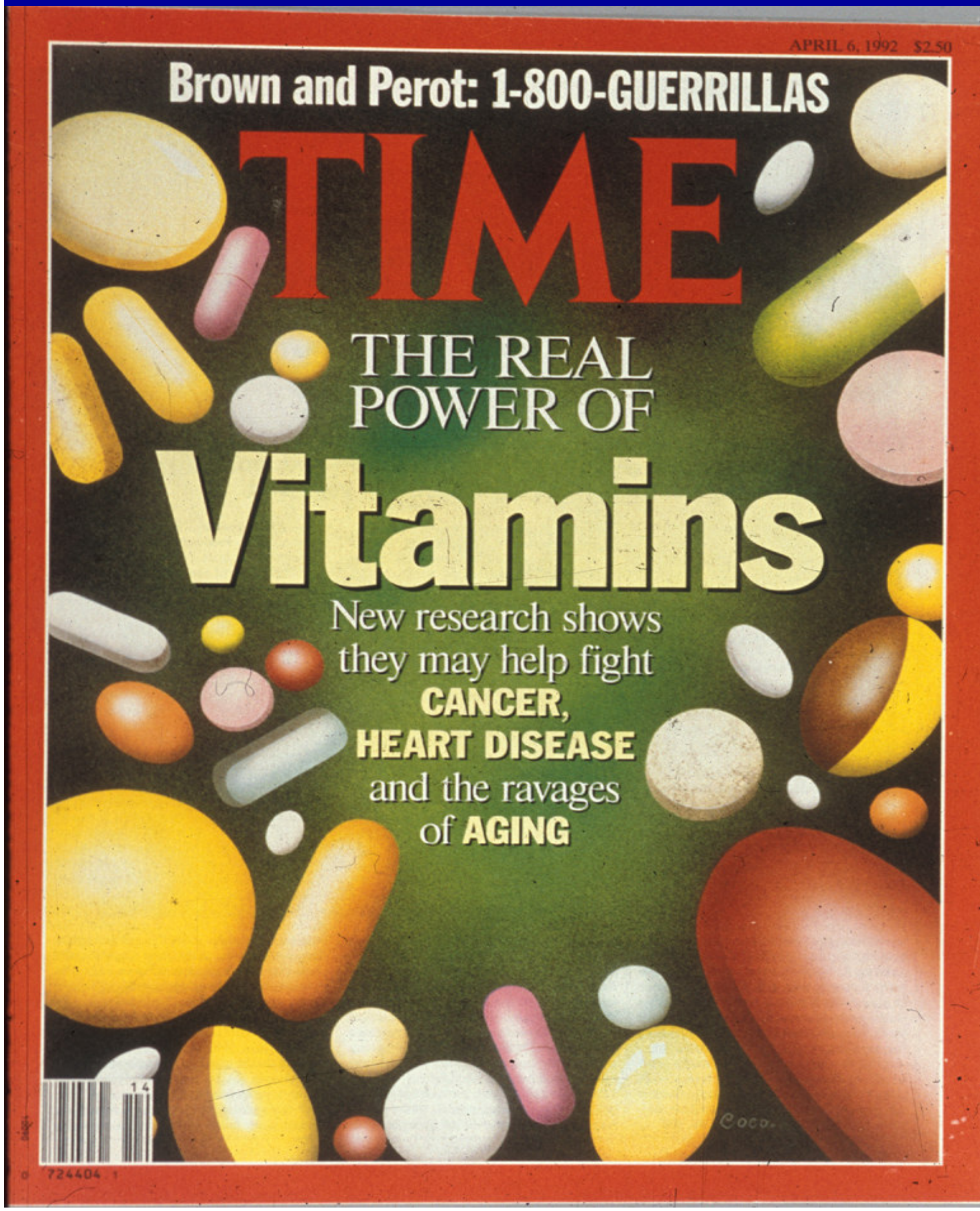
β-Carotene

Friend? 1980-90

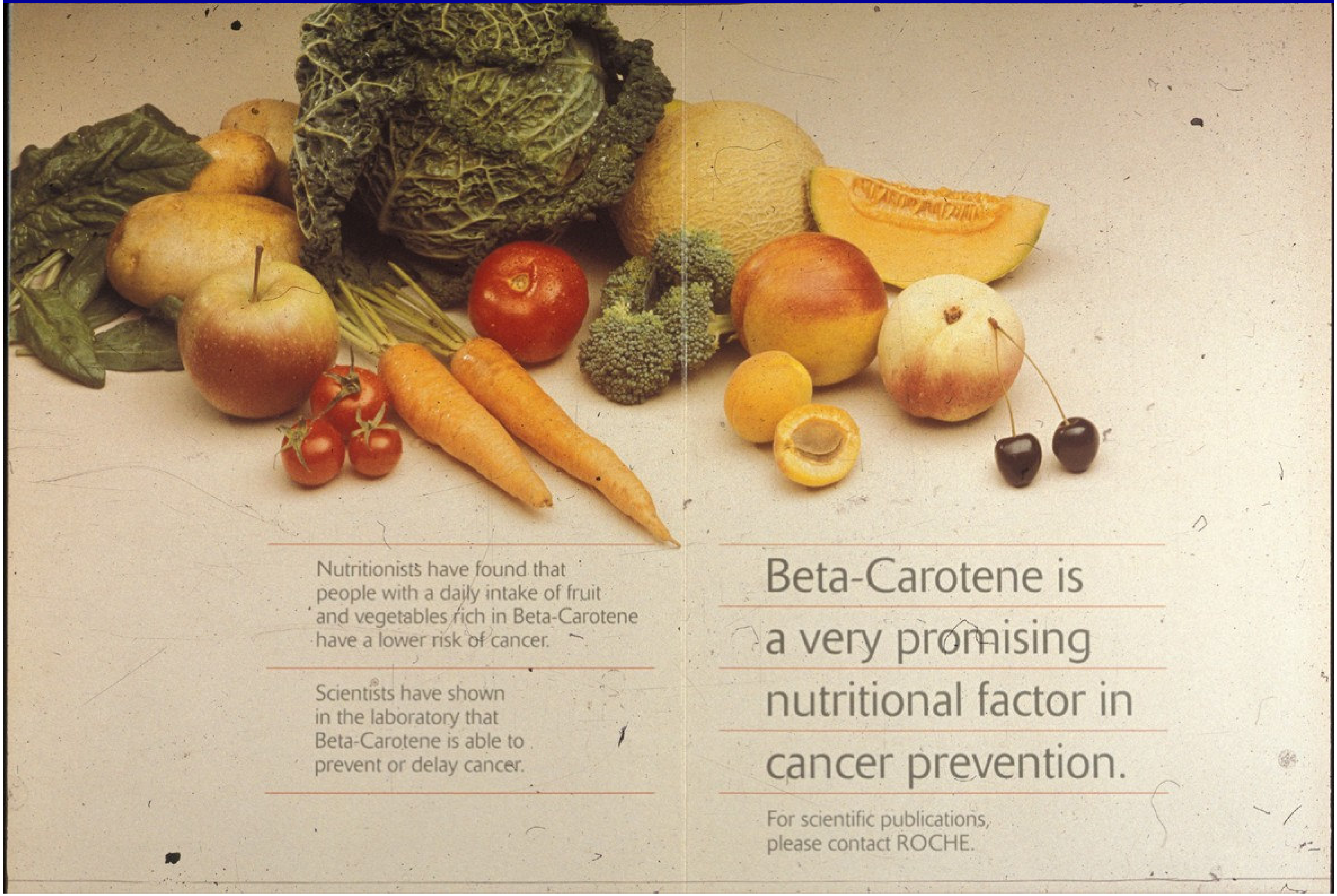
or

Foe? 1990-2000

Vitamins, the boom 1980-1990



“β-Carotene Prevents or Delays Cancer”, Roche 1986



The Rise and Fall of β-Carotene
Omenn 1998

CHEMOPREVENTION OF LUNG CANCER

Table 1 Relative risk comparisons

	Lung cancer		Mortality	
	Relative Risk	95% CI	Relative Risk	95% CI
ATBC	1.18	1.03–1.36	1.08	1.01–1.16
CARET	1.28	1.04–1.57	1.17	1.03–1.33
PHS	0.93	0.69–1.26	1.02	0.93–1.11
Linxian Study	—	—	0.91	0.84–0.99

Annu. Rev. Public Health. 1998. 19:73–99

CHEMOPREVENTION OF LUNG CANCER: The Rise and Demise of Beta-Carotene

Gilbert S. Omenn
Former address: School of Public Health & Community Medicine, University of Washington, Seattle, Washington 98195–7230 and Fred Hutchinson Cancer Research Center, Seattle, Washington 98109–1024; Current address: University of Michigan, Ann Arbor, Michigan 48109–0624; e-mail: gomenn@umich.edu

KEY WORDS: fruits/vegetables, carcinogenesis, vitamin-supplements, antioxidants, vitamin A

ABSTRACT

Beta-carotene and retinoids were the most promising agents against common cancers when the National Cancer Institute mounted a substantial program of population-based trials in the early 1980s. Both major lung cancer chemoprevention trials not only showed no benefit, but had significant increases in lung cancer incidence and in cardiovascular and total mortality. A new generation of laboratory research has been stimulated.

Rational public health recommendations at this time include: 1. Five-A-Day servings of fruits and vegetables, a doubling of current mean intake; 2. systematic investigation of the covariates of extremes of fruit and vegetable intake; 3. discouragement of beta-carotene supplement use, due to adverse effects in smokers and no evidence of benefit in non-smokers; 4. multilevel research to develop and evaluate candidate chemoprevention agents to prevent lung and other common cancers; and 5. continued priority for smoking prevention, smoking cessation, and avoidance of known carcinogens in the environment.

Could Synthetic β-Carotene Be the Real Problem?

Could Synthetic Beta-Carotene Be the Real Problem?

This article is from the September 1996 issue of The Nutrition Reporter■ newsletter.

Smokers who took beta-carotene supplements in recent experiments may have faced a greater risk of lung cancer because they took the synthetic form of the nutrient, a new study suggests. The subtle differences between synthetic and natural beta-carotene do appear to influence how the body uses the nutrient.

Synthetic beta-carotene consists of just the "all-trans" isomer of the nutrient, whereas natural beta-carotene consists of two different isomers, "9-cis" and "all-trans." Isomers have the same molecular formula, but a different arrangement of atoms. They're a little like anagrams, in which the letters of one word can be rearranged to form another, such as "star" and "rats."

It turns out that the natural 9-cis isomer is a more potent antioxidant than the all-trans, according to Ami Ben-Amotz, Ph.D., and Yishai Levy, Ph.D., in the American Journal of Clinical Nutrition (May 1996;63:729-34). That means the natural form has something the synthetic does not.

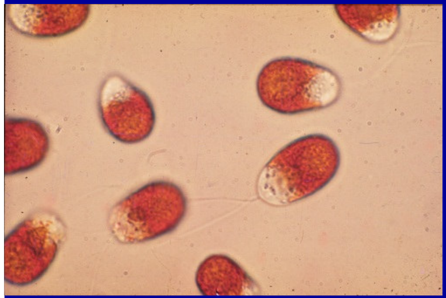
In experiments at Israel's National Institute of Oceanography, the researchers gave young, healthy men supplements of either natural beta-carotene from Dunaliella algae or synthetic beta-carotene. Blood analyses showed the presence of the all-trans isomer of beta-carotene, but not the 9-cis form found in natural beta-carotene. However, the researchers looked for and found 9-cis metabolic byproducts, indicating the presence and activity of the natural isomer.

Ben-Amotz and Levy reported experiments showing that the natural 9-cis isomer was rapidly used up in quenching free radicals and preventing oxidative damage to cell fats. In contrast, much of the all-trans isomer was converted to vitamin A, which is a very weak antioxidant.

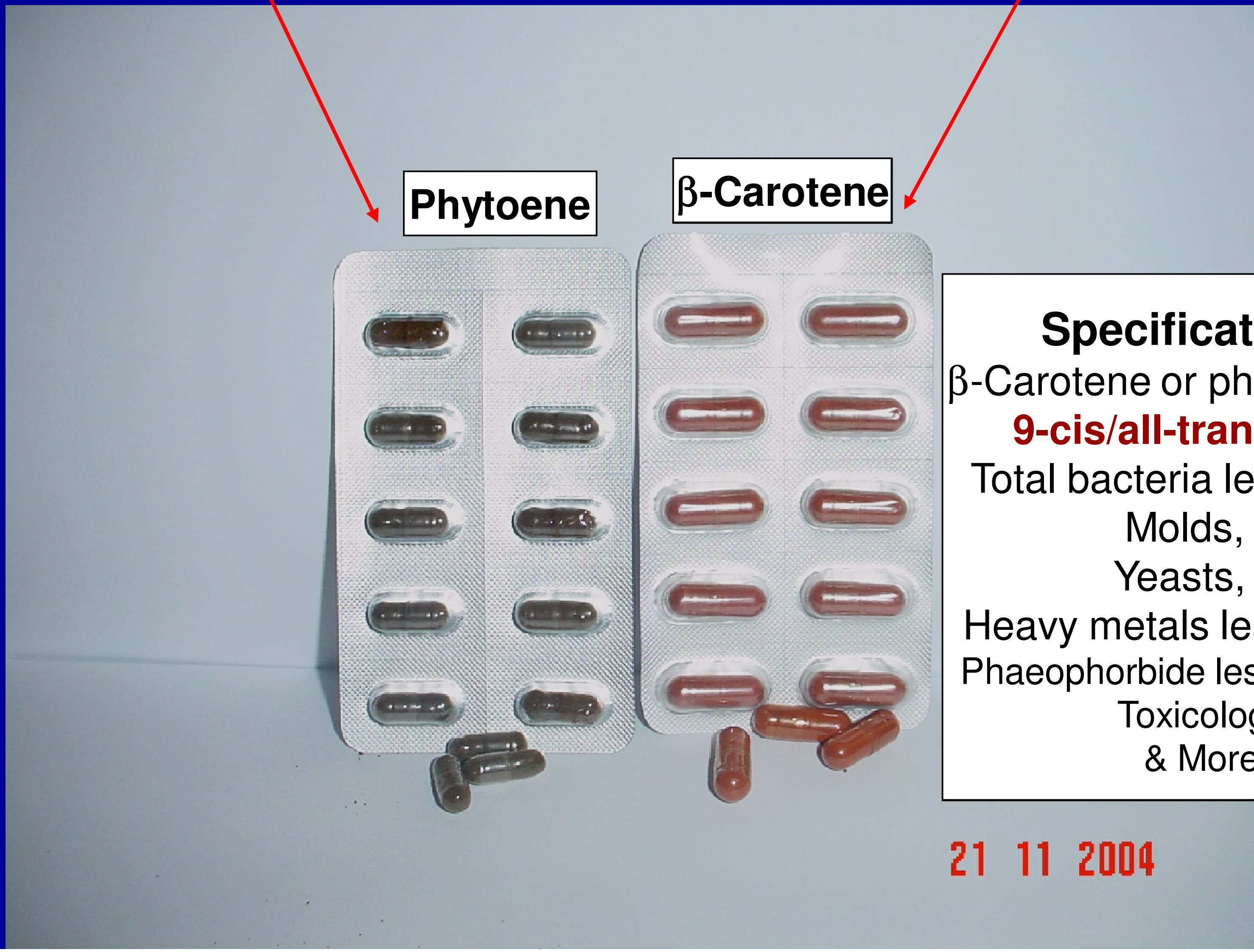
Ben-Amotz and Levy wrote that the differences between natural and synthetic beta-carotene "should provoke a shift in scientific attention to natural sources of carotenoids and their role in cancer prevention." They urged that researchers pay more attention to the different isomers of beta-carotene.

An earlier study, in Free Radical Biology & Medicine (1994;17:77-82) also showed that the natural 9-cis isomer is a more potent antioxidant than the all-trans form.

β-Carotene & Phytoene *Dunaliella* Capsules for Human Studies



Phytoene & β-carotene
Dunaliella Capsules
for human studies



Specifications:

β-Carotene or phytoene, 5%

9-cis/all-trans, 50%

Total bacteria less 3000/g

Molds, 0

Yeasts, 0

Heavy metals less 0.1ppm

Phaeophorbide less 100 mg%

Toxicology

& More

Dunaliella Carotenoids

Nutritional & Medical Effect

1. Antioxidants, cellular level?
2. Pro-retinoic acids, molecular level?
3. Structural?
4. Other?

Dunaliella

Recent Nutritional & Medical Manuscripts

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Research Communications

Availability and antiperoxidative effects of β-carotene from *Dunaliella bardawil* in alcohol-drinking rats

Moshe J. Werman, Ami Ben-Amotz, and Shoshana Mokady

Department of Food Engineering and Biotechnology, Technion; and The National Institute of Oceanography, Haifa, Israel

The present study demonstrated the high bioavailability and antiperoxidative capacity of the natural β-carotene isomer mixture of Dunaliella bardawil compared with synthetic β-carotene under alcohol-induced oxidative stress. Weanling rats were adapted to ethanol by increasing ethanol levels in their drinking water to 30% at 5% intervals per week; other rats received water with no added ethanol. One water-drinking group and one alcohol-drinking group with no dietary carotene were used as controls. Two water-drinking groups were supplemented with 1 g/kg diet β-carotene either from Dunaliella or a synthetic source, and due to reduced food intake, two ethanol-fed groups received 2 g β-carotene per kilogram of diet from each source. Following 3 months of ethanol consumption, both carotene sources were found to prevent ethanol-induced lipid peroxidation as expressed by the hepatic conjugated oxidized dienes level. However, in the algal-fed rats, hepatic carotene and vitamin A levels were higher. In addition to a lower performance of the group fed ethanol and synthetic β-carotene, there were three deaths in this group. (J. Nutr. Biochem. 10:449–454, 1999) *© Elsevier Science Inc. 1999. All rights reserved.*

Keywords: *Dunaliella bardawil*; β-carotene; alcohol; lipid peroxidation; vitamin A; rats

Inflammatory Bowel Diseases®
9(6):372–379 © 2003 Crohn's & Colitis Foundation of America, Inc.

Dietary *Dunaliella bardawil*, a β-Carotene–Rich Alga, Protects Against Acetic Acid–Induced Small Bowel Inflammation in Rats

*Alexandra Lavy, †Yehezkel Naveh, ‡Raymond Coleman, §Shoshana Mokady, and §Moshe J. Werman

**Department of Gastroenterology, B'nai Zion Medical Center; †Department of Pediatrics, Rambam Medical Center; and ‡Departments of Anatomy and Cell Biology, The Bruce Rappaport Faculty of Medicine, and §Food Engineering and Biotechnology, Technion-Israel Institute of Technology, Haifa, Israel*

Summary: *Background:* Reactive oxygen species mediate tissue injury in inflammatory bowel disease. β-Carotene is known as a potent free radical quencher and antioxidant. *Aim:* The authors evaluated the efficacy of prefeeding *Dunaliella bardawil*, rich in β-carotene, to ameliorate acid-induced enteritis in a rat model. *Methods:* Enteritis was induced in female Sprague-Dawley rats by injection of 2 mL acetic acid (0.67 mol/L) to a ligated duodenal loop following 10 weeks of feeding diets containing β-carotene and compared with various controls. The effects of β-carotene were evaluated by changes in myeloperoxidase activity, histology, and histomorphometry. *Results:* Feeding β-carotene resulted in suppressed mucosal myeloperoxidase activity, both basal and that induced by acetic acid injection. Acetic acid treatment induced major histopathologic changes in the duodenal mucosa, including small, irregular, and distorted villi; damage to the epithelium; edema of the lamina propria; accumulation of inflammatory cells; and hemorrhage. β-Carotene treatment prevented these acid-induced histopathologic changes, and this was confirmed by histomorphometry of the villi. *Conclusions:* These results demonstrate the effectiveness of β-carotene in a rat model as a prophylactic dietary measure in reducing t
induced enteritis and raise the possibility
Crohn's disease may benefit from the cons
β-carotene. **Key Words:** Inflammatory
Antioxidants—β-Carotene—Acid-induced

Radiat Environ Biophys (1998) 37: 187–193

ORIGINAL PAPER

Ami Ben-Amotz · Shaul Yatziv · Mordechai Sela
Simon Greenberg · Boris Rachmilevich
Masha Shwarzman · Ze'ev Weshler

Effect of natural β-carotene supplementation in children exposed to radiation from the Chernobyl accident

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Nutrition Research 25 (2005) 1005–1012

NUTRITION RESEARCH

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Selective distribution of β-carotene stereoisomers in rat tissues

Ami Ben-Amotz[a,*], Baruch Volkis[a], Shoshana Mokady[b]

[a]*Israel Oceanographic and Limnological Research, The National Institute of Oceanography, Tel Shikmona, Haifa 31080, Israel*
[b]*Department of Food Engineering and Biotechnology, Technion, Haifa 31080, Israel*

Received 4 February 2005; revised 5 July 2005; accepted 1 September 2005

ORIGINAL ARTICLE

Natural β-Carotene for the Prevention of Post-ERCP Pancreatitis

A. Lavy, MD, A. Karban, MD,† A. Suissa, MD,† K. Yassin, MD,† I. Hermesh, MD,† and A. Ben-Amotz, PhD‡*

Objective: Endoscopic retrograde cholangiopancreatography (ERCP) is a commonly used procedure. Pancreatitis is its most common complication. As the injury may be mediated by oxidative stress, it could be ameliorated by antioxidants.

Methods: We conducted a double-blind trial, giving the patients a single dose of natural β-carotene or placebo, 12 hours prior to procedure, and monitoring them for 24 hours post-procedure for procedure complications, antioxidant levels, and plasma oxidation.

Results: The overall incidence of acute pancreatitis according to our definition was 9.6%. The incidence of pancreatitis was not significantly different between the β-carotene group (10%) and the placebo group (9.4%). Four patients in the placebo group had severe pancreatitis (2.22%), but none in the β-carotene group. This difference is statistically significant.

Conclusion: We did not see a reduction in the incidence of post-ERCP pancreatitis, but there may be some protective effect of treatment with β-carotene regarding the severity of disease.

Key Words: ERCP, antioxidants, pancreatitis, β-carotene

(*Pancreas* 2004;29:e45–e50)

ERCP is a common cause of pancreatic enzyme elevations (up to 70% of the patients) and acute pancreatitis. Acute

The imbalance between the production of free radicals and the antioxidant defenses may be involved in the pathogenesis of many diseases.[7–10] Oxidative stress can cause cell injury and death, leading to necrosis or apoptosis.[11] Any trauma to human tissue will lead to phagocyte recruitment and activation, creating free radicals, arachidonic acid release and enzyme peroxide formation, haem protein release, and metal ion release,[7,12] which all contribute to the oxidative stress.

Such an event may be ERCP.[13] Oxidative stress is postulated to play a crucial role in the initiation of acute pancreatitis, causing exocytic derangement of acinar cells. Further evolution of pancreatic inflammation is caused by the imbalance between intrapancreatic oxidative stress and natural antioxidation mechanisms.[14–16]

Increased consumption of fruits and vegetables was shown to prevent cancer and cardiovascular disease.[17] This effect may be through high contents of antioxidants such as β-carotene. However, studies using artificial β-carotene failed to show beneficial effects.[18,19] The artificial β-carotene is an all-trans isomer and is different from the natural 9-cis all-trans isomer. The later was shown to accumulate in tissues and prevent LDL oxidation, thus showing in vitro antioxidative properties.[19–24]

We used alga-derived β-carotene, which is a mixture of

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ORIGINAL RESEARCH

Antioxidant Vitamin Intake in Adolescent Israeli Girls

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Antioxidants may reduce risks of cardiovascular and neoplastic diseases based on basic, animal and epidemiological research. Therefore, dietary fat-soluble antioxidant vitamin intake and plasma concentration in healthy adolescent girls was investigated. This age was selected since dietary antioxidant protection against major illnesses is a lifelong requirement and because healthy dietary habits are adopted at an early age and are often modified during adolescence. Seventy-one high school girls, age range 15–18 years, were studied by a semi-quantitative food frequency questionnaire. Plasma antioxidants were measured by high-performance liquid chromatography (HPLC) in 16 girls. The diet provided 30% fat with a caloric intake of 34 kcal kg^{-1} *body weight. Vitamin A intake was 2.2 ± 1.0 mg* day^{-1}*, β-carotene intake was 6.2 ± 4.2 mg/day and vitamin E intake was 4.8 ± 2.1 mg* day^{-1}*. Thus, only vitamin E intake was below the recommended allowance for this age range and compared to the USA intake survey at the same age and sex. Plasma antioxidants were all within normal range as compared with other studies. Thus, according to this study, Israeli adolescent girls consume a suboptimal amount of vitamin E in their diet, but sufficient in other fat soluble antioxidants. Though this apparently unbalanced antioxidant intake is not reflected in abnormal plasma concentrations, high intake of vitamin E should be encouraged in order to obtain the maximal benefit from exposure to dietary antioxidants throughout life.*

Keywords: antioxidants, carotenoids, vitamin E, adolescence.

ELSEVIER

Journal of Nutritional Biochemistry 13 (2002) 585–591

THE JOURNAL OF Nutritional Biochemistry

Bioavailability of the isomer mixture of phytoene and phytofluene-rich alga *Dunaliella bardawil* in rat plasma and tissues

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Abstract

Dunaliella bardawil, a β-carotene-accumulating alga was treated by the bleaching herbicide norflurazon to select sub-species rich with a mixture of 9-*cis* and *all-trans* stereoisomers of phytoene and phytofluene. The present study determines the bioavailability of phytoene and phytofluene with their stereoisomers in rats fed on a diet supplemented with *Dunaliella* phytoene-rich spray dried powder. Three groups of female weanling rats, eight animals each, were fed AIN diets for two weeks. The control consumed the diet as is. The experimental group was supplemented with 50 g *Dunaliella* powder to give phytoene/phytofluene at a level of 1 g/kg diet, and the placebo was provided with the oxidized algae free of carotenoids at the same amount. Weight gain and tissues weight of rats fed on the control diet, or on the experimental diets were statistically same. Tissue analyses were carried out by liquid chromatography at the end of two weeks feeding for vitamin A, carotenoids, phytoene and phytofluene and theirs stereoisomers. Liver analyses revealed high hepatic storage of phytoene in the experimental group. Analysis of the other tissues, adrenal, brain, heart, kidney, lung, and spleen detected small amounts of phytoene in the adrenal, kidney and spleen and in the plasma. High-pressure liquid chromatography for stereoisomeric composition was performed to all phytoene-containing tissues. The original algal diet content of 9-*cis*-to-*all-trans* ratio of 1:1 was maintained in the plasma and adrenal while in the liver, spleen and kidney the ratio was reduced to 1:3. The preferential accumulation of *all-trans* phytoene over 9-*cis* phytoene in the liver, spleen and kidney may be interpreted as indicating stronger antioxidative effect of 9-*cis* phytoene over the *all-trans* isomer or alternatively, *in vivo* streoisomerization of 9-*cis* phytoene to the *all-trans* structure.

Keywords: Bioavailability; *Dunaliella*; Phytoene; Phytofluene

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Prevention of exercise-induced asthma by a natural isomer mixture of *β*-carotene

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Background: The unicellular alga *Dunaliella bardawil* was previously shown to contain very high concentrations of *β*-carotene composed of equal amounts of the all-trans and 9-cis stereoisomers which differ in their physicochemical features and antioxidative activity. Due to the controversy regarding the beneficial effect of antioxidants on asthma, the acute effects of *β*-carotene of Dunaliella was assessed on airway hyperreactivity in patients with exercise-induced asthma (EIA).

Methods: Thirty-eight patients with EIA participated in our study to verify the antioxidative effect. The test was based on the following sequence: baseline pulmonary function, 7 minutes exercise session on a motorized treadmill, 8 minutes rest, 1-week oral random, double-blind supplementation of placebo or 64 mg/day *β*-carotene, pulmonary functions at rest, 7 minutes exercise session, 8 minutes rest and again pulmonary functions.

Results: All patients given placebo showed a significant postexercise reduction of more than 15% in their forced expiratory volume in one second (FEV_1). Of the 38 patients who received a daily dose of 64 mg of *β*-carotene for 1 week, 20 (53%) were protected against EIA.

Conclusions: Our results indicate that a daily dose of Dunaliella *β*-carotene exerts a protective effect against EIA in some patients most probably through in vivo antioxidative effect.

Ann Allergy Asthma Immunol 1999;82:549–553.

INTRODUCTION

ter exercise-induced bronchospasm.[8]

acts as an in vivo lipophilic antioxidant more efficiently than does all-trans *β*-carotene.[11] In the present study we examined the effects of natural *β*-carotene on hyper reactive airways at rest and following the physical provocation of exercise.

METHODS

A group of 38 patients with proven EIA participated in the study. There were 26 males and 12 females, ranging in age from 8 to 33 years (mean age 16.3 years). All patients underwent an initial evaluation of pulmonary functions in which all of them showed a reduction of at least 15% in their forced expiratory volume in 1 second (FEV_1) after a 7-minute run on a motorized Quinton treadmill followed by 8 minutes rest. The workload was the same for each patient exercising up to submaximal effort as indicated by an

EFFECT OF DIETARY SUPPLEMENTATION OF β-CAROTENE ON HUMAN MONOCYTE-MACROPHAGE-MEDIATED OXIDATION OF LOW DENSITY LIPOPROTEIN

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[1]Lipid Research Laboratory, Rambam Medical Center and Rappaport Faculty of Medicine and Institute for Research in the Medical Sciences, Technion–Israel Institute of Technology, and [2]National Oceanographic Research Institute, Haifa, Israel

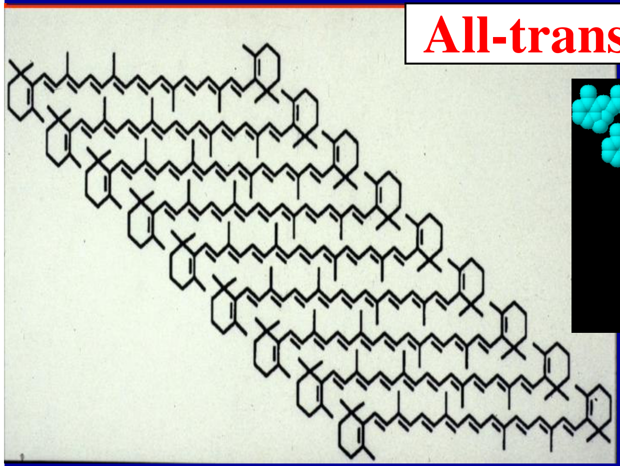
ABSTRACT. Oxidative modification of low density lipoprotein (LDL), a key step in early atherosclerosis, is protected by the lipoprotein-associated antioxidants. The present study analyzes the effect of β-carotene in plasma, in LDL and in monocyte-macrophages, on macrophage-mediated oxidation of LDL. We investigated the effect of dietary supplementation of β-carotene on plasma lipid peroxidation [induced by AAPH (2,2-Azobis-2-amidinopropane hydrochloride)] and on cell-free and cell-mediated oxidation of LDL by human monocyte-derived macrophages (HMDM) in the presence of $CuSO_4$. Significant enrichment with β-carotene was noted in plasma (twofold), in LDL (2.6-fold) and in HMDM (1.6-fold) 2 weeks after dietary supplementation with 180 mg/day of β-carotene. Plasma lipid peroxidation analyzed by conjugated dienes generation decreased by 22% (P <0.01) and LDL susceptibility to oxidation analyzed by malondialdehyde generation decreased by 40% (P <0.01). After β-carotene supplementation, β-carotene-enrichment of HMDM did not affect HMDM capacity to oxidize native LDL, whereas β-carotene enrichment of LDL significantly reduced LDL oxidation. In conclusion, then, our results suggest that β-carotene content of LDL, but not that of the macrophages, is responsible for the inhibition of oxidation of LDL.

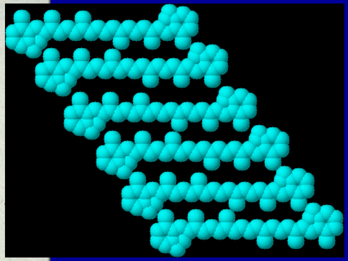
Isr J Med Sci 1996;**32**:473–478

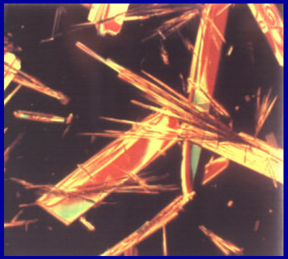
Keywords: hyperlipidemia; β-carotene; low density lipoprotein; macrophage; oxidation

β-Carotene, the Packaging Structural Model

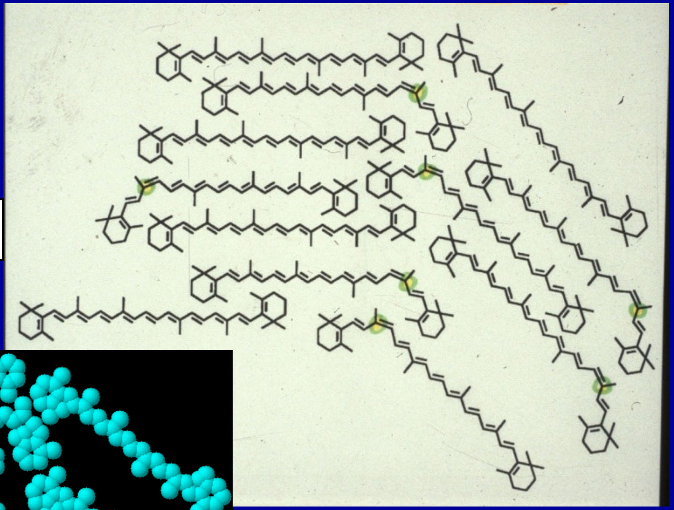
All-trans (crystals, not absorbed)

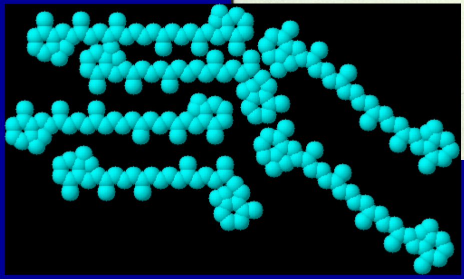




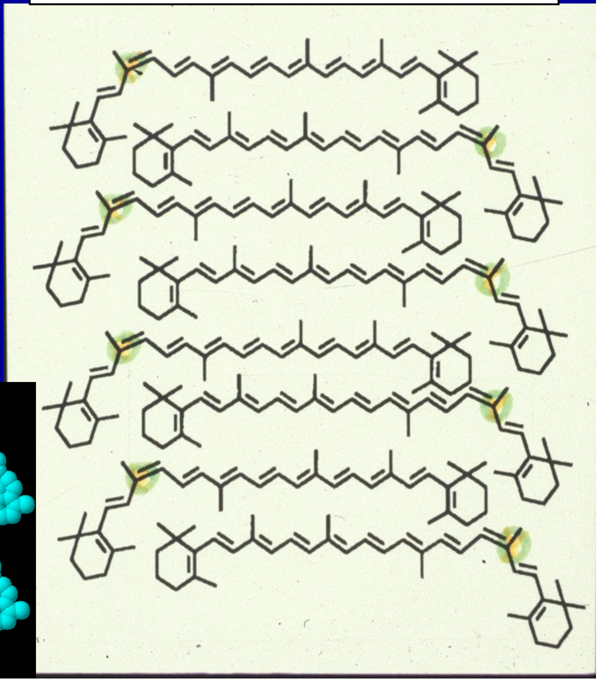


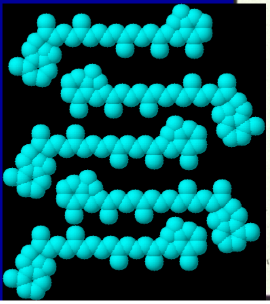
All-trans/9-cis (absorbed)



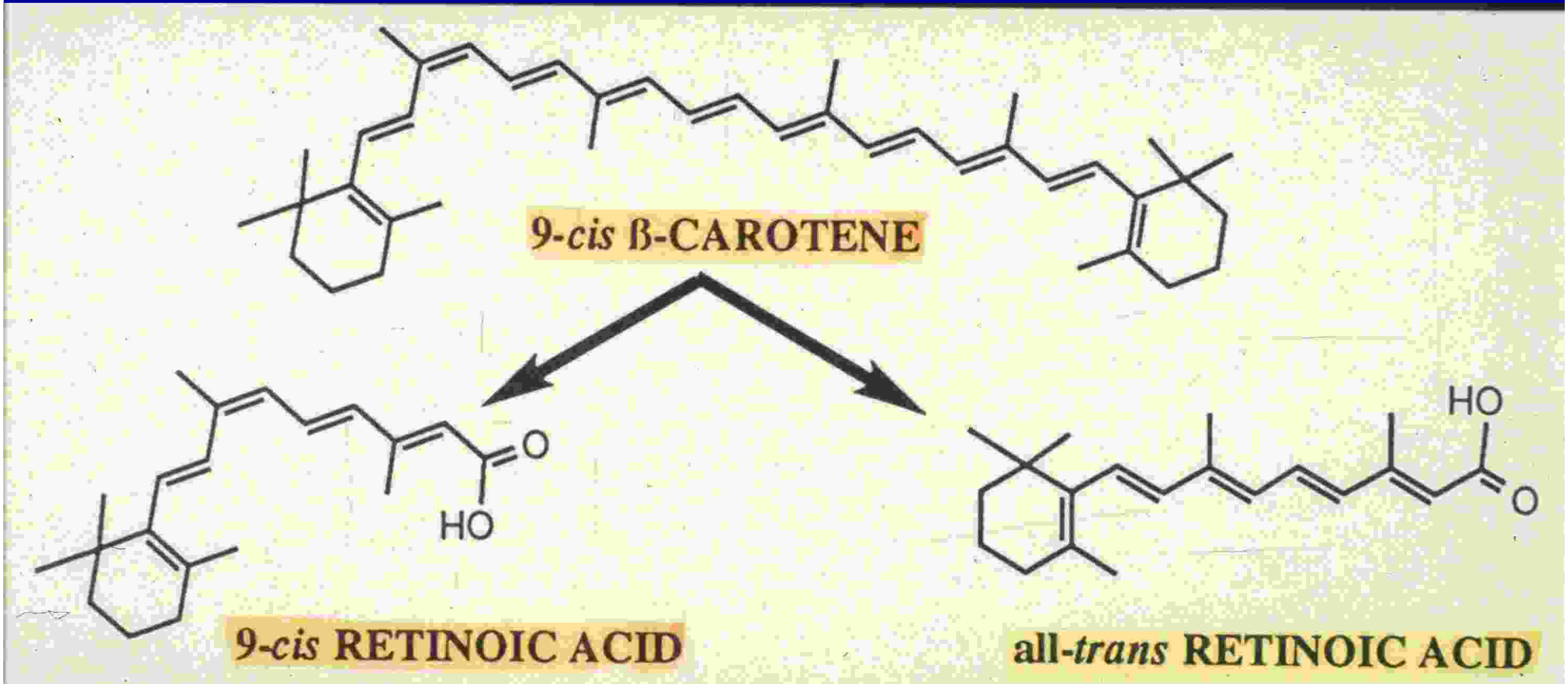


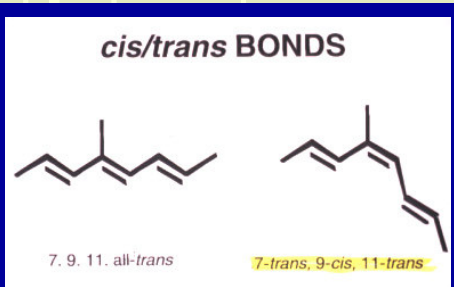
9-cis (absorbed)

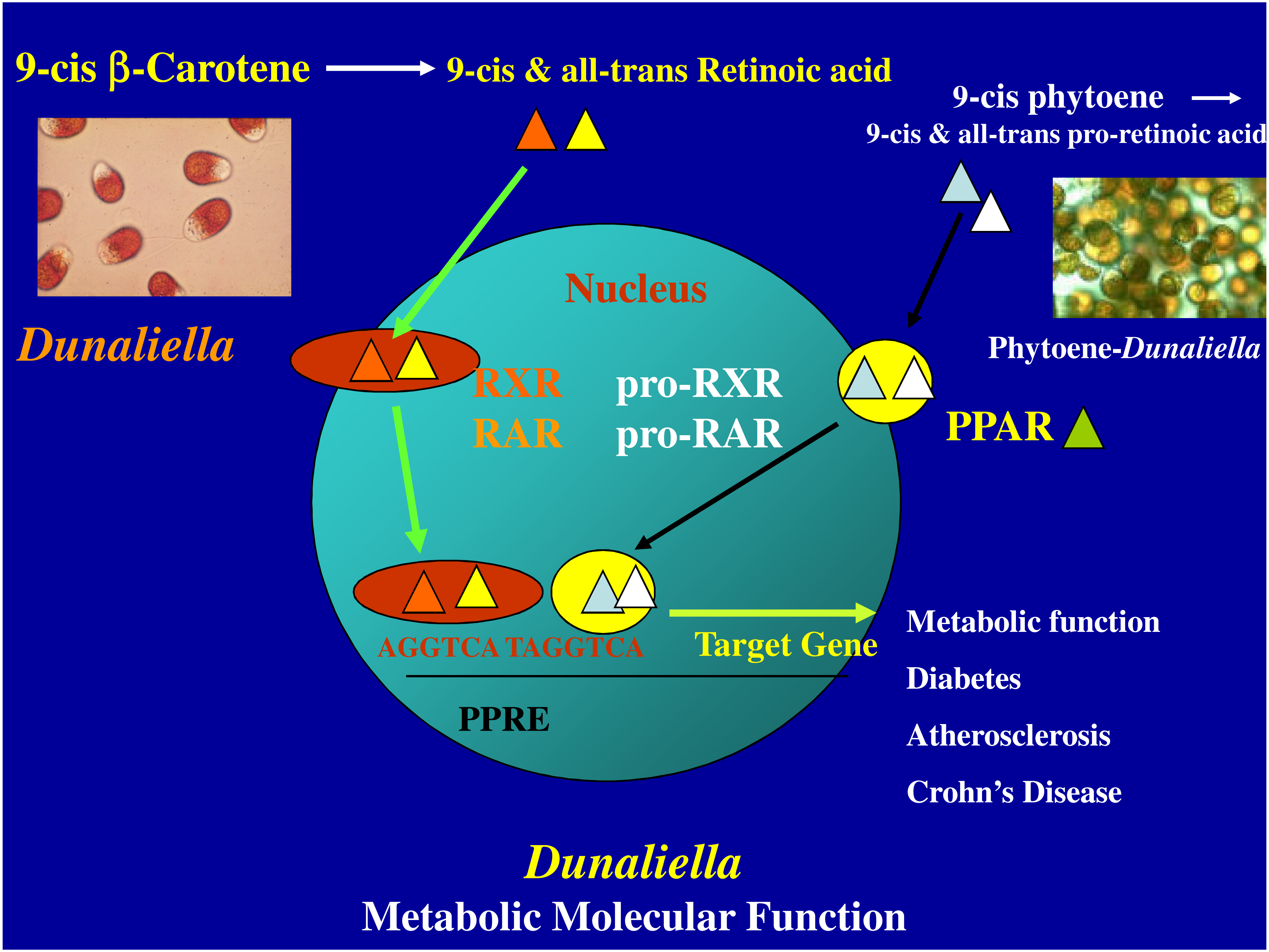




Conversion of 9-cis-β-Carotene to all-trans & cis-Retinoids






9-cis β-Carotene
9-cis & all-trans Retinoic acid
9-cis phytoene
9-cis & all-trans pro-retinoic acid
Dunaliella
Nucleus
RXR
pro-RXR
RAR
pro-RAR
Phytoene-Dunaliella
PPAR
AGGTCA TAGGTCA
Target Gene
PPRE
Metabolic function
Diabetes
Atherosclerosis
Crohn's Disease
Dunaliella
Metabolic Molecular Function

Dunaliella

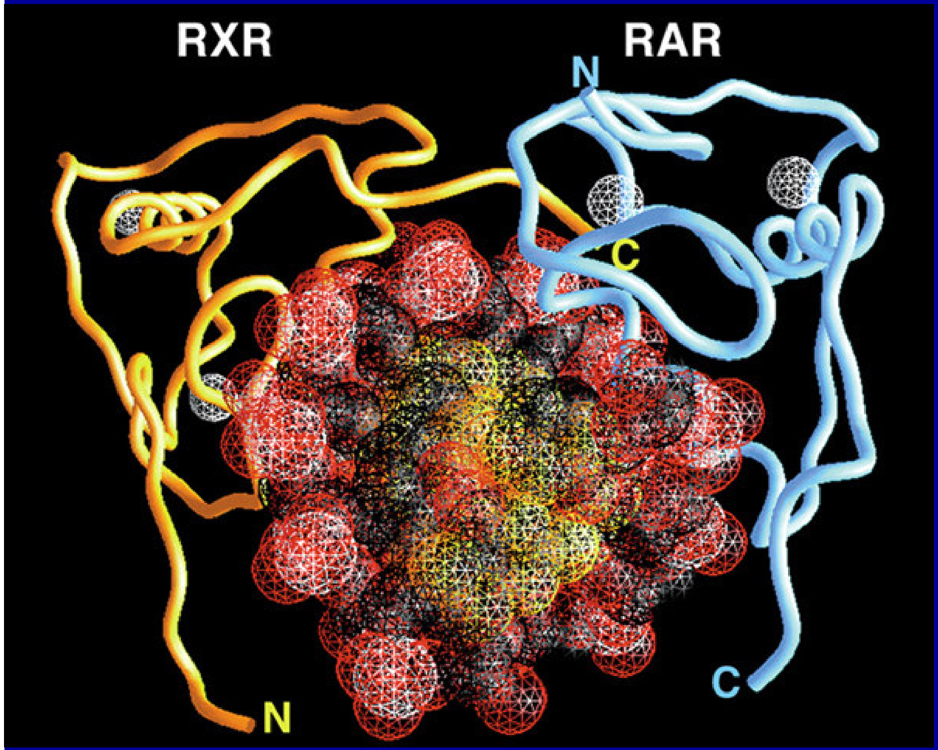
Possible Molecular Cellular Activity

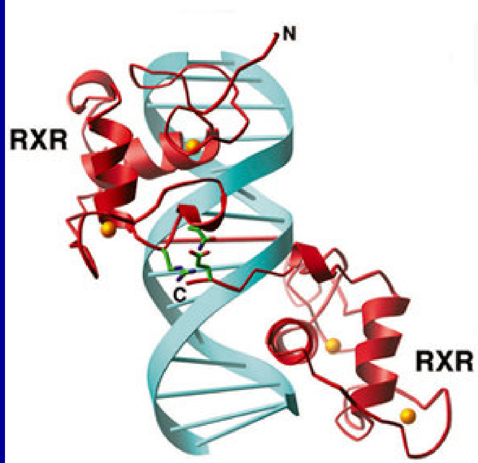
RAR & RXR; pro-RAR & pro-RXR

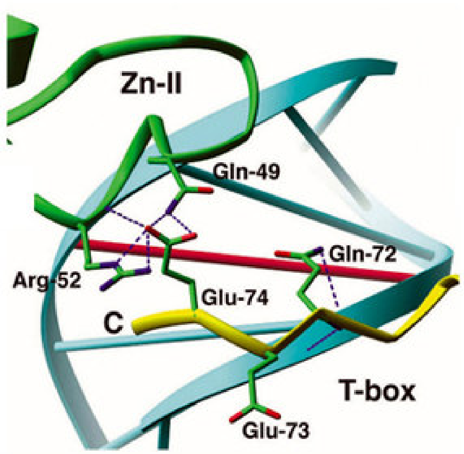
all-trans β-carotene: 2 RAR; 9-cis β-carotene: RAR & RXR

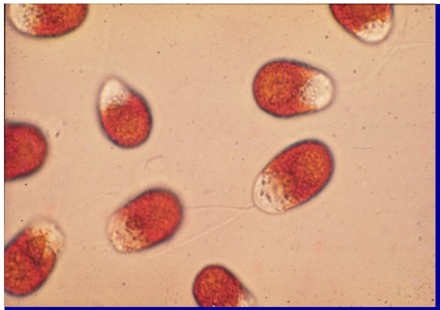
all-trans phytoene: 2 pro-RAR; 9-cis-phytoene: pro-RAR & pro-RXR

Medical molecular heterodimerization with certain drugs



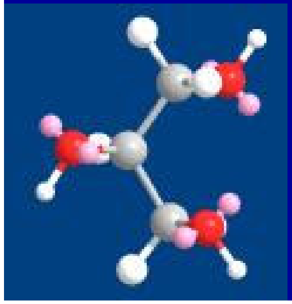


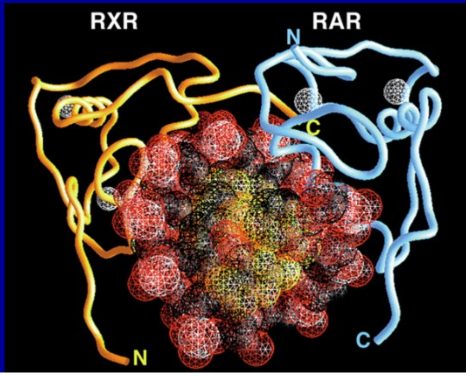




Summary:

The ability to induce, modify and scale up the alga *Dunaliella* to produce glycerol and a series of uncommon new carotenoids opens attractive line in the area of algal biotechnology for energy, nutrition and medicine





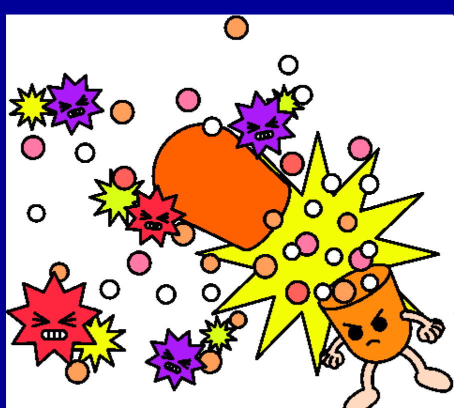
The Future of *Dunaliella*

Energy

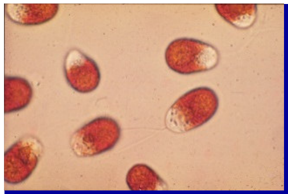
Food

October 2005
USA, DOE
***Dunaliella* Genome**

Medicine



Agriculture



Thank You

Biology, Biochemistry, Physiology: IOLR, WIS, IL
Biotechnology: NBT, IL; **Cognis,** AU; **Lantai, China; others**
Animals: Technion, Sheba, IL
Human: Medical Centers; Sheba, Hadassah, Rambam, Rabin, IL and others