catalyzed by the Na^+,K^+-cotransport (fig. 3C). This effect was observed in 4 experiments on 2 sets of plasma extracts. A maximal inhibitory effect ranging from 65 to 95% was observed with the 1B and 1C fractions. No effect of any of the 1B, 1C or 1D fractions could be observed on the ouabain and bumetanide-resistant Na^+ efflux (fig. 3B) or on the Na^+,Li^+-countertransport (fig. 3D). The plasma extracts which inhibit the Na^+,K^+-ATPase activity possess the properties of endogenous sodium transport inhibitor(s) (ESTI). These ESTI decrease 2 Na^+ transport pathways in human red blood cells; the Na^+,K^+-pump and the Na^+,K^+-cotransport, whereas the passive permeability for Na^+ and the Na^+,Na^+-countertransport remained insensitive to ESTI. There is strong evidence suggesting that the Na^+,K^+-pump and the cotransport system represent different molecular entities[21], with preferential affinities for various inhibitors. The Na^+,K^+-pump is selectively inhibited by ouabain and other digitalis-like compounds[22], and the Na^+,K^+-cotransport by furosemide, ethacrynic acid and other loop diuretics[23]. This could lead to the hypothesis that the plasma may contain molecularly different inhibitors of the 2 Na^+ transport systems.

The effect of plasma extracts on specific ^3H-ouabain binding to human erythrocytes is shown in figure 4. The displacement curve obtained with increasing amounts of plasma extract is dose-dependent and similar to that obtained with increasing concentrations of unlabelled ouabain. The amounts of plasma extract 1B, 1C and 1D which displace the ^3H-ouabain binding by 50% correspond to 300 µl, 660 µl and 520 µl of initial plasma volume, respectively. Such a displacement is obtained by 2×10^{-9} M unlabeled ouabain. The effects of plasma extracts 1 and 1A were not assessed on either ouabain binding or Na^+ fluxes, as they have a high cationic content. The anion plasma vanadate, which is known to inhibit Na^+,K^+-ATPase activity could be eluted off the cellex D. However, the extracts 1B, 1C, and 1D, in addition to inhibiting the enzyme activity, also displace ^3H-ouabain from its binding sites, whereas vanadate up to 10^{-5} M is devoid of this latter effect. Thus, vanadate cannot fully account for the described effects.

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Photoregulation of some enzymes from *Neurospora crassa*

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Summary. Light-grown cultures of *Neurospora crassa* showed photoregulation of a number of enzymes. Proteases and cytosolic malate dehydrogenase showed an increase in activity. There was a decrease in the activity of mitochondrial malate dehydrogenase, isocitrate dehydrogenase and cytosolic glucose-6P-dehydrogenase, isocitrate dehydrogenase and isocitrate lyase.
Key words. Neurospora crassa; enzyme photoregulation; cultures, light-grown; photoregulation, enzyme; carbohydrate metabolism.

Photoregulation of both metabolic processes and development has been shown to play an important role in the life of plants. One of the most striking examples is that of the obligatory light dependent chlorophyll formation in angiosperms[1]. Photoregulation of carotenoid biosynthesis has also been reported for angiosperms, algae, fungi and bacteria and has been extensively reviewed[2-6]. Information is lacking regarding the photoregulation of primary metabolism in fungi. Attempts have been made in the present studies to understand the influence of light on carbohydrate metabolism in *Neurospora crassa*. Light mediated changes in primary metabolism may subsequently help us to understand the regulation of secondary metabolism, thereby leading to an increase in the production of secondary metabolites.

Materials and methods. The carotenogenic strain of *N. crassa* (wild type) obtained from the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi, India, was maintained on Saboraud's agar slants. The synthet-

Table 1. Effect of light on pH, growth, lipid content, carotenoids and activities of proteases and mitochondrial isocitrate dehydrogenase and malate dehydrogenase from *N.crassa*, grown for 72 h at 30 °C

Conditions of growth	pH	Growth (dry wt mg/l)	Lipids (mg/g dry wt)	Protease (U/mg protein) Acidic	Neutral	Alkaline	Isocitrate dehydrogenase (U/mg protein)	Malate dehydrogenase (U/mg protein)
Light	3.7	3400	6.6	82.1	222.4	211.9	8.8	24.1
Dark	3.5	4000	4.7	21.1	93.1	32.1	44.4	31.5

U, Units. Units for isocitrate dehydrogenase and malate dehydrogenase: Amount of enzyme which brings about a change of 0.001 OD at 340 nm/min at 30 °C. Units for protease: umol tryptophan liberated at 37 °C. The values provided here are average values of 4 different sets of experiments.

ic liquid medium employed for the growth contained per liter; Sucrose 25 g; trisodium citrate 2.5 g;$(NH_4)_2SO_4$, 2.5 g; $MgSO_4$, $7H_2O$, 0.5 g; $ZnSO_4$ $7H_2O$, 2.5 mg; $FeCl_3$, 5.0 mg; $CaCl_2$, 10 mg; biotin, 100 µg. The pH was adjusted to 5.6. The culture was grown in 50 ml liquid medium in 250 ml Erlenmeyer flasks on a rotary shaker (150 rpm) at 30 °C in the presence of continuous white fluorescent light (16 lux, fluorescent tubes, 6500 K, 40 watts). For dark conditions of growth, the flasks were placed in black cloth bags. The other conditions of growth with respect to light and dark were identical. The mycelia were harvested by filtration and stored at −5 °C before use. For growth measurement, the mycelia were dried at 50 °C to a constant weight. The growth was expressed as mg dry weight per liter.

The preparation of cell-free extracts for both mitochondrial and cytosolic enzyme assays was carried out as described earlier[7]. The estimation of lipid and carotene was done by the methods of Bragdon[8] and Davies[9], respectively. Protein was estimated by the method of Lowry et al.[10].

The assay methods used for isocitrate dehydrogenase (threo-D-isocitrate NADP oxidoreductase (decarboxylating), EC 1.1.1.42), malate dehydrogenase (L-malate, NAD oxidoreductase, EC 1.1.1.37) glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate: NADP oxidoreductase, EC 1.1.1.49), FDP aldolase (fructose-1-6-diphosphate D-glyceraldehyde-3-phosphate lyase EC 4.1.2.13) and isocitrate lyase (threo-D-isocitrate glyoxylate lyase, EC 4.1.3.1) were the same as that described by Ochoa[11], Ochoa[12], Kornberg and Horecker[13], Jagannathan et al.[14] and Dixon and Kornberg[15], respectively. Proteases were assayed according to the method of Ong and Gaucher[16].

Results and discussion. When *Neurospora crassa* was grown in the presence of continuous fluorescent white light for 72 h, no significant changes were seen in its growth or in the pH of the medium (table 1) but significant changes were observed in the activities of number of enzymes of carbohydrate metabolism. The light-grown culture showed a decrease in the activities of mitochondrial isocitrate dehydrogenase and malate dehydrogenase and an increase in the cytosolic malate dehydrogenase (tables 1 and 2). Light-mediated changes in enzyme activities have been shown earlier for a number of enzymes which are involved mainly in the carotenogenic biosynthetic pathway. Prephytoene pyrophosphate synthetase has been reported to be totally photo-induced in *Mycobacterium* species and the constitutive activity of geranyl pyrophosphate synthetase present in dark-grown cells was increased after exposure to light[17]. Apart from these studies, in a photosynthetic system in angiosperms, Cerff and Kloppstech[18] have demonstrated structural diversity in the cytosolic and chloroplast enzyme glyceraldehyde-3-phosphate dehydrogenase and differential light control of mRNAs coding for the same. The in vivo levels of mRNA coding for the subunits A and B of the chloroplast enzyme were strongly light-dependent and an increase in translational activity was stimulated by continuous white light.

Glucose-6P-dehydrogenase, cytosolic isocitrate dehydrogenase and isocitrate lyase in our studies with *N. crassa* showed decreased activity in the light as compared to dark-grown cultures (table 2). Thus light may also cause a decline in enzyme activities. In *E. coli*[19], several of the active transport systems for nutrients have been reported to be inactivated by intense visible light. FDP aldolase, however, did not show any significant change in our studies with *N. crassa*.

The occurrence of photo-induced carotenogenesis in *N. crassa* is well known[20]. The concentration of lipids was also found to be higher in *N. crassa* grown under light as compared to dark conditions (table 1). Some of the factors which can significantly influence lipid and carotenogenesis are 1) the provision of reducing power, which can be met mainly with glucose-6P-dehydrogenase and, 2) the availability of acetyl CoA, which can be provided by the cleavage of an excess of extramitochondrial citrate in the cytosol. If light increases the accessibility of these factors, carotenogenesis and lipid synthesis can be triggered. But the light-grown culture did not show any increase in the activity of glucose-6P-dehydrogenase in our studies. On the contrary, the activity was found to be less as compared to the dark-grown culture. Likewise, the other enzyme-generating reducing power, cytosolic (NADP) isocitrate dehydrogenase, also showed less activity in light. Cytosolic malate dehydrogenase, however, showed higher activity in the light-grown culture; this enzyme may to some extent provide reducing power. Other enzymes which may also contribute to some extent in this respect have not been studied.

In order to see whether glycolytic pyruvate is more available to synthesize more citrate in the mitochondria, which can then be leaked out into the cytosol in light-grown conditions, one of the glycolytic enzymes, FDP aldolase, was assayed in light- and dark-grown cultures. However, no significant difference was observed. The effect of light on some mitochondrial enzymes was then studied. Mitochondrial isocitrate dehydrogenase showed less activity in light as compared to dark-grown cultures. It is known that the aconitase reaction is reversible

Table 2. Glucose-6-P dehydrogenase, cytosolic malate dehydrogenase, isocitrate dehydrogenase, FDP aldolase and isocitrate lyase activities from *N. crassa* grown in light and dark conditions

Growth conditions	Glucose 6-P dehydrogenase (U/mg protein)	Malate dehydrogenase (U/mg protein)	(NADP) isocitrate dehydrogenase (U/mg protein)	FDP aldolase (U/mg protein)	Isocitrate lyase (U/mg protein)
Light	71	59	21	52	31
Dark	197	35	35	46	53

U: Units, Units for glucose-6-P dehydrogenase, malate dehydrogenase, (NADP) isocitrate dehydrogenase: Amount of enzyme which brings about a change of 0.001 OD at 340 nm/min at 30 °C. Units for FDP aldolase and isocitrate lyase: Amount of enzyme which brings about a change of 0.001 OD at 240 and 324 nm respectively per min at 30 °C. The values provided here are average values from 4 different sets of experiments.

and at equilibrium at pH 7.0; the ratio of citrate: cisaconitate: isocitrate becomes 90:4:6. Thus, for the active catabolism of citrate, a very high level of isocitrate dehydrogenase is required to remove the substrate isocitrate which may otherwise be converted to citrate by the reversible aconitase reaction. If the activity of isocitrate dehydrogenase decreases, citrate wil accumulate. When citrate is present in higher amounts in mitochondria, it may come out in the cytosol and be degraded to acetyl CoaA and oxaloacetate, thus providing acetyl CoA for lipid and carotenogenesis. A low isocitrate dehydrogenase activity of mitochondria from light-grown culture may thus play a significant role in increasing the lipid and carotenoids in light-grown cultures.

Isocitrate lyase activity was found to be lower in light as compared to dark-grown culture, suggesting that the glyoxylate bypass may be operating at higher rate in the dark as compared to the light. If this is the case, under these circumstances, acetyl CoA may be channeled to the glyoxylate bypass and hence may be less available for lipid and carotenogenesis in a dark-grown culture. These are some of the factors which may be playing an important role in light-mediated lipid synthesis and carotenogenesis in *N. crassa*.

The significant role of proteases in cellular regulation is well documented[21,22]. We have also observed significant changes in the intracellular acidic, neutral and alkaline proteases in light and dark grown *N. crassa*. The activities of all these proteases were found to be much higher in light-grown cultures (table 1). Govind et al. have earlier suggested a role of protease in increasing the levels of carotenoids in *Blakeslea trispora*[23]. Whether proteases control carotenogenesis in *N. crassa* is not known at present, though the possibility cannot be ruled out. Proteases may also contribute to the turnover rates of number of enzymes. It is not easy to correlate enzyme activities and the levels of proteases. Further, it is difficult to assume the sequence of events leading to light-mediated control mechanisms in the expression of various enzyme activities. However, attempts have not been made in the present studies to understand the light-mediated control mechanisms in enzyme activities or increases in lipid or carotenogenesis. The present investigation demonstrates the significant changes in the activity of a number of enzymes of carbohydrate metabolism which are considerably influenced when *N. crassa* is grown in light rather than in the dark.

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Autoradiographic investigation of cell proliferation in the adrenal cortex of castrated female rats under the influence of oestradiol

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Summary. Ovariectomy and subsequent treatment with ovarian hormone produces a temporary increase in DNA-synthesizing cells in the zona glomerulosa of the adrenal cortex.
Key words. Rat, female, castrated; oestradiol; ovariectomy; cell proliferation; adrenal cortex, proliferation; DNA-synthesizing cells.

The kinetics of proliferation in the adrenal cortex has already been the subject of numerous investigations[1-7]. In female animals it has been observed that there is a fluctuation in the number of DNA-synthesizing cells related to the oestrous cycle[7]. The aim of this study was to investigate the kinetics of proliferation in the adrenal cortex of castrated female rats which received ovarian hormone.

Material and methods. The investigation was performed in 88 female rats of the Chbb: THOM (SPF) strain. Ovariectomy was performed under Sodium Evipan (1 ml/kg b.wt) anesthesia in all animals when they were 60 days old. Five oestradiol (Ovocyclin = Depotoestradiol, Ciba, Basle) treated animals and 3 control animals treated with saline were investigated at each point in time as indicated in figure 1. Exactly 4 weeks after ovariectomy the animals in the treated group received a single i.m. injection of 2.5 mcg/g b.wt oestradiol.

The animals in the control group were given 0.3 ml of a physiological saline solution. 1 h before sacrifice the animals received a single i.v. injection of 1 μCi/g b.wt of ^{3}H-thymidine. Immediately following sacrifice the uteri and adrenal glands