this communication we have examined the effect of fruit on ribulosediphosphate carboxylase activity and soluble protein content in citrus leaves.

Methods. Cuttings consisting of a lamina, petiole, axillary bud and a 2 cm piece of stem were taken from mature twigs of *C. madurensis* growing in the tropical plant collection at the Faculty of Agriculture, University of Malaya. The cuttings were rooted in a propagation box, and transplanted into a plastic container holding 8 l of coarse river sand. All plants were watered twice daily with a modified Hoagland solution. 4 plants were selected for the experiment on the basis of uniform growth. Flowers developed at the same time in the 4th growth cycle, and were repeatedly removed from 2 plants, while fruit set was allowed on the other two. Leaves were sampled from this 4th growth cycle on both sets of plants 56 days after fruit set, when the developing fruits had reached the phase of cell enlargement.

Ribulosediphosphate carboxylase (EC 4.1.1.39) activity was assayed as follows: leaves were cut into 1 cm strips, and homogenized in pH 8.3 *Tris*-glycine buffer[4] (ratio of tissue to buffer – 1.2 g/10 ml). The final volume of the extract was made to 6 ml, and enzyme activity assayed in a final volume of 0.4 ml: 0.1 ml of leaf homogenate (after filtration through 2 layers of cheese cloth) was added to 0.2 ml of a reaction mixture containing 25 μmoles *Tris* (adjusted to pH 8.0 with HCl), 10 μmoles $MgCl_2$, and 0.26 μmoles EDTA. To this was added 0.05 ml mercaptoethanol (0.31 μmoles), and 0.1 ml $Na^{14}CO_3$/$NaHCO_3$ solution (10 μCi–0.17 μmole/22.3 μmoles). This mixture was incubated at 30 °C for 20 min, and the reaction initiated by adding 0.1 ml of ribulose-1.5-diphospahte (1.5 μmoles). Samples were withdrawn from the reaction mixture at 2 min intervals for 10 min, and the reaction terminated by pipetting 50 μl portions into vials containing 5 ml of an ethanol (95% v/v) – formic acid (90% v/v) mixture (1:1 v/v). Counting was performed in a dioxane based fluor at 53% efficiency.

Results and discussion. Ribulosediphosphate carboxylase activity – expressed as amount of CO_2-fixed per g leaf fresh weight per min – is presented in the Table. Results are means of duplicate determinations performed on separate lots of paired plants, and standard errors are included. Both enzyme activity and soluble protein contents are higher in the leaves of fruiting than in non-fruiting plants. These differences are significant at the 99% confidence level (*t*-test).

Effect of fruit on the amount of $^{14}CO_2$ fixed per unit fresh weight

Sample	Amount (μmoles) of $^{14}CO_2$ fixed/g fresh weight/min.	Soluble protein (mg/g fresh weight)
Fruiting plants	0.39 ± 0.05	22.3 ± 1.2
Non-fruiting plants	0.18 ± 0.02	17.2 ± 1.3

The possibility exists that the observed differences in enzyme levels are the result of a hormone imbalance caused by the removal of fruit. Auxins, cytokinins, ethylene, and gibberellins have all been detected in citrus fruits[5], and various workers have shown that ribulosediphosphate carboxylase activity can be hormonally regulated[2,6,7]. However, the possibility should not be excluded that the flux of carbohydrate from leaves to fruits has a regulatory effect on enzyme activity for LENZ and KÜNTZEL[8] have reported considerable variation in sugar and starch contents between leaves of fruiting and non-fruiting plants.

Increases in soluble protein, though significant, do not parallel the increases in enzyme activity. It is therefore suggested, that both the amount and specific activity of ribulosediphosphate carboxylase increases in the leaves of fruiting plants, and further investigations are necessary to determine the relative importance of each process.

Zusammenfassung. An fruchtenden und entblüteten Stecklingen von *Citrus madurensis* wurde an vergleichbar inserierten Blättern die Aktivität von Ribulose-Diphosphat Carboxylase und der Gehalt an löslichen Proteinen bestimmt. Sowohl die Aktivität von RuD Carboxylase als auch der Gehalt an löslichen Proteinen waren in Plättern fruchtender höher als in solchen nicht fruchtender Pflanzen.

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Inhibition of Protein Synthesis in Ehrlich Ascites Tumor Cells by Irradiation (365 nm) in the Presence of Skin-Photosensitizing Furocoumarins

The Ehrlich ascite tumor cells lose their in vivo ability to induce tumors after irradiation with long wavelength UV- light (365 nm) in the presence of skin-photosensitizing furocoumarins[1]. The tumor cells photoinactivated as above behave as the untreated control cells with regard to the exclusion of vital dye and oxygen uptake[2]; on the contrary their nucleic acids synthesis is strongly inhibited[3].

These results are related to the capacity of the furocoumarins to photoreact by a C_4-cycloaddition with the pyrimidine bases of nucleic acids, DNA and RNA[4–7]; the formation of covalent linkages between furocoumarins and DNA by irradiation of Ehrlich ascite cells in the presence of ^{3}H-psoralen was previously demonstrated[8].

This paper deals with the protein synthesis after photosensibilization by furocoumarins using the Ehrlich ascite cells as a model.

The cells (2–3×10^6/0.1 ml) suspended in saline solution containing the furocoumarin were irradiated in Petri dishes with a Philips HPW 125 lamp (365 nm; irradiation intensity 1.07×10^{15} quanta/cm²/sec as determined with a chemical actinometer[9]) and washed 3 times with ice-cold Hank's saline solution. The washed cells were then resuspended in the latter medium ($4\text{–}5 \times 10^6$ cells/0.1 ml) and incubated at 37°C in the presence of labelled amino acids (0.5 µCi/ml; equimolar mixture of fourteen ^{14}C-amino acids, each being of specific activity 10 mCi/m*M*; The Radiochemical Centre, Amersham, England).

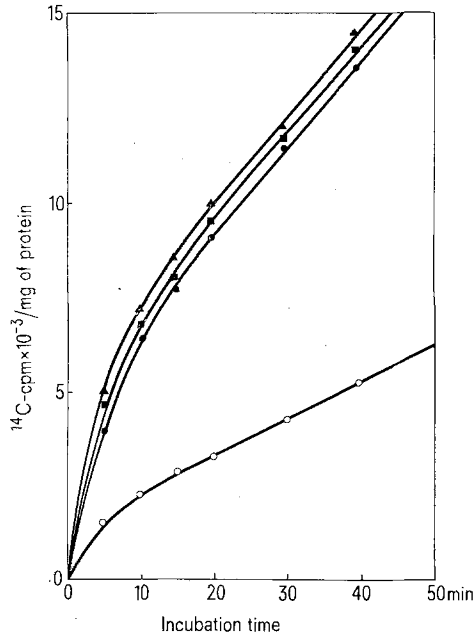
At fixed times, aliquots of reaction mixture containing about 2.5–3 mg of protein, evaluted according to the procedure of LOWRY[10], were treated with 5% trichloroacetic acid, filtered on glass-fibre dishes Whatman GF/C, and the radioactivity determined using a liquid scintillation counting system Beckman LS 150 and a toluene-based solution. Specific activity of controls after incubation of 30 min was about 12–14,000 cpm/mg of protein.

As seen in the Figure, after irradiation in the presence of psoralen, the incorporation of labelled amino acids proceeds at a slower rate in comparison with the controls. By contrast, the cells irradiated without psoralen, or incubated in the dark with psoralen, behave as the untreated controls. Further results obtained with other furocoumarins as well as different experimental conditions are given in the Table.

The protein synthesis is inhibited at larger furocoumarin concentrations and higher radiation doses than those already observed in the inhibition of DNA and RNA synthesis[3]. Under the same conditions, the greatest inhibition was observed with 8-methylpsoralen, followed by psoralen and xanthotoxin. It is worth observing that the same order of activity was already observed in the skin-photosensibilization[11] as well as in the ability to photoreact in vitro with the nucleic acids[6].

We recall that in a cell-free system, using *E. coli* ribosomes previously irradiated in the presence of some furocoumarins, among which were psoralen and 8-methylpsoralen, RODIGHIERO, CHANDRA and WACKER[12] found an inhibition in the incorporation of ^{14}C-phenylalanine of the same extent for all the tested substances.

Further research is in progress with the aim of explaining whether the inhibition observed in the Ehrlich ascite tumor cells is an expression of damage to ribosomes or of some modification of the genetic control of protein synthesis as a consequence of the furocoumarins photobinding to DNA.



^{14}C-aminoacids incorporation into proteins of Ehrlich ascite tumor cells after exposure to: (-■-■-■-), nothing, untreated controls; (-▲-▲-▲-), psoralen alone, 1 µg/10^6 cells; (-●-●-●-), UV-irradiation alone, 41.73×10^{18} quanta; (-○-○-○-), psoralen, 1 µg/10^6 cells + UV-irradiation, 41.73×10^{18} quanta.

Protein synthesis in Ehrlich ascite tumor cells after irradiation in the presence of skin-photosensitizing furocoumarins

Furocoumarin	µg/10^6 cells	Quanta $\times 10^{-18}$	Percent inhibition $\pm \Sigma$	P^a
Psoralen	0.12	38.8	10 ± 0.95	0.05 – 0.01
	1	4.86	< 5	> 0.05
	1	9.73	< 5	> 0.05
	1	19.4	25 ± 3.87	< 0.001
	1	41.73	65 ± 1.33	< 0.001
Xanthotoxin	1	38.8	25 ± 4.14	< 0.001
8-methylpsoralen	0.12	38.8	71 ± 1.08	< 0.001
	1	38.8	93 ± 0.38	< 0.001

The cell suspensions containing the furocoumarin were irradiated at 365 nm and then incubated with ^{14}C-amino acids mixture; the TCA-insoluble radioactivity was determined. [a] *P* has been calculated according to the Students *t*-test.

Riassunto. La sintesi proteica nelle cellule del tumore ascitico di Ehrlich viene inibita dall'irradiazione a 365 nm in presenza di furocumarine fotosensibilizzatrici cutanee (psoralene, xantotossina e 8-metilpsoralene).

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