ribosomal RNA from the ribosomal subunits and the determination of its specific radioactivity, have been described previously[6,10]. The rate of nuclear and mitochondrial DNA synthesis was estimated by administering ($^3$H-methyl) thymidine (15 Ci/mmole; 22 μCi/250 g body wt.) to the rats at 25 h, then removing the liver at 26 h post-operative. The specific radioactivity of the DNA (cpm/μg of deoxyadenosine) in the purified nuclear and mitochondrial fractions[11] was determined as previously described[6], the DNA being estimated with diphenylamine reagent using deoxydenosine (AdR) as standard.

*Results and discussion.* The rate of ribosome formation which is maximal at 19 h after partial hepatectomy remains elevated throughout peak DNA synthesis which occurs at approximately 25 h post-operative. The dependency of the elevated ribosome formation on nuclear DNA synthesis (Table I) was estimated by administering a single dose of hydrocortisone (40 mg/kg/dose of hydrocortisone sodium succinate) at 19 h, or 2 doses of either hydroxyurea (250 mg/kg/dose) or cytosine arabinoside (250 mg/kg dose) at 19 h and 23 h followed by the measurement of the rate of DNA synthesis over the interval 25 to 26 h after partial hepatectomy. Hydroxyurea, cytosine arabinoside and hydrocortisone inhibited DNA synthesis by approximately 70%, 80% and 63%, respectively, throughout the 19 to 26 h period of observation. Despite this marked inhibition there was no significant effect on either ribosome formation, or mitochondrial DNA synthesis.

The effect on nuclear DNA synthesis, of inhibiting mitochondrial DNA synthesis (Table II) was tested by administering a single dose (1–3 mg/kg/dose) of ethidium bromide at 19 h post-operation. These dosages gave a maximal inhibition of 50% over the 6 h period, a value which is comparable to that obtained in cell culture systems[12]. However, despite this significant inhibition, nuclear DNA synthesis was unaffected.

Other experiments (results not shown) indicated that the above results were not significantly affected by pool dilution effects since similar differential effects were observed when 6-$^{14}$C orotic acid was used as the single labelled precursor for both DNA and RNA synthesis as previously described[5,6]; mitotic counts at 31 h post-operation also paralleled the relative rates of DNA synthesis as measured by tritiated thymidine incorporation[5]. In conclusion, the enhanced synthesis, processing and transport of ribosomal RNA in the regenerating liver proceeds independently of nuclear DNA synthesis. Furthermore, mitochondrial and nuclear DNA synthesis are not tightly coupled, in agreement with an earlier study[13] based on the differential effect cycloheximide on these 2 processes; however some form of loose coupling must exist to maintain a relatively constant number of mitochondria per cell[14].

*Zusammenfassung.* In der regenerierenden Rattenleber wird eine beschleunigte Bildung von Ribosomen und eine vermehrte Synthese von mitochondrialem DNA beobachtet, welche unabhängig von der nuklearen DNA-Synthese über eine 6 stündige Periode der S-Phase erfolgt. Während dieser Zeit geht die nukleare DNA-Synthese unabhängig von der mitrochondrialen DNA-Synthese vor sich.

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Table II. Dependence of nuclear DNA synthesis on mitochondrial DNA synthesis

| Treatment | Mitochondrial DNA (cpm/μg AdR) | Nuclear DNA (cpm/μg AdR) |
|---|---|---|
| Controls | 26.5 ± 4.6 | 101.5 ± 15.0 |
| Ethidium bromide | | |
| 1 mg/kg | 18.9 ± 4.1 | 103.0 ± 11.4 |
| 2 mg/kg | 14.7 ± 2.4 | 99.0 ± 14.6 |
| 3 mg/kg | 13.7 ± 0.4 | 105.4 ± 8.7 |

The values (± standard errors) are based on 5–15 rats.

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## Nucleic Acids and Protein Synthesis in Mice Tumor and Normal Cells after Irradiation at 365 nm in the Presence of Psoralen

The cells of some experimental mice tumors, such as the Ehrlich ascite carcinoma or the Graffi virus leukaemia, lose their ability to transmit the tumor by tranplantation into susceptible animals when irradiated with long wave ultra-violet light (365 nm) in the presence of skin-photosensitizing furocoumarins[1,2]. These drugs yield a photo-$C_4$-cycloaddition to the pyrimidine bases of the nucleic acids, DNA and RNA[3–6], behaving both as monofunctional and bifunctional reagents[7]. Using the Ehrlich ascite tumor as a model, we have previously established that the cells so treated behave like the controls with regard to sopravital stains and oxygen uptake[8], while on the contrary their nucleic acids and protein synthesis are strongly inhibited[9,10].

We have now extended these experiments to other tumor and normal cells of the mouse, studying the macromolecular synthesis after UV-irradiation in the presence of the furocoumarin psoralen, the parent compound. The cells examined are the Ehrlich tumor and the sarcoma 37, both in ascite form and transferred into NCL mice, the P1534 and Graffi virus leukaemias, transplanted into $DBA/_2$ and $C_{57}BL/6$ mice respectively, and the spleen cells of these last two mice strains.

The methods are the same as previously described[9,10]. The cells ($2 \times 10^6$/0.1 ml in balanced saline solution containing the psoralen) were irradiated on crushed ice with a Philips HPW 125 lamp (365 nm; irradiation intensity $1.07 \times 10^{15}$ quanta/cm$^2$/sec); after washing, the cells were incubated at 37 °C ($4 \times 10^6$/0.1 ml in Hank's solution) in the presence of the suitable radioactive precursor (3 μCi/ml of $^3$H-thymidine, 2 Ci/m$M$, or of $^3$H-uridine, 4.6 Ci/m$M$; 0.5 μCi/ml of an equimolar mixture of fourteen $^{14}$C-amino-

acids, 10 mCi/m$M$; the Radiochemical Centre, Amersham, England).

The nucleic acids were extracted by the hot 10% sodium chloride method after 30 min of incubation and their specific activity determined using a modified Bray's fluid[11] with a Beckman LS-150 liquid scintillation counter and the diphenilamine[12] or orcinol[13] reactions.

To study the protein synthesis, aliquots containing about 1 mg of protein (evaluted according to LOWRY[14]) were removed at fixed times from the incubation mixture, precipitated with 5% trichloroacetic acid on Wathman GF/C filters and counted in a toluene-based solution.

The results obtained by studying the nucleic acids synthesis are summarized in Table I; as may be observed, the psoralen exhibits different photosensitizing activity upon the DNA synthesis. It is to be noted that the greater diversity occurs between cells arising from mice of different strains. On the contrary, psoralen inhibits the RNA synthesis with practically the same strength; only in the Graffi leukaemia cells it is suppressed even at small radiation doses.

Indeed, these cells behave in very singular way; their DNA synthesis is considerably resistant and is depressed only at higher psoralen concentrations. In actual fact, it contains the Graffi RNA tumor virus[15] and therefore the observable molecular events are the result of both cellular and viral activity. It is possible that this peculiar situation may be related to the different results obtained by the psoralen photosensibilization.

Table I. Photosensitizing effect of psoralen on the nucleic acids synthesis

| Cells | Psoralen concentration | | $D_{50}$ (quanta $\times 10^{-18}$) | | $\frac{D_{50}\,DNA}{D_{50}\,RNA}$ |
|---|---|---|---|---|---|
| | μg/ml | μg/$10^6$ cells | DNA | RNA | |
| Ehrlich tumor | 3.7 | 0.18 | 9.8 | 14 | 0.7 |
| | 20 | 1 | 1.5 | | |
| Sarcoma 37 | 3.7 | 0.18 | 8.5 | 9 | 0.94 |
| Graffi leukaemia | 3.7 | 0.18 | 78 | 1.7 | 45.8 |
| | 20 | 1 | 9.5 | | |
| P1534 leukaemia | 3.7 | 0.18 | 18 | 8 | 2.25 |
| $C_{57}BL/_6$ spleen cells | 3.7 | 0.18 | 58 | 13 | 4.46 |
| $DBA/_2$ spleen cells | 3.7 | 0.18 | 20 | 11 | 1.82 |

The cell suspensions containing psoralen were irradiated with graded doses of UV-radiation (365 nm) and then incubated with $^3$H-thymidine or $^3$H-uridine; the nucleic acids were extracted with 10% NaCl and their specific activity determined. The $D_{50}$ is the UV-radiation dose, calculated by probit analysis, that at a fixed psoralen concentration, yields a 50% inhibition.

Table II. Photosensitizing effect of psoralen on the protein synthesis

| Cells | Inhibition ± standard error (%) | $P$ |
|---|---|---|
| Ehrlich tumor | 65 ± 1.33 | < 0.001 |
| Sarcoma 37 | 46 ± 2.3 | < 0.001 |
| Graffi leukaemia | 5 ± 2.5 | > 0.05 |
| P1534 leukaemia | 6 ± 0.58 | > 0.05 |
| $C_{57}BL$ spleen cells | 31 ± 1.04 | < 0.001 |
| $DBA_2$ spleen cells | 63 ± 0.34 | < 0.001 |

The cell suspensions containing psoralen (20 μg/ml; 1 μg/$10^6$ cells) were irradiated at 365 nm ($39 \times 10^{18}$ quanta) and then incubated with $^{14}$C-amino-acid mixture; the TCA-precipitable radioactivity was then determined. P has been calculated according to the Students $t$-test.

Table II shows the data related to the protein synthesis; as we have already observed, high psoralen concentrations and large radiation doses are required for the protein synthesis inhibition. With the Ehrlich carcinoma, the sarcoma 37, as well the $C_{57}$ BL/6 and $DBA/_2$ spleen cells, significant values of inhibition are obtained; by contrast, in the Graffi and P1534 leukaemia cells the protein synthesis is entirely unaffected by psoralen.

In these experiments $^{14}$C-aminoacid incorporation was observed in the absence of RNA synthesis; in fact, at psoralen concentrations and radiation doses used to study the protein synthesis, in all examined cells the RNA synthesis is fully abolished. In this situation, the $^{14}$C-amino-acid incorporation should be directed by pre-existing and not degraded messenger RNAs; therefore their inhibition appears related to a damage to ribosomes, very probably by psoralen photobinding to RNA, and not to an interference in the transcription process.

At present we are investigating whether the failed inhibition of protein synthesis observed in the leukaemic cells is in any way related to cell transformation.

In conclusion, the results show clearly that to study a skin-photosensitizing furocoumarin it is necessary to bear in mind not only its photochemical behaviour but also the properties of the biological substrates employed.

*Riassunto.* L'irradiazione a 365 nm in presenza di psoralene, furocumarina fotosensibilizzatrice cutanea non sostituita, influisce in modo diverso sulla sintesi macromoleculare in alcune cellule tumorali e normali del topo.

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