ducing plasma flow rates and glomerular filtration. The ROS clearance of Experiment 3 is however similar to that of the repeatedly etherized mice in Experiment 2. This is probably because the mice were kept in the ether/air mixture for no more than 10 sec after becoming unconscious. In some preliminary experiments however, using longer etherization, there was considerable slowing of clearance rates.

Finally, the TdR $t^{1/2}$ values may be overestimated due to the presence of intermediate products i.e. thymidine, dihydrothymine, β-ureidoisobutyric acid and B-aminoisobutyric acid. These intermediate reactions are rapid[2] so the amounts involved are small compared with the THO end product. It was hoped that CdR clearance would be similar since CdR is excreted directly by the kidney[15,16]. In some systems however CdR can be converted to deoxyuridine and thence to uracil and TdR which can be degraded to CO_2 and water[17].

The TdR clearance rates determined are experimentally convenient because administration of TdR, followed by suitable hourly booster doses, should maintain peripheral plasma levels above 1 m*M*. Just 1 injection of CdR should then release any block of DNA synthesis so that any cell cycle perturbation should be well defined. The effects of concentrated TdR on the cell kinetics of mouse femoral bone marrow are being investigated.

Résumé. Le TdR concentré, avec le ^{3}HTdR comme traceur, fut injecté i.p. dans des souris de souche CBA. De temps en temps, jusqu'à 4 h après l'injection, on enregistra l'activité totale du ^{3}H et du THO dans le plasma. Dans le sang du sinus retroorbital, la production de THO était linéaire, mais la clearance du TdR exponentielle ($t^{1/2}$ = 45–50 min). Par contre, dans les échantillons de sang cardiaque, les courbes étaient modifiées par un maximum augmenté, 10 min après l'injection. Avec le temps la courbe de $\log_{10}$ CdR devint de forme quadratique, indiquant sans doute la présence d'une grande quantité de produits intermédiaires de dégradation.

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Interrelationship Between Ribosome Formation and Nuclear and Mitochondrial DNA Synthesis in the Regenerating Liver

The regenerating liver of the young adult rat is characterized by an early increase in ribosome formation[1] which is maximal (e.g. a 4-fold increase) by the time nuclear DNA synthesis is initiated at approximately 19 h postoperatively[2]; nuclear DNA synthesis and mitosis subsequently peak at 24 h and 31 h, respectively[2]. In the present investigation the degree of integration between ribosome and cellular DNA synthesis and between DNA synthesis in the various cellular organelles during liver regeneration is assessed by use of inhibitors which affect principally either nuclear, or mitochondrial DNA biosynthesis[3,4]. Hydrocortisone[5,6], hydroxyurea[7] and cytosine arabinoside[8] are used to inhibit nuclear DNA synthesis, while ethidium bromide[9] is used as a specific inhibitor of mitochondrial DNA synthesis.

Materials and methods. The specific radioactivity of the ribosomal RNA during a specific period of the nuclear DNA synthetic period of the regenerating tissue was estimated by administering to the rats by i.p. injection of 6-^{14}C orotic acid (50 mCi/mmole; 20 μCi/250 g body wt.) at 25 h after partial hepatectomy[9]; the livers were removed for processing 1 h later. The isolation of the

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Table I. Dependence of ribosome formation and mitochondrial DNA synthesis on nuclear DNA synthesis

Treatment	Nuclear DNA (cpm/μg AdR)	Ribosomal RNA (cpm/μg RNA)	Mitochondrial DNA (cpm/μg AdR)
Controls	101.5 ± 15	254 ± 10	26.5 ± 4.6
Hydroxyurea	28.7 ± 11	267 ± 7	27.5 ± 8.6
Cytosine arabinoside	20.0 ± 7	242 ± 7	26.1 ± 3.7
Hydrocortisone	37.6 ± 11	374 ± 18	26.4 ± 1.3

The values (± standard errors) are based on 5–15 rats. (Similar results were obtained when the rates of synthesis of both RNA and DNA were estimated from the rate of incorporation of label from 6-^{14} orotic acid).

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