speed is also quite different from that observed in extracts of barley seed[19] and pea cotyledon[20], where it is always slower than the α-amylase. But it is possible that the root β-amylase is rather different from that of the seeds, which is formed of several subunits and sometimes bound to protein-residues[19].

Present results indicate that *Lens* roots contain at least 3 amylases. In *Pisum*, the electrophoresis on polyacrylamide gel revealed 2 bands with amylase activity, which are not only located in the roots, but are found also in the other parts of the plant[3]. The analysis of the products of the enzymatic degradation seems to indicate that only the β-amylase is present in the axis of pea, whereas the cotyledons contain both α- and β-amylase[21]. But it must be noted that such plant contains also amylopectin 1,6-glucosidase[11], which was not found in the extracts of lentil roots.

If the physiological role of the amylases is now well established in seeds, it is not the case in other organs like roots. For instance, the ultrastructural observations on the destarching of amyloplasts in lentil root statenchyma[9] following a treatment with gibberellic acid, and in wheat coleoptile treated with GA_3 or kinetin – this latter correlated with an increase of amylase activity[22] – allow the hypothesis of the intervention of one or more amylases in the hormonal control of starch metabolism.

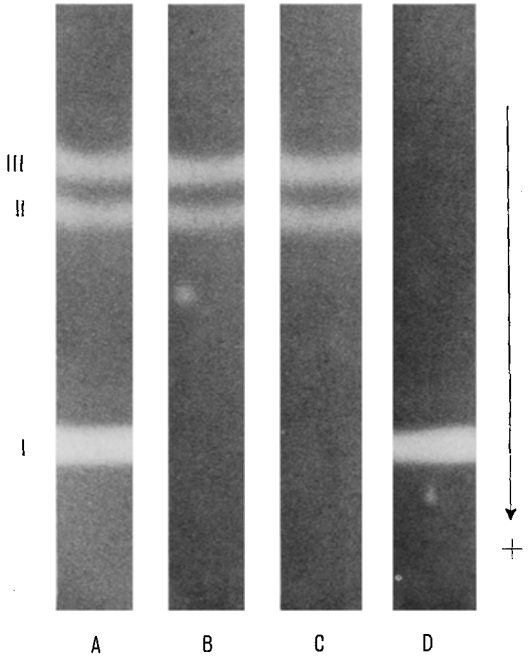

Fig. 2. Zymogram of the electrophoresis on 7% polyacrylamide gel; central parts of gel slices. A) total extract; B) 70°C heated extract; C) gel incubated with PMC; D) gel incubated with EDTA.

Résumé. Les amylases de la racine de *Lens culinaris* sont éluées en un seul pic par chromatographie sur gel de Sephadex G-50 et G-100. Par contre, l'électrophorèse en plaque de gel de polyacrylamide révèle 3 zones d'activité amylasique. Deux d'entre elles sont constituées d'isozymes d'α-amylase; la troisième pourrait être une β-amylase, bien qu'elle ne possède pas toutes les propriétés généralement attribuées à cette enzyme.

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Clearance of Concentrated Thymidine and Deoxycytidine from the Plasma of CBA Mice

Tritiated thymidine (^{3}HTdR) is commonly used as a labelled precursor of DNA both in vivo and in vitro and data is available on clearance from plasma at the concentrations used (usually $\sim 10^{-7}M$)[1,2]. At much higher concentrations (above $10^{-3}M$), TdR is used in vitro to induce synchrony of mitosis[3–5]. Phosphorylation of TdR produces large amounts of thymidine triphosphate which inhibits the cytosine diphosphate to deoxycytosine diphosphate pathway so that removal of TdR releases cells from a block of DNA synthesis[6,7]. Before attempting similar studies in vivo clearance rates of concentrated TdR were needed and results are presented here. As deoxycytidine (CdR) can bypass a TdR block in vitro[8–10] some data is also included on its clearance.

Materials and methods. Animals: CBA mice were used (F36-41), originally from Carshalton M.R.C. Laboratory Animal Centre. Animals were weighed to ± 0.1 g and in Experiment 1 the age recorded.

Inocula. TdR and CdR (Sigma) in Hank's BSS were membrane sterilised before i.p. injection. ^{3}HTdR (S.A. 5 Ci/mM) and ^{3}HCdR (S.A. 25 Ci/mM) were from Amersham and used at 5μCi/ml of inoculum.

Procedure. Blood was obtained in heparinized microhaematocrit tubes from the retroorbital sinus (ROS) under ether anaesthesia, or from cardiac puncture following cervical dislocation. The cells were spun down and two aliquots of either 0.1 ml cardiac plasma or 10 μl (Shandon microcaps) ROS plasma were taken, one for estimation of total ^{3}H activity and the other for tritiated water (THO) determination. As water is the most common ^{3}H labelled end product of ^{3}HTdR degradation, it was assumed that the difference would represent TdR, with only small quantities of intermediate products.

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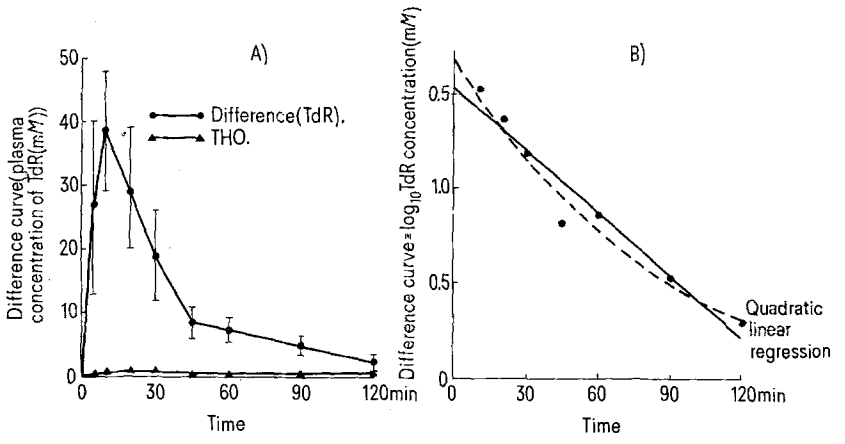

Fig. 1. A) Clearance curve of TdR from cardiac deproteinized mouse plasma (15 mice per point ± 95% confidence limits). B) Linear ($t^1/_2 = 28$ min) and quadratic functions fitted to $\log_{10}$ concentration data of A).

THO determination. Each aliquot of plasma was diluted with carrier water to 0.1 ml and placed in a petri dish on a 60 °C hotplate[11]. The lid was cooled by an acetone/dry ice mixture, so that the frozen distillate containing THO could be collected.

Total ^{3}H activity determination. In Experiment 1 the protein in 0.1 ml plasma was precipitated by the addition of 0.1 ml 10% trichloracetic acid. The precipitate was spun down and 0.1 ml clear supernatant placed in scintillation fluid. When microhaematocrit tubes were used, they were scratched with a diamond, broken above the buffy coat and 10 µl. plasma placed directly into scintillation fluid. The small amount of protein precipitate produced little quenching.

Scintillation counting. Samples were dissolved in Bray's scintillation fluid and counted on a Nuclear Chicago MK II counter. Results were corrected for quenching by the channels ratio method.

Results. Experiment 1. Clearance of TdR from cardiac blood. Mice of widely different weights (14-27 g) and ages (8–40 weeks) were used, and given 300 mM TdR i.p. at 0.02 ml/g body weight. After various times (5–120 min) groups of 16 mice were sacrificed and cardiac blood taken.

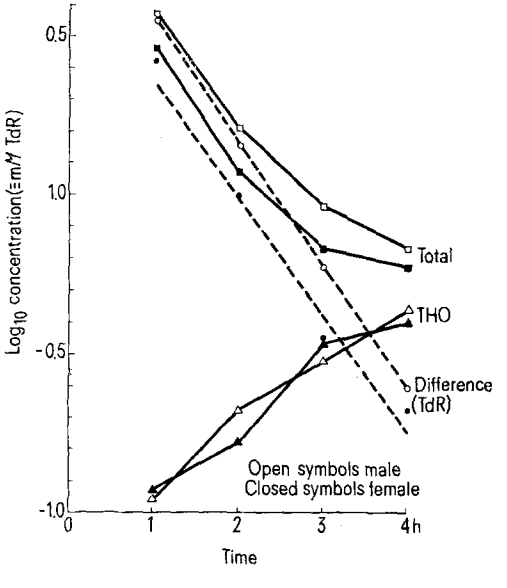

Fig. 2. Clearance of TdR from ROS using whole plasma from male ($t^1/_2 = 46$ min) and female ($t^1/_2 = 48$ min) CBA mice.

Total ^{3}H less pooled THO activity was averaged and plotted as a 'difference curve' (Figure 1A). THO rises eventually to 10% of the total activity at 2 h. Earlier the THO curve shows a peak coincident with the 'difference curve' peak but not representing more than 1.5% of the total at this time. Presumably this represents contaminant THO in the administered ^{3}HTdR. The 'difference curve' when plotted as $\log_{10}$ concentration against time approximates to a regression line $y(\log_{10}\text{m}M) = 1.5129 - 0.0109\,x$ (min) i.e. a $t^{1/2}$ of 27.6 min (Figure 1B). This is a very short $t^{1/2}$ in view of the high TdR concentration reached. A quadratic with significant curvature ($0.05 > p > 0.02$) can also be fitted to the data $y(\log_{10}\text{m}M) = 1.6775 - 0.01877x + 0.00006096x^2$(min) indicating that clearance is not simply exponential.

Regressions were also calculated within each point against weight and age. There were no significant regressions with age but with weight all the slopes were positive with 2 significantly different from zero (at 10 min $p > 0.001$ and at 60 min $0.002 > p > 0.001$). Thus in all the following experiments the mouse weights were standardized.

Experiment 2. Clearance of TdR from ROS blood. This experiment was performed because the very rapid cardiac clearance might not be representative of venous blood elsewhere. 10 male mice (wts. 20–24 g) and 10 females (wts. 16–18 g) were injected with 0.04 ml/g body wt. of 150 mM TdR. Samples of ROS blood were taken hourly up to 4 h and total ^{3}H determined. Second samples were pooled within each hourly group for THO determination. It is apparent that THO increases linearly showing saturation of the degredative pathways but the 'difference' curve is exponential with a $t^{1/2} = 46$ min for males and 48 min for females (see Figure 2). Male and female slopes are not significantly different but both are significantly less than the slope in Experiment 1 ($p > 0.001$). THO data was pooled with that from other unpublished experiments (when hourly booster injections were given up to 6 h) and shows a good fit to the linear regression: $y(\equiv \text{m}M\ \text{TdR}) = 0.000655 + 0.10715x$ (h) (S.E. ± 0.00668).

Experiment 3. Comparison of cardiac and ROS plasma clearance rates. Female mice (18-22 g) were injected with TdR as in Expt. 2 but blood samples were taken from both the ROS and heart of the same mouse. 3 mice were used at each sampling (Figure 3). A very large peak is apparent early in the cardiac 'difference' curve compared with that of the ROS but later the 2 curves converge and are similar after about 60 min. THO represents only a small proportion of total activity until 3–4 h. If

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the 'difference' values are plotted as $\log_{10}$ (see inset Figure 3) and using data from 20 min to 3 h only, then cardiac blood has a $t^{1/2} = 39.06$ min (95% confidence limits 32.81–48.24 min) and sinus blood has a $t^{1/2} = 50.06$ min (42.77–60.33 min). The slopes of these 2 curves are significantly different from that of Experiment 1. The sinus blood slope here shows a significant difference ($0.05 > p > 0.02$) when compared with that of females in Experiment 2, but there is no difference when compared with males. When quadratics are fitted to the data then analysis of variance shows no significant quadratic terms.

Experiment 4. Clearance of deoxycytidine. A block of DNA synthesis by concentrated TdR can be bypassed in vitro by the addition of 10^{-5}–$10^{-6}M$ CdR. In this experiment 0.02 ml/g body wt. of 1.5×10^{-2} MCdR with 5 μCi/ml ^{3}HCdR, was injected into male mice (20–24 g). This corresponds, when uniformly diluted, to $3 \times 10^{-4}M$ and this level was thought suitable to compare with in vitro because of loss of CdR by excretion and degredation.

ROS blood was taken at various times after injection and total ^{3}H and THO activity determined on the pooled samples from 3 animals at each time (Figure 4). When $\log_{10}$ concentrations are plotted, the line from 20–120 min fits the quadratic $y(\log_{10}\text{m}M) = 0.2369 - 0.018072x + 0.00007166x^2$ (min), and analysis of variance shows a highly significant quadratic term ($p < 0.001$). In this experiment intermediate degradation products could be expected to comprise a larger portion of the 'difference' curve compared with TdR clearance because the starting concentration was 20 times lower. It appears however that concentrations of CdR would remain above $10^{-6}M$ for at least 2 h and this would be long enough for TdR to drop below 1 mM, (using similar TdR inocula to the previous experiments) at which blocking of DNA synthesis is no longer complete in vitro.

Discussion. These experiments show that TdR clearance rate can apparently vary at different sites. ROS clearance followed a simple exponential form while cardiac clearance showed high concentrations 10–20 min after injection which eventually fell, after 2–3 h, near to the sinus values. The mean 10 min cardiac value in Experiment 1 (~38 mM) was not significantly different from the 10 min cardiac value (~ 24 mM) in Experiment 3, and this reflects the considerable variation at these high levels especially compared with the ROS values. Presumably this is due to differential rates of uptake between animals, particularly because i.p. injections depend on variables of blood supply and intraperitoneal mixing. In Experiment 1 the TdR concentration versus mouse weight data showed the most significant +ve regression 10 min after injection i.e. when peak values were observed. Small size differences at this time will therefore increase variation of mean TdR levels.

Another possible variable is the etherization in Experiment 2. Ether is known to produce oligouria in man[12,13] and dog[14] by increasing tubular reabsorption while re-

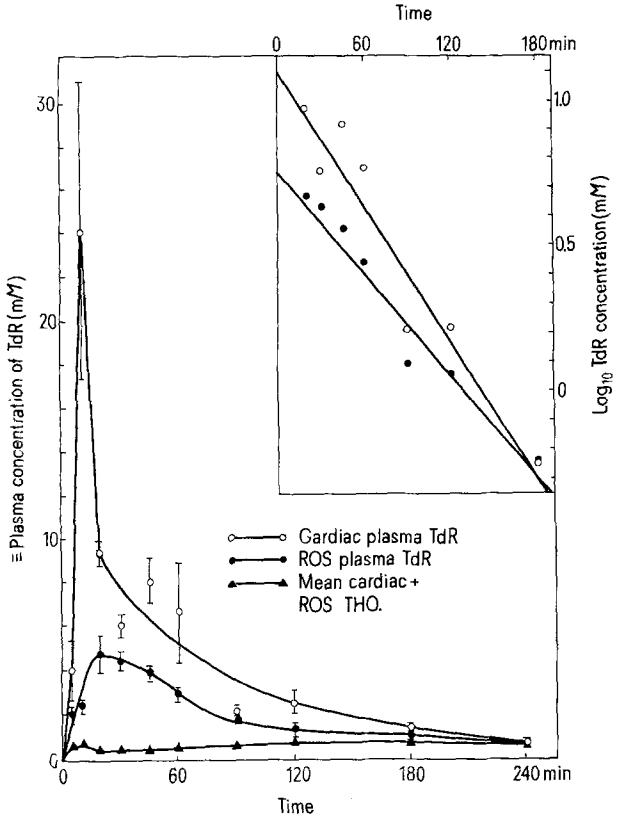

Fig. 3. Clearance of TdR from both ROS and cardiac blood of female mice (3 mice per point ± S.E.). Inset is data 20–180 min plotted as $\log_{10}$ concentration with fitted linear regressions ($t^{1}/_{2}$ cardiac = 39 min, $t^{1}/_{2}$ ROS = 50 min).

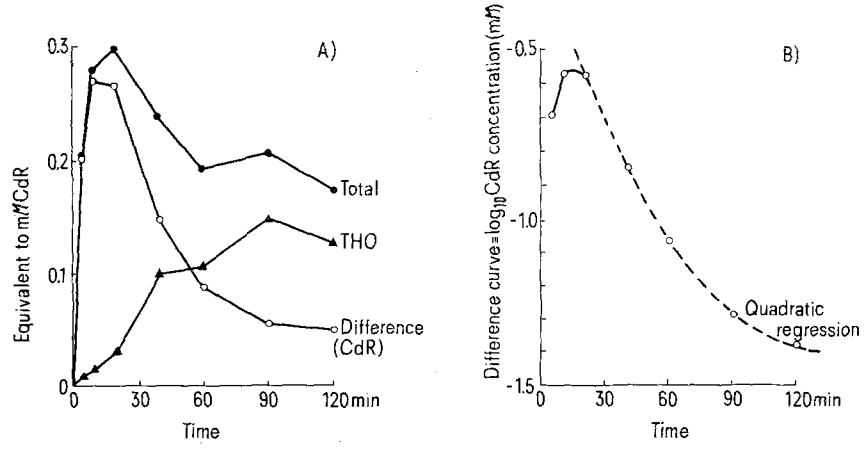

Fig. 4. A) Clearance of CdR from ROS blood of female mice. B) Same data, plotted as $\log_{10}$ concentration with fitted quadratic regression (3 mice per point).

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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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[19] This work was supported financially by the North West Cancer Research Fund (Great Britain).

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).