with the former reagent there is an incorporation of the L-amino acid in the $[Fe_3O]^{7+}$ unit while with the latter no incorporation of L-amino acid in the $[Fe_3O]^{7+}$ unit is observed.

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RNA polymerase activities in the isolated perfused rat liver

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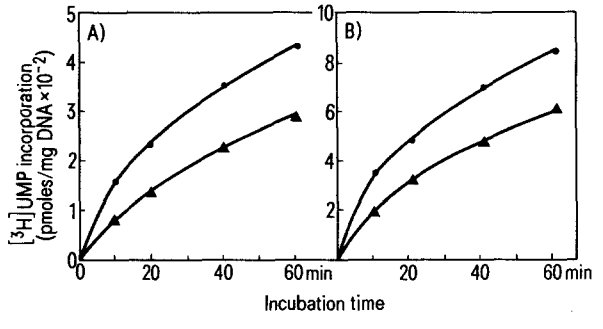
Summary. The routine procedure for the isolation of rat liver induces a significant fall in RNA polymerase I and II activities which are rapidly restored to the control levels during perfusion.

The technique of isolated liver perfusion offers a convenient model of an integrated metabolic system where cell and tissue organization is preserved, and regulatory mechanisms are still operating under physiological conditions, but without the interference of other organs[1]. In addition, the perfusion of liver with a recirculated medium offers the advantage over in vivo studies that serial sampling of the perfusion fluid can be made and portions of the liver can be removed at any stage during the perfusion for biochemical determinations and for ultrastructural analysis[1,2].

Recently it has been demonstrated that the isolated perfused liver technique is suitable for studying hepatic RNA synthesis as evaluated by the incorporation of orotic acid into different species of RNA[3-5]. In this paper we report studies on the levels of nuclear RNA polymerase I and II activities (the enzymes responsible for rRNA and mRNA synthesis, respectively) during perfusion of the isolated rat liver.

Our results demonstrate that at the start of perfusion the levels of RNA polymerase I and II activities are significantly below control values and are rapidly restored during perfusion.

Materials and methods. Male Wistar rats (160–200 g), fasted overnight, were anesthetized with Pentothal (50 mg/kg b.wt) and opened for liver isolation and perfusion[5]. The perfusion medium was Krebs-Ringer bicarbonate buffer, pH 7.4, containing bovine serum albumin (3%, w/v), and washed rat erythrocytes to give a haematocrit of 20%. The perfusate also contained: glucose 50 mg, heparin 5000 units, penicillin 1500 units, streptomycin 1.5 mg, lactate 1 mM, pyruvate 0.1 mM and an amino acid mixture corresponding to the normal rat plasma concentration in a final volume of 50 ml[6]. In addition, from the start of perfusion a concentrated amino acid solution was continuously infused into the perfusate reservoir to avoid a decrease in the perfusate amino acid concentration during



Time-course of nuclear RNA polymerase I (A) and II (B) activities in nuclei isolated from the liver portion removed at the start of perfusion (▲) and from the remaining liver perfused for 4 h (●). RNA polymerase activities are expressed as [^{3}H] UMP pmoles/mg DNA/20 min. The values represent the arithmetical mean of 8 experiments.

Nuclear RNA polymerase I and II activities in nuclei isolated from perfused rat livers and controls

Experimental conditions	RNA polymerase I	RNA polymerase II
Control (4)	218±36	421±71
Liver sample removed at the start of perfusion (15)	118±14	288±37
Lobectomized liver perfused for 2 h (3)	193±38	383±49
Lobectomized liver perfused for 4 h (13)	210±25	432±46
Intact liver perfused for 4 h (8)	196±32	454±51

RNA polymerase activities are expressed as [^{3}H] UMP pmoles/mg DNA/20 min. The values represent the arithmetic mean ± SD of the number of experiments given in parentheses. Control values are from livers rapidly removed from rats stunned and killed by decapitation.

the experiment. The rate of infusion was selected to supply the liver with the normal rat plasma amino acid level every 15 min. Liver viability was checked by bile production and urea and glucose output in the perfusate. The oxygenation state of the liver was evaluated by the lactate/pyruvate ratio in the perfusion medium[7]. The perfusion was carried out at 37 °C for 4 h with a flow rate of 1.5 ml/g/min. When indicated, at the start of perfusion a portion of the median lobe (approximately 20% of the liver) was removed and rapidly frozen. The perfusion of lobectomized livers was continued for 2 or 4 h. At the end of perfusion all livers were washed with 30 ml of ice-cold 0.25 M sucrose, pH 7.5, and rapidly frozen. Livers rapidly removed from rats stunned and killed by decapitation were used as controls. RNA polymerase activities were determined using liver nuclei as previously described[8]. DNA was determined by the method of Burton[9].

Results and discussion. The figure shows the time-course of RNA polymerase I and II activities in nuclei isolated from the liver portion removed at the start of perfusion and from the remaining liver perfused for 4 h. It can be seen that the 4-h perfusion of rat liver results in a net increase in the level of RNA polymerase I and II activities when compared to the polymerase levels of the liver portion removed at the start of perfusion.

The results shown in the table clearly demonstrate that the increase in polymerase activities during perfusion is not due to the initial removal of a liver portion since the RNA polymerase levels of the intact liver perfused for 4 h equal those of the initially lobectomized perfused liver. However, RNA polymerase levels in the liver portion removed at the start of perfusion are significantly below RNA polymerase levels in normal rat liver. These data could indicate that during the routine procedure for the isolation of rat liver there is a significant fall in RNA polymerase I and II activities which is followed by a rapid restoration to the level observed in control liver. As can be seen in the table, the recovery of RNA polymerase I and II activities during liver perfusion is rather rapid, appearing as early as 2 h after the start of perfusion. Moreover, this effect seems to be related to liver viability. Actually we have observed that when liver perfusion was accompanied by any procedure which diminished the portal blood supply or elevated the lactate/pyruvate ratio in the perfusate the restoration of RNA polymerase activities did not occur.

The fall in RNA polymerase activity is concomitant with the slight but significant disaggregation of rat liver polysomes observed by others during routine surgery for the preparation of rat liver for perfusion[3,10,11]. Nevertheless, while disaggregation of polysomes is counteracted by high levels of amino acids in the perfusing medium, we have observed that RNA polymerase restoration also takes place without amino acid addition to the perfusate (data not shown).

Clearly, the mechanism responsible for the variations of RNA polymerase activities reported above remains to be elucidated. However, the possibility that other enzyme activities may be affected in a similar fashion during rat liver isolation and perfusion cannot be excluded. In the light of this fact, when a liver sample is removed at the start of perfusion and used as a reference for the remaining tissue a comparison is being made between samples of the same liver which are in different functional states.

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Karyomorphology of two species of *Tor* (Pisces; Cyprinidae) with a high number of chromosomes

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Summary. The karyotypes of *Tor khudree* and *Tor tor*, occurring in the Himalayan streams in India, are described. As reported earlier for *T. putitora*, the diploid complements in these 2 congeneric species also comprise 100 chromosomes. The modal number being 2n = 50 in this family, *Tor* appears to be of tetraploid origin.

The occurrence of 100 chromosomes in the hillstream cyprinid *Tor putitora*[2] led me to examine cytologically some congeneric species of *Tor* to see if they also possessed a similarly high number of chromosomes. The present report embodies findings on the somatic chromosomes of 2 species of *Tor*, viz. *khudree* and *tor*.

Materials and methods. 7 adult specimens, 4 males and 3 females, of *Tor khudree* and 5 adult specimens, 3 males and 2 females, of *Tor tor* were captured respectively from the Tawi river off Jammu in the Jammu and Kashmir State and the Bhimtal lake in the Uttar Pradesh State in India. The colchicinized specimens were processed for observation of the somatic and germinal chromosomes by employing the citrate flame-drying method described elsewhere[3]. The morphology of the chromosomes has been described following Levan et al.[4]. In the karyotypes (figs 1 and 2) the biarmed (meta-, submeta- and subtelo-centric = M, SM, ST) and the rod-shaped (acrocentric = A) chromosomes have been arranged separately in decreasing order of lengths.

Results. As the preparations from kidney in *T. khudree* and gill materials in *T. tor* happened to yield better spreads, suitable for morphometrical analysis, kidney metaphase complements in the former and gill metaphase complements in the latter species were analyzed. The metaphase complements in the majority of cells examined in both *T. khudree* and *T. tor* had 100 chromosomes although the numbers varied between 96 and 104 in some others (table). Karyotypes of kidney metaphase complements of *T. khudree* (fig. 1) consisted of 50 pairs of chromosomes comprising 8 pairs of M (Nos. 5, 9, 10, 12, 13, 16, 20, 24), 14 pairs of SM (Nos. 1–4, 6–8, 11, 14, 17, 18, 21–23), 3 pairs of ST (Nos. 15, 19, 25) and 25 pairs of A (Nos. 26–50) chromosomes in both the sexes. Karyotypes of gill metaphase