showed an extra CO absorption peak at 1742 cm^{-1}. The MS and IR data suggested the presence of an acetate group in A. The final structure of A was determined on the basis of ^{1}H-NMR study. The PMR-spectra of A and B showed some resemblance, although several significant differences were observed. In the spectrum of A an A_3X quartet (J = 7 Hz) centred at 6.36 (δ in ppm), a singlet (3H) at 2.05 and a doublet at 1.60 (J = 7 Hz) were detected, whereas in the spectrum of B an A_3X_2 quartet at 2.75 (J = 7.2 Hz) and a triplet at 1.13 (J = 7.2 Hz) were measured. The CH_3 signal at 2.05 was absent in this spectrum. It was concluded from the PMR and spectral data that A can only have the structure shown in the figure (2,7-dimethoxy-5-hydroxy-6-(1-acetoxyethyl)-1,4-naphthoquinone). Since the molecule has an asymmetric C atom, it was interesting to investigate the optical activity of the pigment. A specific rotation of −46.0° was determined. The stereochemistry at the asymmetric C atom has remained unsolved. A good distinction between the 2 compounds can also be obtained by gas-liquid chromatography without derivatization. Retention times of 3.80 and 4.75 min were measured for B and A respectively using a 1.7 m glass column packed with 3% SE-30 on Gas-Chrom Q (225 °C isothermally; gas flow rate 30 ml/min).

Compound A was the major pigment of the strain M 56, whereas B was the main product of CBS 131.78. The yield was 38 mg A and 103 mg B from 1.47 g EtOAc extract of CBS 131.78 and 660 mg A and 305 mg B from 3.4 g EtOAc extract of M. 56. Other strains of *H. toruloidea* were also examined for their capability of forming A and B. The results are summarized in the table. Recently Campbell and Mulder[6] described the strains M 38, M 48, M 52 and M 56 (type culture) and some other isolates as *Scytalidium hyalinum*. The genus *Scytalidium* was chosen since the isolates exhibited the same mode of conidial production as *S. lignicola viz.* arthroconidia. They also suggested that the 'torula' state of *H. toruloidea* would be better placed in *Scytalidium*. A pycnidial form which is shown by the more typical strains of *H. toruloidea* has not been seen in *S. hyalinum*.

Physico-chemical data. Compound A: m.p. 160–163 °C (dec.), $[\alpha]_{589}^{25} = -46.0°$ (c 0.3 in $CHCl_3$); mol. wt 320.0912, calc. for $C_{16}H_{16}O_7$ 320.0896; transition $320^+ \rightarrow 260^+ + 60$ (AcOH) found 260.0702, calc. 260.0685; λ_{max} (MeOH): 220 (logε 4.51), 257 sh (4.14), 263 (4.15), 306 (3.97), 425 nm (3.55); ν_{max} (CCl_4): 1742, 1690, 1632 cm^{-1}; PMR (90 MHz, $CDCl_3$): 1.60 ($-CH_3$, d, J = 7 Hz), 2.05 ($-CO-CH_3$, s), 3.90 ($-OCH_3$, s), 4.00 ($-OCH_3$, s), 6.01 (<C=CH-, s), 6.36 (<CH-, q, J = 7 Hz), 7.23 (aromatic H, s), 12.80 δ_{TMS} (-OH, s).

Compound B: m.p. 187–190 °C; mol. wt 262.0864, calc. for $C_{14}H_{14}O_5$ 262.0841; λ_{max} (MeOH): 220.5 (logε 4.47), 258 sh (4.22), 263.5 (4.24), 306 (4.00), 424 nm (3.64); ν_{max} (CCl_4): 1688, 1632 cm^{-1}; PMR (90 MHz, $CDCl_3$): 1.13 ($-CH_3$, t, J = 7.2 Hz), 2.75 ($-CH_2-$, q, J = 7.2 Hz), 3.90 ($-OCH_3$, s), 3.97 ($-OCH_3$, s), 5.99 (<C=CH-, s), 7.20 (aromatic H, s), 12.50 δ_{TMS} (-OH, s).

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Effect of tryptophan on hepatic nuclear free and engaged RNA-polymerases in young and adult rats

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Summary. Whereas in young rats (2 weeks old), administration of typtophan produced marked enhancement in the activity of both engaged and free polymerases of nuclei, in adult rats (10 weeks old) only the engaged polymerases showed higher activities following tryptophan force-feeding.

In the light of recent observations that a single tube-feeding of L-tryptophan enhances hepatic protein synthesis[2–5], several investigators, besides ourselves, have studied the activity of hepatic nucelar RNA polymerases[6–8]. The results show that tryptophan stimulates both Mg^{2+} (polymerase I) and $Mn^{2+}/(NH_4)_2SO_4$-dependent (polymerase II) activities. However, in these experiments endogenous DNA-template-directed RNA synthesis in nuclei was measured, which essentially reflects the activity of different polymerases, bound to chromatin. In recent years it has been demonstrated that, in intact liver, nuclei RNA polymerases exist both as 'engaged' (chromatin-bound) and 'free' states[9–11], and free polymerase population can account for as much as 50% of the total polymerase activity[11]. In view of large populations of 'engaged' and 'free' RNA polymerases in nuclei, we decided to investigate the activity of different polymerases in these 2 population groups in young and adult rat livers after a single tube-feeding of tryptophan.

Materials and methods. Young and adult (2 and 10 weeks old) male Wistar rats were fasted for 24 h, and were then fed by stomach-tube either L-tryptophan (30 mg/100 g) or an equivalent volume of water, and killed 1 h later. Livers were excised and homogenized immediately in 10 vols. of cold 2.3 M sucrose 3.3 mM $CaCl_2$. Nuclei were isolated according to the procedure of Yu and Feigelson[12]. DNA concentration was measured by the method of Burton[13].

RNA-polymerase activity was measured in a 0.25 ml incubation medium containing (μmole): Tris-HCl (pH 8.0), 25, 2-mercaptoethanol 5; $MnCl_2$ 0.4; $(NH_4)_2S/_4$ (pH 8.0 with NH_3) 16.25; each of ATP, GTP and CTP 0.0625; UTP 0.00625; and 0.5 μCi of [5,6-^{3}H]UTP (41 Ci/mmole, Radiochemical Centre, Amersham, England.) When required, as in the case for exogenous template, 4 μg actinomycin-D and 10 μg poly (dA-dT) were also added to the incubation medium; α-amanitin was added at a concentration of 2 μg/ml. The reaction was initiated by addition of 0.05 ml of nuclei and incubated for 10 min at 37 °C. The reaction was terminated by addition of $Na_4P_2O_7$ and trichloroacetic acid (TCA) as described earlier[14]. The acid-insoluble material was collected on glass fibre filter (GF/C), and processed for radioactivity measurements as described previously[15].

Results and discussion. In the present investigation, all incubations were performed in the presence and absence of α-amanitin, a fungal toxin which specifically inhibits polymerase II activity[16,17]. Although polymerase III activity is

resistant to the concentration of α-amanitin used in the present study, nuclei have been reported to contain 5–10 times more plymerase I activity than polymerase III[18]. Thus in the presence α-amanitin, polymerase activity in isolated nuclei was considered as mostly due to polymerase I. The activity of polymerase II was taken as the difference between RNA synthesis in the absence (total polymerase) and presence (polymerase I) of α-amanitin. In the present study, addition of α-amanitin caused a 35–50% inhibition, regardless of whether RNA synthesis in nuclei was directed by endogenous DNA template or exogenous poly(dA-dT), reflecting the activity of nuclear engaged and free polymerases, respectively. This observation is similar to that earlier reported by Yu[10], suggesting further that both engaged and free RNA polymerases are, in fact, derived from a single population of RNA polymerase molecules.

Table 1. Effect of a single tube-feeding of tryptophan on the activity of engaged RNA-polymerases in young and adult rat liver nuclei

Rats and treatment	RNA-polymerase activity pmoles [^{3}H]UMP incorporated/mg DNA		
	Minus α-amanitin (total)	Plus α-amanitin (polymerase I)	Polymerase II
Young (2-week): control	158 ± 10 (100)	101 ± 15 (100)	57 (100)
Tryptophan	422 ± 22* (267)	278 ± 24* (275)	144 (253)
Adult (10-week): control	284 ± 30 (100)	182 ± 15 (100)	102 (100)
Tryptophan	459 ± 6* (162)	298 ± 17* (164)	161 (158)

In each group, livers from 3 to 6 rats were pooled for isolation of nuclei. RNA-polymerase activities were measured in the presence of endogenous DNA template as described in Materials and methods. The results are expressed as means ± SEM except for polymerase II which were calculated from the mean values. The figures in the parentheses represent percentage of the respective water-fed control. * Statistically different compared to the respective water-fed control at the level of $p < 0.001$ as judged by Student's t-test.

Table 2. Effect of a single tube-feeding of tryptophan on the activity of free RNA-polymerases in young and adult rat liver nuclei

Rats and treatment	RNA-polymerase activity pmoles [^{3}H]UMP incorporated/mg DNA		
	Minus α-amanitin (total)	Plus α-amanitin (polymerase I)	Polymerase II
Young (2-week): control	294 ± 40 (100)	170 ± 5 (100)	124 (100)
Tryptophan	566 ± 11* (193)	314 ± 22* (185)	252 (204)
Adult (100-week): control	661 ± 6 (100)	332 ± 5 (100)	329 (100)
Tryptophan	650 ± 25 (93)	329 ± 5 (99)	322 (97)

Incubations were carried out in the presence of poly(dA-dT) and actinomycin-D as described in Materials and methods. The results are expressed as means ± SEM, except for polymerase II which were calculated from the mean values. The figures in the parentheses represent percentage of the respective water-fed control. * Statistically different compared to the respective water-fed control at the level of $p < 0.025$ or lower as judged by Student's t-test.

The results presented in table 1 reveal that administration of tryptophan to both young and adult rats increased the endogenous template-directed RNA synthesis in isolated liver nuclei in vitro, compared to the respective control, suggesting that the tryptophan-mediated stimulation of RNA synthesis could be due in part to enhanced activity of engaged polymerases. It was also observed that, whereas in the young rats the activity of liver polymerase I and II was 175 AND 153% higher than in the control, in adult rats, the activities for the same were 64 and 58% above the control. The results indicate that the responsiveness of liver to tryptophan with respect to RNA synthesis is different at various stages of development. An explanation for this phenomenon would be that, at the end of 24 h fasting, tryptophan concentration in the liver of the young animals was comparatively lower than that of the adult rat, a condition which might make the liver of the young rats more susceptible to tryptophan stimulus.

The observation of increased RNA synthesis in isolated nuclei following tryptophan administration might be due to higher capacity of DNA template for RNA synthesis or increased polymerase level. In intact nuclei, these 2 possibilities are distinguished by studying the activity of nuclear free enzymes with exogenous templates such as poly(dA-dT) in the absence of functional endogenous template which can be inhibited by addition of actinomycin-D in the reaction medium[9–11]. We employed this technique to determine whether tryptophan would alter endogenous DNA template or RNA polymerase level. It was assumed that if nuclei from tryptophan-treated livers still showed higher activities, it would indicate that the effect is upon the enzyme protein and not on the DNA template. The results presented in table 2 show that in young rat livers tryptophan increased the activity of free polymerase I and II by 85 and 104%, respectively, whereas in adult rats the activity of the enzyme remained the same as that of the control.

The present results suggest that in 2 weeks old, young rat livers the enhancement in the activity of RNA polymerases following tryptophan force-feeding could in part, may be due to higher enzyme protein level, whereas in adult rats tryptophan appears to affect the functional capacity of DNA template.

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